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Article

Diabetic Kidney Disease Associated with Chronic Exposure to Low Doses of Environmental Cadmium

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Abstract

Accumulating evidence suggests that exposure to pollution from environmental cadmium (Cd) contributes to diabetic kidney disease as indicated by albuminuria and a progressive decrease in the estimated glomerular filtration rate (eGFR). This study examined the effects of Cd exposure on eGFR and the excretion rates of albumin (E_{alb}) and β2-microglobulin ($E_{\beta 2M}$) in 65 diabetics and 72 controls. Excretion of Cd (E_{Cd}) was a measure of exposure, while excretion of N-acetylglucosaminidase (E_{NAG}) reflected the extent of kidney tubular cell injury. In participants with an elevated excretion of $E_{\beta 2M}$, the prevalence odds ratios (POR) for a reduced eGFR rose 6.4-fold, whereas the POR for albuminuria rose 4.3-fold, 4.1-fold, and 2.8-fold in those with a reduced eGFR, diabetes, and hypertension, respectively. By using covariance analysis, which adjusted for the interactions, 43% of the variation in E_{alb} among diabetics could be explained by female gender (η^2 = 0.176), E_{NAG} (η^2 = 0.162), hypertension (η^2 = 0.146), smoking (η^2 = 0.107) and body mass index (η^2 = 0.097), while the direct contribution of E_{Cd} to E_{alb} variability was minimal (η^2 = 0.005). Results from a mediation analysis inferred that Cd could indirectly contribute to albuminuria and a falling eGFR through inducing additional tubular cell injury, leading to reduced reabsorption of filtered protein, albumin and β2M included.

Keywords: albuminuria; cadmium; diabetic kidney disease; estimated glomerular filtration rate; hypertension; N-acetylglucosaminidase

1. Introduction

Prediabetes and diabetes (DM) are respectively designated when fasting plasma glucose (FPG) concentrations ≥ 110 and ≥ 126 mg/dL [1]. Persistent albuminuria and a progressive decline in an estimated glomerular filtration rate (eGFR) are the complications of diabetes, known as diabetic kidney disease (DKD) [2–4]. The severity of DKD has been based on albumin excretion rate (E_{alb}), expressed as urinary albumin-to-creatinine ratio (ACR) where microalbuminuria and macroalbuminuria are described [3,4]. Notably, however, about 30% of DKD patients had abnormal eGFR together with normal ACR figures; <20 mg/g creatinine (cr) in men and <30 mg/g cr in women [4–6]. A wide spectrum of DKD has increasingly been recognized, suggesting involvement of different pathogenic factors. Dietary exposure to low doses of the metal contaminant cadmium (Cd) has emerged as one of the potential contributors to DKD onset and its progression; the mechanism, however, remains unclear.

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The kidney and liver are the two organs in the body that produce and release glucose into the circulation [7–10]. In addition, the kidney is responsible for filtration, and retrieval of 160 to 180 g of glucose each day, mediated by the sodium glucose co-transporter 1 (SGLT1) and SGLT2 [10]. Increased abundance of the glucose transporters may raise the renal threshold for glucose excretion [8]. The deterioration of eGFR can be attenuated by SGLT2 inhibitors [11].

Acquired Cd accumulates mostly within the kidney tubular cells, where its levels increase through to the age of 50 years but decline thereafter due to its release into the urine as the injured tubular cells die [12]. Thus, excretion of Cd (E_{Cd}) reflects kidney accumulation of Cd and its nephrotoxicity [13]. A prospective cohort study causally linked a fall of eGFR at a high rate to E_{Cd} [14]. Furthermore, increased risks of prediabetes, diabetes and albuminuria were associated with E_{Cd}, while FPG correlated with E_{Cd} [15–19]. Excretion of N-acetylglucosaminidase (NAG) and kidney injury molecule-1 have also been related to E_{Cd} [20–22].

Intriguingly, the excretion rates of NAG (E_{NAG}) and β2-microglobulin ($E_{\beta 2M}$) were enhanced in DM [23–27], while glycemic risk indices were associated with an elevated E_{NAG} that preceded albuminuria [28]. This study explored how Cd exposure induces albuminuria and a falling eGFR by examining associations of E_{alb}, E_{NAG} and eGFR in Cd-exposed DM (n = 65) and Cd-exposed controls (n = 72). Also, it was to show the effects of adjusting E_{alb}, E_{NAG}, $E_{\beta 2M}$ and E_{Cd} to creatinine excretion (E_{cr}) that obscured Cd effects.

2. Results

2.1. Descriptive Data on the Controls and Diabetics

Characteristics of the controls and diabetes are enlisted in Table 1.

Table 1. Descriptive characteristics of controls and diabetics.

Variables	All subjects *n* = 137	CTRL *n* = 72	DM		*p*
			< 10 yrs, *n* = 37	≥ 10 yrs, *n* = 25	
Women, %	78.4	79.2	75.7	80.0	0.894
Smoking, %	10.4	11.1	10.8	8.0	0.905
Mean age (range), years	59.5 (41–80)	61.2 (43–80)	58.3 (42–75)	56.6 (41–78)	0.073
Mean BMI (range), kg/m²	25.6 (15–48)	24.6 (15–48)	26.7 (15–36)	26.6 (20–35)	0.016
FPG, mg/dL	130 (61)	94 (11)	172 (76)	170 (58)	<0.001
FPG ≥ 110 mg/dL, %	49.3	11.1	91.9	96.0	<0.001
FPG ≥ 126 mg/dL, %	39.6	1.4	81.1	88.0	<0.001
eGFR, mL/min/1.73 m²	79.7 (15.9)	78.5 (13.3)	83.7 (17.1)	77.2 (20.3)	0.169
[a] Reduced eGFR, %	11.9	9.7	8.1	24.0	0.116
Mean SBP (range)	138 (103–187)	134 (107–173)	140 (106–184)	144 (103–176)	0.030
Mean DBP (range)	84 (61–106)	84 (62–103)	85 (65 – 106)	86 (61–101)	0.399
Hypertension, %	54.2	44.9	54.1	80.0	0.011
$[\beta_2M]_s$, mg/L	5.96 (3.32)	5.02 (2.28)	7.44 (4.20)	6.4 (3.59)	0.022
$[\beta_2M]_u$, µg/L	67 (58)	42 (37)	84 (58)	114 (73)	<0.001
E_{Cd}/E_{cr}, µg/g cr	1.00 (1.87)	0.99 (1.94)	0.72 (1.54)	1.41 (2.11)	0.267
E_{alb}/E_{cr} (ACR), mg/g cr	39.8 (103)	12.4 (26.1)	59.8 (115)	89.2 (177)	0.002
Microalbuminuria [b], %	22.4	8.3	35.1	44.0	<0.001
Macroalbuminuria, %	3.7	0	8.1	8.0	0.046
$(E_{Cd}/C_{cr}) \times 100$, µg/L filtrate	0.87 (1.70)	0.86 (1.69)	0.55 (1.17)	1.37 (2.25)	0.215
$(E_{alb}/C_{cr}) \times 100$, mg/L filtrate	37.2 (107)	9.76 (19.2)	51.4 (96.8)	95.2 (205)	0.003
Microalbuminuria [c], %	26	12.9	33.3	52.0	<0.001
Macroalbuminuria, %	4.5	0	10.8	8	0.023

CTRL, control; DN, diabetes; BMI, body mass index; FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DMP, diastolic blood pressure; β_2M, β2-microglobulin; Cd, cadmium;

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cr, creatinine; E_{cr}, excretion of cr; alb, albumin; C_{cr}, creatinine clearance. Continuous variables are presented as arithmetic mean and standard deviation (SD) values. [a] Low eGFR was defined as eGFR ≤60 mL/min/1.73 m^2. [b] Micro(macro)albuminuria was defined as E_{alb}/E_{cr} ≥ 20 (300) mg/g cr in men and ≥ 30 (300) mg/g cr in women. [c] Micro(macro)albuminuria was defined as E_{alb}/C_{cr} ≥ 0.2 (2) mg/L filtrate. For all tests, $p \leq 0.05$ identifies statistical significance.

This cohort had 30 men and 107 women with the overall mean age (range) 59.7 (41–80) years and the overall mean BMI (range) of 25.6 (15-48) kg/m^2. Respective percentages (%) of smoking, reduced eGFR and hypertension were 10.4, 11.9 and 54.2. Nearly half (49.3%) had FPG ≥ 110 mg/dL, while 39.6 % had FPG ≥ 126 mg/dL. The overall % E_{cr}-based albuminuria was 22.4 and 26 for C_{cr}-based albuminuria.

Across the three study groups, % smoking and reduced eGFR were similar, while % hypertension was highest, middle, and lowest in the ≥ 10-yr DM (80), < 10-yr DM (54.1) and CTRL (44.9). C_{cr}-based microalbuminuria was highest, middle, and lowest in the ≥ 10-yr DM (52), <10-yr DM (33.3) and CTRL (12.9), while % macroalbuminuria were 0, 10.8 and 8. The variations in age, eGFR, DBP, E_{Cd}/E_{cr}, and E_{Cd}/C_{cr} across the three groups were insignificant. The variables showing significant variations across the three study groups were BMI, FPG, SBP, $[\beta_2M]_s$, $[\beta_2M]_u$, E_{alb}/E_{cr} , and E_{alb}/C_{cr}.

2.2. Comparing Cd Exposure and other Measured Variables in Three Study Groups

The distribution of E_{Cd}, eGFR, FPG ($[Glc]_p$) and serum β_2M are presented in Figure 1.

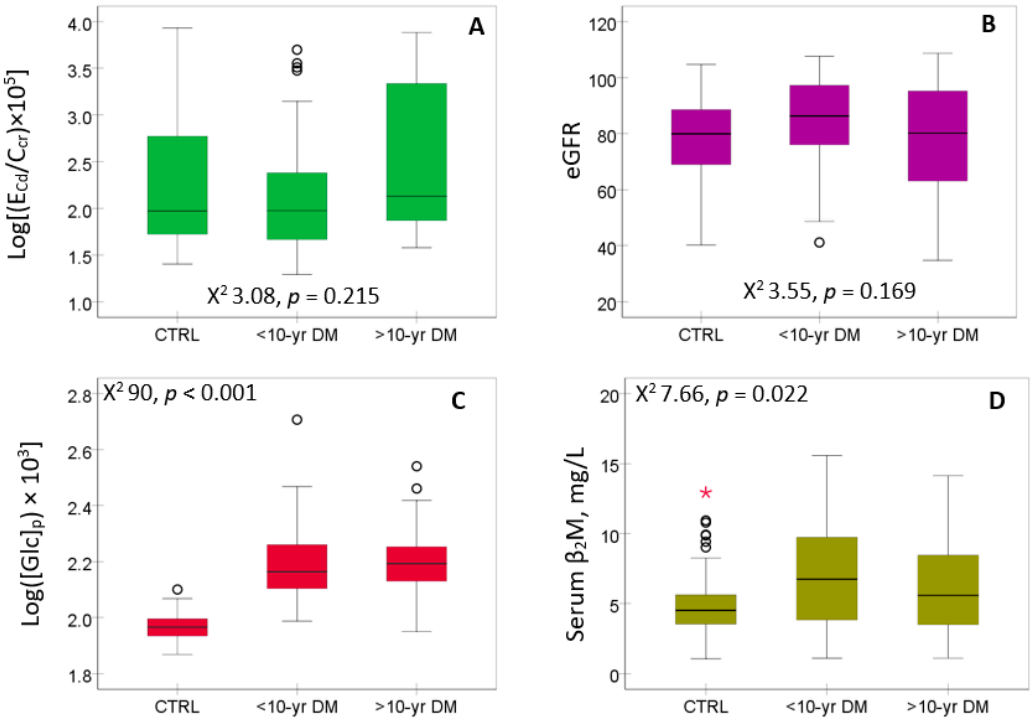

Figure 1. Urinary Cd excretion rates and levels of eGFR, FPG and serum β_2M in controls and diabetics. Boxplots of data on E_{Cd}/C_{cr} (**A**), eGFR (**B**), $[Glc]_p$ (**C**) and serum levels of β_2M (**D**) in controls and subjects with diabetes for < 10 and ≥ 10 years, respectively. Each box represents 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.

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Neither E_{Cd}/C_{cr} (Figure 1A) nor eGFR (Figure 1B) show a significant variation across the three study groups, while FPG (Figure 1C) and serum β_2M (Figure 1D) were higher in diabetics than controls.

Figure 2 presents the distribution of data on kidney tubular cell injury (E_{NAG}/C_{cr}) and its function, assessed with E_{alb}/C_{cr}, $E_{\beta 2M}/C_{cr}$ and $FrTD_{\beta 2M}$.

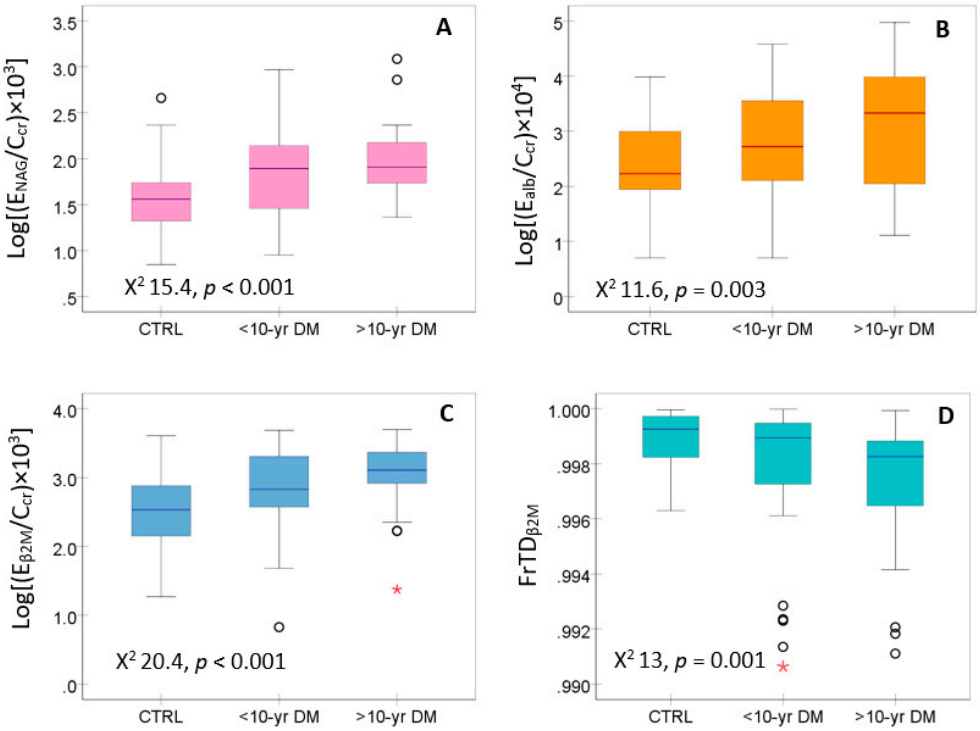

Figure 2. Excretion rates of NAG, albumin and β_2M and the fractional tubular degradation of β_2M in controls and diabetics. Boxplots of data on E_{NAG}/C_{cr} (**A**), E_{alb}/C_{cr} (**B**), $E_{\beta 2M}/C_{cr}$ (**C**) and $FrTD_{\beta 2M}$ (**D**) in controls and subjects with DM for < 10 and ≥ 10 years, 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.

E_{NAG}/C_{cr} (Figure 2A), E_{alb}/C_{cr} (Figure 2B) and $E_{\beta 2M}/C_{cr}$ (Figure 2C) were elevated in the diabetics, compared to controls, while the $FrTD_{\beta 2M}$ was reduced in the diabetics compared to controls (Figure 2D).

2.3. Logistic Regression Modeling of Albuminuria and reduced eGFR

Logistic regression model analysis was used to define the determinants of the prevalence odds ratios (POR) for albuminuria and reduced eGFR using C_{cr}-based data (Table 2).

Table 2. Determinants of the prevalence odds ratios for albuminuria and reduced eGFR.

Independent Variables	Albuminuria [a]		Reduced eGFR [b]	
	POR (95% CI)	*p*	POR (95% CI)	*p*
Age, years	0.988 (0.936, 1.043)	0.667	1.153 (1.055, 1.260)	0.002
BMI	0.988 (0.899, 1.086)	0.799	1.049 (0.920, 1.197)	0.473
Log[(E_{Cd}/C_{cr})], µg/L filtrate	0.870 (0.471, 1.608)	0.658	1.542 (0.679, 3.502)	0.301
Smoking	0.384 (0.062, 2.384)	0.304	1.866 (0.119, 29.24)	0.657
Gender	2.749 (0.769, 9.831)	0.120	3.195 (0.218, 46.85)	0.397
Reduced eGFR	4.294 (1.185, 15.56)	0.027	–	–

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Diabetes	4.081 (1.601, 10.40)	0.003	1.883 (0.509, 6.969)	0.343
Hypertension	2.786 (1.091, 7.114)	0.032	–	–
$E_{\beta 2M}/C_{cr} \geq 3$ μg/L filtrate	–	–	6.372 (1.137, 35.71)	0.035

[a] Albuminuria was based on $E_{alb}/C_{cr} \geq 0.2$ mg/L filtrate in women and men. [b] Reduced eGFR was defined as eGFR ≤ 60 mL/min/1.73 m². POR, prevalence odds ratios; CI, confidence interval; BMI, body mass index; Cd, cadmium; E_{Cd}, excretion of Cd; cr, creatinine; Ccr, creatinine clearance; β₂-microglobulin, β_2M.

Age, BMI, E_{Cd}/C_{cr}, smoking and gender had little effects on the POR for albuminuria. However, this parameter was increased 4.3-fold, 4.1-fold, and 2.8-fold in those with reduced eGFR ($p = 0.027$), diabetes ($p = 0.020$), and hypertension ($p = 0.032$). Age and $E_{\beta 2M}/C_{cr} \geq 3$, μg/L filtrate were associated with 1.15-fold and 6.4-fold increases in the POR for reduced eGFR, respectively.

For E_{cr}-based data (Table S1), the POR for albuminuria was increased 5.7-fold, 7.1-fold, and 3.7-fold in women ($p = 0.015$), those with diabetes ($p < 0.001$) and hypertension ($p = 0.027$). Notably, however, an association of E_{cr}-based albuminuria with reduced eGFR did not reach a statistically significance level ($p = 0.052$). Age was the only variable associated with reduced eGFR (POR 1.052, $p = 0.002$) (Table S2).

Results of covariance analysis of E_{alb} and eGFR are provided in Tables 3 and 4.

Table 3. Covariance analysis for albumin excretion rates.

Independent variables	Log_{10} [(E_{alb}/C_{cr})], μg/L filtrate							
	CTRL, $n = 44$		DM, $n = 56$		Women, $n = 77$		Men, $n = 23$	
	η^2	p	η^2	p	η^2	p	η^2	p
Age	0.073	0.117	0.016	0.395	0.007	0.493	0.010	0.709
BMI	0.050	0.196	0.097	0.033	0.002	0.724	0.011	0.699
E_{Cd}/C_{cr}	0.076	0.110	0.005	0.652	0.000028	0.965	0.038	0.470
$FrTD_{\beta 2M}$	0.228	0.004	0.018	0.365	0.008	0.466	0.050	0.405
$Log_{10}[(E_{NAG}/C_{cr})]$	0.061	0.153	0.162	0.005	0.054	0.050	0.268	0.040
Gender	0.115	0.046	0.176	0.003	–	–	–	–
Smoking	0.194	0.008	0.107	0.025	–	–	0.000044	0.981
HTN	0.210	0.006	0.146	0.008	0.121	.003	0.109	0.212
Gender × HTN	0.044	0.226	0.122	0.016	–	–		
Smoking × HTN	0.021	0.408	0.178	0.003	–	–	0.035	0.489
Unadjusted (adjusted) R^2	0.422 (0.246)	0.028	0.535 (0.431)	<0.001	0.237 (0.172)	0.003	0.434 (0.111)	0.301

η^2 = eta squared; HTN, hypertension; ×, represents an interaction term; R^2, coefficient of determination. η^2 indicates the fraction of E_{alb}/C_{cr} variation explained by each independent variable. Adjusted R^2 indicates the fraction of total variation of E_{alb}/C_{cr} explained by all independent variables. For all tests, p-values ≤ 0.05 indicate statistically significant levels.

Table 4. Covariance analysis for eGFR.

Independent variables	eGFR							
	CTRL, $n = 44$		DM, $n = 56$		Women, $n = 77$		Men, $n = 23$	
	η^2	p	η^2	p	η^2	p	η^2	p
Age	0.026	0.364	0.183	0.003	0.099	0.008	0.019	0.626
BMI	0.005	0.694	0.005	0.653	0.018	0.265	0.047	0.439
E_{Cd}/C_{cr}	0.063	0.151	0.009	0.520	0.008	0.445	0.066	0.357
$Log_{10}[(E_{NAG}/C_{cr})]$	0.301	0.001	0.199	0.002	0.222	<0.001	0.307	0.032
$Log_{10}[(E_{\beta 2M}/C_{cr})]$	0.018	0.454	0.140	0.010	0.072	0.023	0.031	0.532
FPG	0.013	0.526	0.054	0.119	0.109	0.005	0.147	0.158
Gender	0.053	0.192	0.009	0.520	–	–	–	–
Smoking	0.058	0.169	0.019	0.366	–	–	0.032	0.527
HTN	0.005	0.680	0.020	0.348	0.014	0.324	0.004	0.829

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Gender × HTN	0.005	0.698	0.003	0.741	–	–	–	–
Smoking × HTN	0.016	0.476	0.049	0.140	–	–	0.110	0.227
Unadjusted (adjusted) R^2	0.510 (0.341)	0.007	0.683 (0.603)	<0.001	0.564 (0.520)	<0.001	0.562 (0.258)	0.151

eGFR, estimated glomerular filtration rate; η^2 = eta squared; HTN, hypertension; ×, represents an interaction term; R^2, coefficient of determination. η^2 indicates the fraction of the eGFR variation explained by a corresponding independent variable. Adjusted R^2 indicates the fraction of total variation in eGFR explained by all independent variables in the first column. For all tests, p-values ≤ 0.05 indicate statistically significant levels.

As shown in Tables 3 and 4, variables altogether explained significant fractions of E_{alb} and eGFR variability in CTRL, DM, and women, but they explain a little E_{alb} variation in men was insignificant. Results of covariance analyses of E_{alb} and eGFR using E_{cr}-based data are provided in Tables S3 and S4.

In CTRL (Table 3), E_{alb} was influenced by $FrTD_{\beta 2M}$ ($\eta^2 = 0.228$), hypertension ($\eta^2 = 0.210$). smoking ($\eta^2 = 0.194$) and gender ($\eta^2 = 0.115$). In DM, E_{alb} in diabetes was influenced by gender ($\eta^2 = 0.176$), E_{NAG} ($\eta^2 = 0.162$), hypertension ($\eta^2 = 0.146$), smoking ($\eta^2 = 0.107$), and BMI ($\eta^2 = 0.097$), while E_{Cd} and $FrTD_{\beta 2M}$. showed minimal effects.

In CTRL (Table 4), E_{NAG} explained the largest fraction eGFR variation ($\eta^2 = 0.301$). The eGFR variation among DM was explained by E_{NAG} ($\eta^2 = 0.199$), $E_{\beta 2M}$ ($\eta^2 = 0.183$) and age ($\eta^2 = 0.140$). In women, eGFR was explained by E_{NAG} ($\eta^2 = 0.222$), FPG ($\eta^2 = 0.109$), and $E_{\beta 2M}$ ($\eta^2 = 0.072$).

2.4. Bivariate Analysis of E_{alb}

Table 5 provides results of the Spearman's rank correlation analysis.

Table 5. Bivariate relationship analysis of DKD indicators.

Variables	Spearman's rank correlation coefficient							
	E_{Alb}/C_{cr}	Age	BMI	eGFR	FPG	E_{Cd}/C_{cr}	E_{NAG}/C_{cr}	$E_{\beta 2M}/C_{cr}$
Age	0.085							
BMI	0.076	−0.262**						
eGFR	−0.136	−0.356**	0.161					
FPG	0.273**	−0.222**	0.184*	0.089				
E_{Cd}/C_{cr}	0.106	0.078	−0.083	−0.227**	0.166			
E_{NAG}/C_{cr}	0.327**	−0.115	0.002	−0.467**	0.278**	0.328**		
$E_{\beta 2M}/C_{cr}$	0.265**	0.170*	−0.066	−0.515**	0.306**	0.496**	0.534**	
$FrTD_{\beta 2M}$	−0.237**	−0.096	0.048	0.434**	−0.215*	−0.527**	−0.536**	−0.891**

BMI, body mass index; eGFR, estimated glomerular filtration rate FPG, fasting plasma glucose concentration; $FrTD_{\beta 2M}$, fractional tubular degradation of β_2-microglobulin. * Significant correlation at the 0.05 level. ** Significant correlation at the 0.01 level.

E_{alb} varied inversely with $FrTD_{\beta 2M}$ ($r = -0.237$) and directly with FPG ($r = 0.273$), E_{NAG} ($r = 0.327$) and $E_{\beta 2M}$ ($r = 0.265$). A correlation between E_{alb} and E_{Cd} was insignificant. Apparently, the strength of E_{alb}/E_{NAG} correlation was the highest, compared to other 4 variables tested. Consequently, scatterplots and regression analysis were used to further examined E_{alb} and E_{NAG}/C_{cr} relationship in subgroups (Figure 3).

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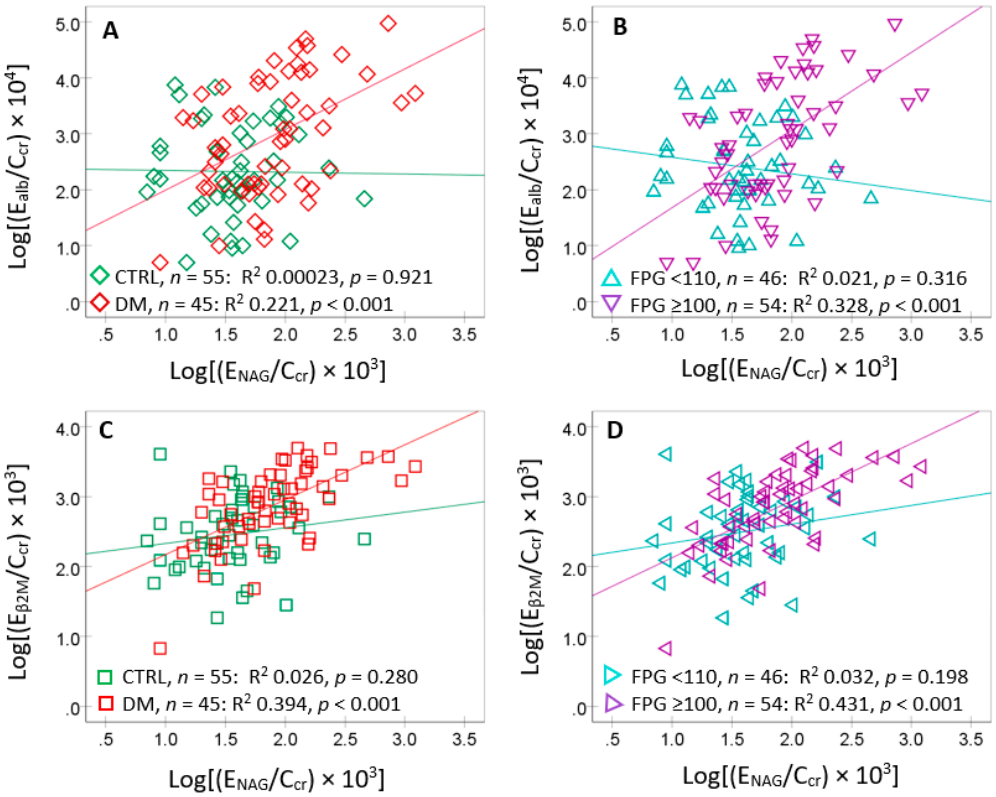

Figure 3. Excretion of albumin and β2M in relation to the excretion of NAG. Scatterplots related E_{alb}/C_{cr} to E_{NAG}/C_{cr} in controls and diabetics (**A**) and those with FPG <110 and ≥110 mg/dL (**B**). Scatterplots related $E_{\beta 2M}/C_{cr}$ to E_{NAG}/C_{cr} to controls and diabetics (**C**) and those with FPG <110 and ≥110 mg/dL (**D**).

E_{alb} showed a moderate association with E_{NAG} in diabetics but not in controls (Figure 3A). It was strongly associated only with those who had FPG ≥110 mg/dL (Figure 3B). E_{alb} showed a strong association with $E_{\beta 2M}$ in diabetics (Figure 3C) and those with FPG ≥110 mg/dL (Figure 3D). The associations of E_{alb} with $E_{\beta 2M}$ in controls and those with FPG <110 mg/dL were insignificant (Figure 3C,D).

Given the particularly strong correlation strengths of eGFR versus E_{NAG} (r = −0.467) in the correlation analysis (Table 5), scatterplots and regression analysis were used to further examines the correlations of eGFR with E_{NAG}/C_{cr} in subgroups (Figure 4).

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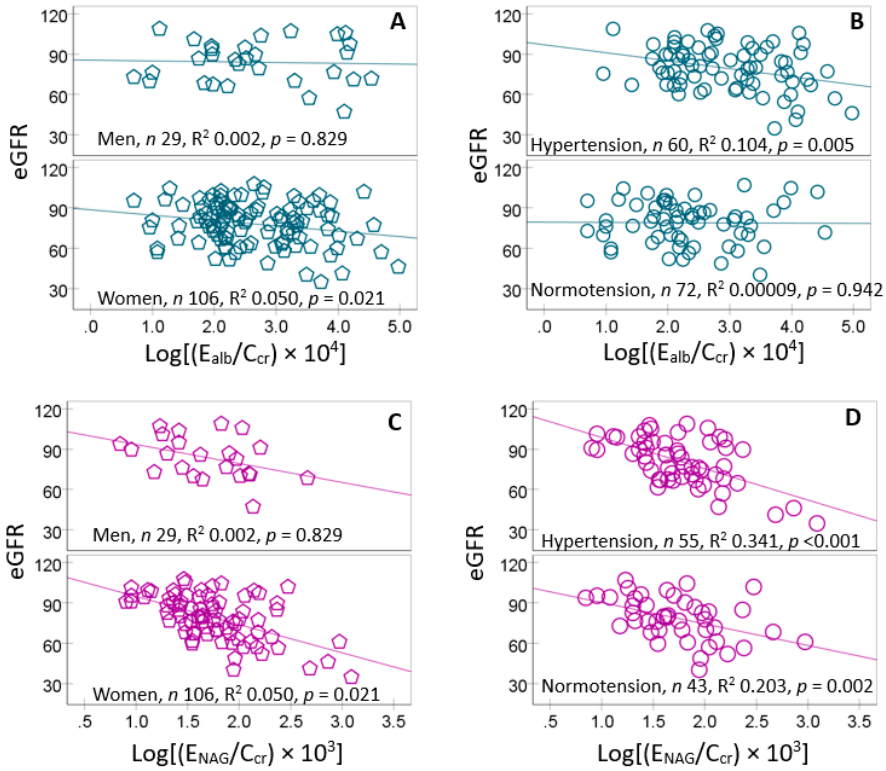


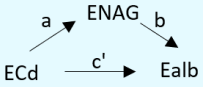
Figure 4. Reduction in eGFR in relation to the excretion of albumin and NAG. Scatterplots related eGFR to E_{alb}/C_{cr} in men and women (**A**) subjects with and without hypertension (**B**). Scatterplots related eGFR to E_{NAG}/C_{cr} in men and women (**C**) subjects with and without hypertension (D).

eGFR was inversely associated with E_{alb} only in women (Figure 4A) and those with hypertension (Figure 4B). It showed also inverse association with E_{NAG} in women only (Figure 4C). In those with hypertension eGFR showed a more closely inverse association with E_{NAG}, compared to the normotensive group.

2.5. The Indirect Effects of Cd on E_{alb} and eGFR

Because Cd explain minimally the variations in E_{alb} and eGFR (Tables 4 and 5), a simple mediation model analysis was conducted to determine whether Cd could indirectly influence E_{alb} and eGFR (Figure 5).

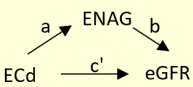
Model A



Sobel test			
Parameter	β	Bootstrapped 95% CI	*p*
a	0.140	0.030, 0.255	<0.008
b	0.825	0.416, 1.262	<0.001
c'	0.032	−0.187, 0.265	0.785
a*b	0.12	–	0.046

(**a**)

Model B



Sobel test			
Parameter	β	Bootstrapped 95% CI	*p*
a	0.140	0.042, 0.241	0.012
b	−19.28	−25.60, −12.04	<0.001
c'	−1.094	−5.061, 2.754	0.583
a*b	−2.70	–	0.030

(**b**)

Figure 5. Mediating effects of Cd by tubular injury and impaired functions. The Sobel test for the indirect effects of E_{Cd} on E_{alb} (**a**) and eGFR (**b**) mediating through E_{NAG}. E_{Cd}, excretion of cadmium; E_{alb}, excretion of albumin; E_{NAG}, excretion of NAG; $E_{\beta 2M}$, excretion of β2M; CI; bootstrapped confidence interval.

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In Figure 5, E_{NAG} was depicted as the mediator of Cd effect on E_{alb} (model A) and eGFR (model B). In both models A and B, the Sobel test of the direct effects of Cd were insignificant, reflected by β values and *p*-values for the c' parameter. The significant indirect effect (a*b) inferred that E_{NAG} mediated fully the effects of Cd on E_{alb} and eGFR (Figure 4A,B).

3. Discussion

The present Thai cohort with a modest sample size (65 DM and 72 CTRL) has provided several insights into the effects of low-dose Cd in combination with a high fasting plasma glucose (one form of metabolic stress). Results of logistic regression modeling and covariance analyses of the two major outcomes; albuminuria and a reduced eGFR have revealed the dominant tubular basis of the nephrotoxicity from a low-dose Cd.

3.1. Effects of Cd on FPG and Albuminuria

The levels of Cd exposure experienced by cohort participants were insufficient to induce DM, evident from an insignificant correlation between E_{Cd} and FPG ($r = 0.166$, $p = 0.053$) (Table 5), and the E_{Cd} geometric mean below 1 μg/g cr. A study from Korea reported an association between increased risk of DM with E_{Cd} 2-3 μg/g cr, where a significant correlation was found between E_{Cd} and FPG [15]. In a study on U.S. population, E_{Cd} of 1–2 μg/g cr was associated with 24-48% increases in risk of prediabetes and diabetes adjusting for potential confounders [16]. Furthermore, macroalbuminuria was present only in the DM group although the DM and CTRL groups both had E_{Cd} in the same range (Table 1, Figure 1). Thus, the diabetogenic effects of Cd at the exposure levels in this cohort participants appeared to be minimal.

Notably, microalbuminuria (E_{cr}-based) was found in both CTRL and DM; 8.3% of CTRL had microalbuminuria, while 35.1% of the <10-yr DM and 44% in the ≥10-yr DM had microalbuminuria (Table 1). These data suggest E_{alb} was particularly sensitive to Cd, consistent with the studies from Spain and China, where the increment of albuminuria risk was observed at E_{Cd} below 0.5 μg/g cr [18,19]. Compared to the ACR-based, the % microalbuminuria (C_{cr}-based) were higher in the CTR (12.9), the <10-yr DM (33.3), and the ≥10-yr DM (52.0) groups, indicating the tendency to underestimate the nephrotoxicity of Cd when E_{alb} was adjusted to E_{cr}, illustrated in SM (Tables S1-S4).

3.2. Albuminuria and Reduced eGFR: The Tubular Rule

In an analysis of risk factors for albuminuria (Table 2), diabetes, reduced eGFR and hypertension independently increased the risk for albuminuria. Age, BMI, E_{Cd}/C_{cr}, smoking and gender had little effects on the prevalence odds for albuminuria in the present cohort. An impaired tubular function, indicated by $E_{\beta 2M}/C_{cr} \geq 3$ μg/L filtrate and age were independent risk factors for reduced eGFR. Using Ecr-based data, the relationship between a reduced eGFR and risk of albuminuria was obscured as was the relationship between tubular dysfunction and a reduced eGFR (Tables S1 and S2).

Covariance analyses (Tables 3 and 4) provide further evidence for the tubular roles in albuminuria onset and eGFR reduction. In CTRL, $FrTD_{\beta 2M}$ accounted for the largest fraction of the variation in E_{alb} ($\eta^2 = 0.228$), while E_{NAG} explained the largest fraction of the eGFR variation ($\eta^2 = 0.301$). In DM, gender contributed the most to the variation in E_{alb} ($\eta^2 = 0.176$) followed by E_{NAG} ($\eta^2 = 0.162$), hypertension ($\eta^2 = 0.146$), smoking ($\eta^2 = 0.107$), and BMI ($\eta^2 = 0.097$). E_{NAG} explained the largest fraction of eGFR variation in DM ($\eta^2 = 0.199$), followed by $E_{\beta 2M}$ ($\eta^2 = 0.183$) and age ($\eta^2 = 0.140$).

The above findings are in line with the SPRINT Trial, where clinical and demographic characteristics were found to be associated with unique profiles of tubular damage, which indicated under-recognized patterns of kidney tubule disease among individuals with reduced eGFR [29].

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3.3. Mediation Analysis for the Indirect Effects of Cd

As shown in Table 5, E_{alb} correlated more strongly with E_{NAG} than with $FrTD_{\beta 2M}$, FPG and $E_{\beta 2M}$, while a correlation between E_{alb} and E_{Cd} was insignificant. In subgroup analysis (Figure 3), the E_{alb}/E_{NAG} association was present only in DM and those with FPG ≥ 110 mg/dL. Of relevance, a cross-sectional study from Japan (DM = 245, CTRL= 39) showed that E_{alb}/E_{cr} and E_{NAG}/E_{cr} were associated with glycemia risk index, and the elevation of E_{NAG}/E_{cr} occurred before the onset of albuminuria.

As also shown in Table 5, eGFR inversely correlated with E_{NAG} (r = −0.467), and its inverse association with E_{NAG} was found in women together with an inverse association of eGFR with E_{alb} (Figure 5). The small number of men (n = 29) compared to women (n = 106) was a likely explanation for the absence of $eGFR/E_{NAG}$ and $eGFR/E_{alb}$ associations among men. A further research study with enough men is required. By using mediation analysis (Figure 5), Cd indirectly influenced E_{alb} and eGFR, while the tubular damage, assessed with E_{NAG}, was identified as the full mediator of Cd effects on these parameters.

In summary, covariance analysis of albuminuria and a reduced eGFR suggests that the contribution of Cd exposure to albuminuria and eGFR reductions both were minimal (Tables 3 and 4). Consequently, mediation analysis was used to investigate the indirect effects of Cd (Figure 5). Results of the mediation analysis inferred that tubular injury (E_{NAG}) could mediate the Cd effects on both albuminuria and a falling eGFR. These findings underscore the essential tubular role in maintaining plasma glucose. and the injury to tubular cells from any causes can potentially influence risk of hyperglycemia. The kidney is known for its producing and releasing glucose into the circulation as well as its filtration and reabsorption of glucose, mediated primarily by SGLT2 [7–10]. In clinical trial, reduction of glucose reuptake by SGLT2 inhibitors attenuated the loss of eGFR [11].

4. Materials and Methods

4.1. Participants

Individuals with diabetes (n = 65) and non-diabetic controls (n = 72) in the present study were chosen from a pre-existing cohort of 88 persons with diagnosed diabetes and 88 without diabetes [30]. They met the enrollment criteria, which included living at current addresses for at least 40 years and attending annual health checkups at the Pak Poon municipality health center, Nakhon Si Thammarat Province, Thailand. Exclusion criteria were non-resident status, pregnancy, breast feeding and those with records of ill-health status such as heart disease, stroke, and cancer.

Participants gave written informed consent and they received study objectives, study protocols, potential risks and benefits. Sociodemographic information, educational attainment and occupation were collected using structured interview questionnaires as were health status, family history of diabetes, use of dietary supplements, alcohol consumption, and smoking status [31].

4.2. Collection, Storage and Compositional Analysis of Blood and Urine Samples

Whole blood and urine samples were collected in the morning after an overnight fast at the Pak Poon health center. Urine samples were collected in metal-free polypropylene collection cups. For the glucose assay, blood samples were collected in heparinized tubes, using fluoride as an inhibitor of glycolysis. For analysis of blood metals, ethylene diamine tetra acetic acid was an anticoagulant. All test tubes, bottles, and pipettes used in metal analysis were acid-washed and rinsed thoroughly with deionized water.

Blood and urine samples were kept on ice during transportation to the laboratory, where sample aliquots were prepared. The pH of urine aliquots was adjusted to > 6 using 1M NaOH to prevent the degradation of β_2M in acidic conditions. Aliquots of urine, whole blood, serum, and plasma were stored at −80°C for later analysis.

Graphite furnace atomic absorption spectrometry (GBC Scientific Equipment, Hampshire, IL, USA) was employed to determine Cd concentrations in urine and whole blood samples. To prepare

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samples for AAS, blood samples were deproteinized with 5% HNO_3 as previously described [32]. For the instrumental calibration, the certified reference material of nine elements (Centipur®, Merck KGaA, Darmstadt, Germany) was used. Duplicate samples were analyzed for consistency checks. Blank samples were included for contamination monitoring. Reference urine and whole blood metal control levels 1, 2, and 3 (Lyphocheck, Bio-Rad, Hercules, CA, USA) were used for accuracy and precision assurance and quality control purposes.

The limit of detection (LOD) for blood Pb was 3 µg/dL and 0.1 µg/L for blood Cd. The LOD of urinary Cd was 0.1 µg/L. For urine and blood samples containing Cd and Pb concentrations below the LOD figures, the concentrations assigned were the LOD values divided by the square root of 2 [33].

The urinary NAG assay was based on colorimetry, using 4-nitrophenyl N-acetyl-β-D-glucosaminide as a substrate (Merck KGaA, Darmstadt, Germany). Urinary and serum concentrations of β_2M were determined using the human beta-2 microglobulin/β_2M ELISA pair set (Sino Biological Inc., Wayne, PA, USA). The lower limit of β_2M detection was 3.13 pg/mL. The oxidase–peroxidase method (Glu Colorimetric Assay Kit, Elabscience, Catalog No: E-BC-K234-M, Houston, TX, USA) was used to determine plasma glucose concentrations [34]. Jaffe's alkaline picrate method was used to determine urinary and plasma creatinine concentrations [35]. All assay gave the coefficients of variation (CV) within acceptable clinical chemistry standards.

4.3. Correction for Differences in Urine Dilution

Because urine samples were collected at a single time point (voided urine), a correction for differences among people in urine volume (dilution) was undertaken. Accordingly, E_{alb}, E_{NAG}, $E_{\beta 2M}$ and E_{Cd} were normalized to creatinine clearance (C_{cr}), using the equation; $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = alb, NAG, β_2M or Cd; $[x]_u$ = 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 [36]. This C_{cr}-normalization is unaffected by variation in muscle mass, while correcting for both dilution and functioning nephrons.

E_{alb}, E_{NAG}, $E_{\beta 2M}$ and E_{Cd} were normalized to creatinine excretion (E_{cr}), using the equation; $E_x/E_{cr} = [x]_u/[cr]_u$, where x = alb, NAG, β_2M or Cd; $[x]_u$ = 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. Results of logistic regression and covariance analysis conducted with Ecr-based data are provided in SM (Tables S1–S4).

E_{cr}-normalization corrects for difference in urine dilution only and it is affected by a large variation in muscle mass, especially between men and women. E_{cr}-adjustment can create non-differential errors/imprecisions that can obscure or even nullify the dose-response relationships [37]. Previously, the risk of abnormal $E_{\beta 2M}$ values was not significantly related to E_{Cd} when E_{Cd} and $E_{\beta 2M}$ were adjusted to E_{cr} [38].

4.4. Computaion for eGFR and Fractional Tubular Degrdation of β_2M

Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations were used to compute eGFR [39]. 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 fractional tubular degradation of filtered β_2M ($FrTD_{\beta 2M}$) (equation 1) was computed using rate of glomerular filtration of β_2M ($F_{\beta 2M}$), mg/d (equation 2) and amount of β_2M undergoing tubular degradation per volume of glomerular filtrate ($TD_{\beta 2M}/C_{cr}$), mg/L filtrate) (equation 3) [40].

Equation 1: $FrTD_{\beta 2M} = (TD_{\beta 2M}/C_{cr})/[\beta_2M]_s$

Equation 2: $F_{\beta 2M} = eGFR[\beta_2M]_s$

Equation 3: $TD_{\beta 2M}/C_{cr} = [\beta_2M]_s - E_{\beta 2M}/C_{cr}$

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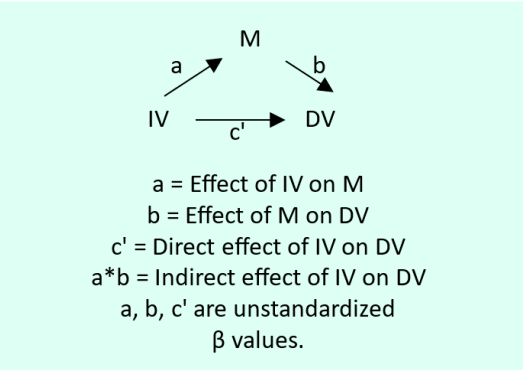
4.5. Analysis for the Indirect Effects of Cd

The Baron and Kenny method was used to examine the indirect and/or direct effects Cd on E_{alb} and eGFR [41–43]. The mediation model with one mediator is depicted in Scheme 1.



Scheme 1. A simple mediation model with one mediator. IV, independent variable; DV, dependent variable; M, mediator.

The statistical parameters a, b, and c' are β coefficients describing the effect of IV on M, the effect of M on DV and the direct effect of IV on DV, respectively. The product a*b indicates the indirect effect of IV on DV, mediated by M. The Sobel test is employed to define a statistical significance level of the indirect effect (a*b).

4.6. Statistical Analysis

Data were analyzed with IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). The Kruskal–Wallis's test was employed to assess the variation in any continuous variable across three study groups; CTRL, <10-yr DM and ≥ 10-yr DM. The Pearson chi-squared test assessed differences in percentages across the three study groups. The one-sample Kolmogorov–Smirnov test assessed deviation from a normal distribution of any continuous variable. Logarithmic transformation was applied to E_{Cd}/C_{cr}, E_{alb}/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.

The POR values for albuminuria and a reduced eGFR were obtained using logistic regression modeling, which adjusted for potential confounders (Tables 2). The univariate of analysis of covariance was undertaken to identify the contributors to the variation in E_{alb} and eGFR (Tables 3 and 4). Spearman's rank correlation analysis was used to assess bivariate relationships of nine variables: E_{alb}/C_{cr}, age, BMI, eGFR, FPG, E_{Cd}/C_{cr}, E_{NAG}/C_{cr}, $E_{\beta 2M}/C_{cr}$ and $FrTD_{\beta 2M}$ (Table 5).

5. Conclusions

Exposure to low-level Cd, insufficient to induce abnormally high plasma glucose concentrations, is not associated with an increased risk of albuminuria or reduced eGFR. However, in the presence of diabetes and/or hyperglycemia, exposure to low-level Cd could enhance albumin excretion rates, while aggravating eGFR reduction through inducing tubular cell damage and functional impairment.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to a corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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
alb	albumin
E_{alb}	urinary excretion rate of alb
NAG	N-acetylglucosaminidase
E_{NAG}	urinary excretion rate of NAG
cr	creatinine
C_{cr}	creatinine clearance
β_2M	β_2-microglobulin
$F_{\beta 2M}$	rate of glomerular filtration of β_2M
$E_{\beta 2M}$	urinary excretion rate of β_2M
$TD_{\beta 2M}$	rate of tubular degradation of β_2M
$FrTD_{\beta 2M}$	fractional tubular degradation of filtered β_2M

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