

Research Article

Open Access



Homeostasis of β_2 -microglobulin in diabetics and non-diabetics with modest cadmium intoxication

Kenneth R. Phelps¹, Supabhorn Yimthiang^{2,3}, Phisit Pouyfung⁴, Tanaporn Khamphaya³, David A. Vesey^{5,6}, Soisungwan Satarug⁶

¹Research Service, Stratton Veterans Affairs Medical Center and Albany Medical College, Albany, NY 12208, USA.

²Environmental Safety Technology and Health, School of Public Health, Walailak University, Nakhon Si Thammarat 80160, Thailand.

³Occupational Health and Safety, School of Public Health, Walailak University, Nakhon Si Thammarat 80160, Thailand.

⁴Department of Community Health, Faculty of Public Health, Mahidol University, Bangkok 20100, Thailand.

⁵Department of Kidney and Transplant Services, Princess Alexandra Hospital, Brisbane 4102, Australia.

⁶Centre for Kidney Disease Research, Translational Research Institute, Woolloongabba, Brisbane 4102, Australia.

Correspondence to: Dr. Kenneth R. Phelps, Research Service, Stratton Veterans Affairs Medical Center and Albany Medical College, Stratton VAMC, Research Service, 113 Holland Avenue, Albany, NY 12208, USA. E-mail: kennethrphelps@icloud.com

How to cite this article: Phelps, K. R.; Yimthiang, S.; Pouyfung, P.; Khamphaya, T.; Vesey, D. A.; Satarug, S. Homeostasis of β_2 -microglobulin in diabetics and non-diabetics with modest cadmium intoxication. *J. Environ. Expo. Assess.* 2025, 4, 23. <https://dx.doi.org/10.20517/jeea.2025.09>

Received: 12 Feb 2025 **First Decision:** 26 Jun 2025 **Revised:** 17 Jul 2025 **Accepted:** 30 Jul 2025 **Published:** 8 Aug 2025

Academic Editors: Ata Rafiee, Stuart Harrad **Copy Editor:** Xing-Yue Zhang **Production Editor:** Xing-Yue Zhang

Abstract

Beta-2-microglobulin (β_2 M) is released into plasma from nucleated cells, filtered by glomeruli, and degraded by proximal tubular cells (PTCs). Normally, < 1% of filtered β_2 M is excreted in urine. Intoxication of PTCs by cadmium (Cd) reduces degradation and increases excretion of β_2 M (TD_{β_2M} and E_{β_2M}). Diabetes may exacerbate these effects or produce them independently. Herein, we normalized fluxes to creatinine clearance (C_{cr}) to quantify amounts of β_2 M excreted and degraded per volume of filtrate (E_{β_2M}/C_{cr} and TD_{β_2M}/C_{cr}). We then performed a case-control study of diabetics (DM, $n = 65$) and non-diabetics (CTRL, $n = 72$) with modest Cd exposure. β_2 M influx (I_{β_2M} , equated with β_2 M filtration rate), serum β_2 M ($[\beta_2M]_s$), and TD_{β_2M}/C_{cr} were higher in DM. Fractional tubular degradation of filtered β_2 M ($FrTD_{\beta_2M}$) emerged as the least confounded descriptor of PT β_2 M processing, and low values of $FrTD_{\beta_2M}$ were seen in a subset of diabetics with minimal Cd intoxication. $FrTD_{\beta_2M}$ varied inversely with E_{Cd}/C_{cr} in DM and directly with estimated GFR (eGFR) in DM and CTRL. In both groups, E_{β_2M}/C_{cr} was inversely related to eGFR and $FrTD_{\beta_2M}$ but at any value of $FrTD_{\beta_2M}$, E_{β_2M}/C_{cr} varied by a large multiple. We conclude that in subjects with modest Cd intoxication, I_{β_2M} was variable in DM and CTRL and higher in DM; $FrTD_{\beta_2M}$ was related inversely to E_{Cd}/C_{cr} in DM and directly to eGFR in DM and CTRL; and E_{β_2M}/C_{cr} varied inversely with eGFR and $FrTD_{\beta_2M}$ in both groups. E_{β_2M}/C_{cr}



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



did not depict $\text{FrTD}_{\beta_2\text{M}}$ precisely. We recommend $\text{FrTD}_{\beta_2\text{M}}$ as an indicator of proximal tubular dysfunction.

Keywords: Cadmium, cadmium nephropathy, beta-2-microglobulin, beta-2-microglobulin influx, beta-2-microglobulin degradation, beta-2-microglobulin excretion, diabetes

INTRODUCTION

Cadmium (Cd), a ubiquitous environmental contaminant, is present in many foodstuffs^[1,2]. Most people acquire Cd primarily from the diet, and they retain it in multiple organs, most notably the kidneys and liver^[1-3]. Inhalation of cigarette smoke and polluted air are additional exposure routes^[4,5]. Within the kidneys, Cd accumulates disproportionately in proximal renal tubules, where bulk reabsorption of filtrate and its constituents occurs. In time, the metal may interfere with the reabsorption of some filtered substances^[6].

Previously, we presented evidence that Cd appears in urine after being released into filtrate from injured tubular cells^[7]. Given this evidence, we argued that the excretion rate of Cd (E_{Cd}) should be normalized to a function of *nephron mass* to depict the amount of Cd exiting the kidneys per nephron^[7]. GFR is such a function, and creatinine clearance (C_{cr}) is an attractive surrogate for GFR because $E_{\text{Cd}}/C_{\text{cr}}$ can be determined without a timed urine collection^[7,8]. $E_{\text{Cd}}/C_{\text{cr}}$ is more physiologically informative than the ratio of urine concentrations of Cd and cr ($[\text{Cd}]_{\text{u}}/[\text{cr}]_{\text{u}}$), the conventional expression of Cd excretion, because E_{cr} and thus $[\text{cr}]_{\text{u}}$ are functions of muscle mass, which is unrelated to renal Cd handling^[9].

Beta-2 microglobulin ($\beta_2\text{M}$), the light-chain component of class I histocompatibility complexes A, B, and C, is shed continuously by nucleated cells. The protein has a molecular weight of 11,800 daltons; consequently, it is extremely if not completely filterable by human glomeruli^[10-12]. In the S1 segment of the proximal tubule (PT), the brush border receptor megalin mediates endocytosis of most or all filtered $\beta_2\text{M}$; within the tubular cell, the protein undergoes lysosomal degradation, and expelled fragments are taken up in segments S2 and S3 of the PT^[13]. We recently described evidence from Cd-intoxicated subjects that filtered albumin not subjected to transcytosis in S1 is processed identically to $\beta_2\text{M}$ ^[14]. In addition, it is possible that complexes of Cd-albumin and Cd- $\beta_2\text{M}$ also undergo endocytosis through the mediation of the high-affinity/low-capacity lipocalin 2 receptor (SLC22A17) in distal tubular cells^[15,16]. Renal tubules normally process at least 99% and as much as 99.9% of filtered $\beta_2\text{M}$ ^[17-19].

For more than 55 years, increased excretion of $\beta_2\text{M}$ ($E_{\beta_2\text{M}}$) has been employed as an indicator of proximal tubular dysfunction^[20]. Indeed, if the filtration rate of $\beta_2\text{M}$ ($F_{\beta_2\text{M}}$) has not risen, the presence of excessive $\beta_2\text{M}$ in urine necessarily implies a reduction in the degradation of the protein. A report from the Food and Agriculture Organization of the United Nations provided evidence that $E_{\beta_2\text{M}} > 300$ g/g of creatinine (cr) is accompanied by $E_{\text{Cd}} > 5.24$ $\mu\text{g/g}$ creatinine (<https://apps.who.int/iris/handle/10665/44521>, accessed on January 7, 2025). The value of $[\beta_2\text{M}]_{\text{u}}/[\text{cr}]_{\text{u}}$ is widely employed to identify significant Cd nephrotoxicity. Remarkably, $\beta_2\text{M}$ degradation itself is rarely quantified in practice or research, and to the best of our knowledge, it has not been linked systematically to other parameters of $\beta_2\text{M}$ homeostasis. Proximal tubular processing of $\beta_2\text{M}$ is depicted in [Diagram 1](#).

The individual and combined effects of diabetes mellitus (DM) and Cd on excretion of albumin and $\beta_2\text{M}$ have received substantial attention over the past three decades^[21-25]. In general, DM is thought to cause proteinuria of glomerular origin, as judged from albumin excretion, and Cd is thought to cause proteinuria of tubular origin, as judged from $\beta_2\text{M}$ excretion, but these assumptions may not be entirely defensible.

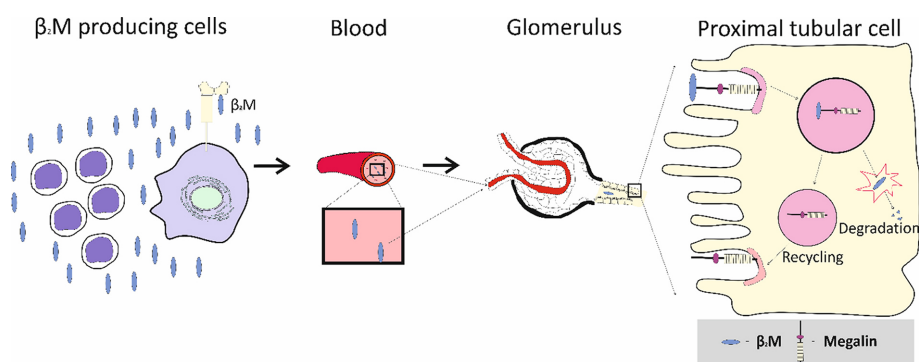


Diagram 1. Proximal tubular processing of β_2 M. β_2 M is released to plasma from nucleated cells and filtered completely with plasma by glomeruli. Most or all filtered β_2 M is degraded in segment S1 of the proximal tubule; resulting fragments are expelled and processed in segments S2 and S3 (not shown). Additional β_2 M may also be taken up in the distal nephron through the mediation of the lipocalin 2 receptor. β_2 M: β_2 -microglobulin.

Diabetes reportedly promotes excretion of β_2 M and other small proteins^[22,26,27], and albuminuria is strongly associated with β_2 -microglobulinuria in cadmium nephropathy^[14]. Effects of diabetes and Cd on β_2 M homeostasis require further clarification.

Herein, we introduce a conceptual framework for quantifying the components of β_2 M homeostasis, and we examine the effects of diabetes and a modest body burden of Cd on those components. We document surprising variability of influx of β_2 M into plasma and a reduction in tubular degradation of β_2 M in some diabetics with minimal Cd toxicity. We also examine shortcomings of E_{β_2M} as an indicator of tubular handling of β_2 M and suggest a better alternative for that purpose. Abbreviations are listed in Table 1.

EXPERIMENTAL

Study design

This investigation employed a case-control design. A purposive sampling technique was used to recruit type 2 diabetics and controls in equal numbers. Recruitments occurred during annual checkups at a local health center in Pakpoo Municipality, Nakhon Si Thammarat Province, Thailand. Diabetics and controls were matched for age, gender, and locality, and all subjects were at least 40 years old.

Exclusion criteria were non-resident status, current pregnancy and/or breastfeeding, and hospital records or a physician's diagnosis of an advanced chronic disease, including heart disease, stroke, and cancer. Control subjects were in good general health and met all exclusion criteria. Sociodemographic data, educational attainment, occupation, health status, family history of diabetes, and smoking status were ascertained by structured interview questionnaires. After exclusion of outliers and subjects with missing data, 65 diabetics and 72 controls were included for analysis.

Diabetes was defined as fasting plasma glucose [Glc]_p levels ≥ 126 mg/dL or a physician's diagnosis. Some patients with DM took metformin. Special diets were not employed in diabetics or controls. Reported use of dietary supplements was 3% among controls and 7% among diabetics^[28].

Technical considerations

Participants were asked to fast overnight, and collection of blood and urine samples was carried out at the Pakpoo health center on the morning of the following day. For the glucose assay, blood samples were collected in tubes containing fluoride that inhibited glycolysis. Blood samples for Cd analysis were collected

Table 1. Abbreviations

Abbreviation	Meaning	Calculation	Units
DM	Group with diabetes mellitus	n/a	n/a
CTRL	Control group without diabetes	n/a	n/a
GFR	Glomerular filtration rate	Footnote	mL/min or L/d
eGFR	Estimated GFR	Reference 26	mL/min/1.73m ²
V _u	Urine flow rate	n/a	mL, dL, or L/d
cr	Creatinine	n/a	n/a
[cr] _s	Serum creatinine concentration	n/a	mg/dL
[cr] _u	Urine creatinine concentration	n/a	mg/dL
E _{cr}	Rate of urinary creatinine Excretion	$[cr]_u(V_u)$	mg/d
C _{cr}	Creatinine clearance	$E_{cr}/[cr]_s$	mL/min; L/d
Cd	Cadmium	n/a	n/a
[Cd] _u	Urinary Cd concentration	n/a	µg/L
[Cd] _b	Blood Cd concentration	n/a	µg/L
E _{Cd}	Urinary excretion rate of Cd	$[Cd]_u V_u$	g/d
E _{Cd} /C _{cr}	Amount of Cd excreted per volume of glomerular filtrate	$[Cd]_u [cr]_s / [cr]_u$	g/L
β ₂ M	β ₂ -microglobulin	n/a	n/a
[β ₂ M] _p	Plasma concentration of β ₂ M	n/a	mg/L
[β ₂ M] _s	Serum concentration of β ₂ M	n/a	mg/L
[β ₂ M] _u	Urine concentration of β ₂ M	n/a	g/L
F _{β₂M}	Rate of glomerular filtration of β ₂ M	$eGFR[\beta_2M]_s$	mg/d
E _{β₂M}	Urinary excretion rate of β ₂ M	$V_u[\beta_2M]_u$	g/d
TD _{β₂M}	Rate of tubular degradation of β ₂ M	$F_{\beta_2M} - E_{\beta_2M}$	mg/d
E _{β₂M} /C _{cr}	Amount of β ₂ M excreted per volume of glomerular filtrate	$[\beta_2M]_u [cr]_s / [cr]_u$	g/L
TD _{β₂M} /C _{cr}	Amount of β ₂ M undergoing tubular degradation per volume of glomerular filtrate	$[\beta_2M]_s - E_{\beta_2M}/C_{cr}$	mg/L
FrTD _{β₂M}	Fractional tubular degradation of filtered β ₂ M	$(TD_{\beta_2M}/C_{cr})/[\beta_2M]_s$	Decimal fraction
GSC	Glomerular sieving coefficient	$[x]_{filtrate}/[x]_{plasma}$	Decimal fraction
SLR	Simple linear regression	n/a	n/a
MLR	Multiple linear regression	n/a	n/a

GFR is most accurately measured as inulin clearance (C_{in}), i.e., $E_{in}/[in]_s$, where E_{in} is the urinary excretion rate of inulin, and [in]_s is the serum inulin concentration. Measurement of C_{in} requires a constant intravenous infusion of inulin. n/a: Not applicable.

in separate tubes containing ethylenediamine tetra-acetic acid (EDTA) as an anticoagulant. To prevent the degradation of β₂M in acidic conditions, an alkaline (NaOH) solution was added to adjust the pH of urine samples to > 6 before their storage. Blood and urine samples were kept on ice and transported within one hour to the laboratory of Walailak University, where samples of plasma and serum were prepared. Aliquots of urine, whole blood, serum, and plasma were stored at -80 °C for later analysis.

The plasma glucose assay was based on the oxidase-peroxidase method (Glu Colorimetric Assay Kit, Elabscience, Houston, TX, USA)^[29]. Assays of creatinine in urine and plasma were based on Jaffe's alkaline picrate method, as described previously^[30]. The human beta-2 microglobulin/β₂M ELISA pair set (Sino Biological Inc., Wayne, PA, USA) was employed to determine serum and urine concentrations of β₂M ([β₂M]_s and [β₂M]_u), with a lower limit of detection of 3.13 pg/mL. We assumed that [β₂M]_s and the plasma concentration ([β₂M]_p) were equal.

Urinary and whole blood Cd concentrations ([Cd]_u, [Cd]_b) were determined with GBC System 5000 graphite furnace atomic absorption spectrometry (AAS) (GBC Scientific Equipment, Hampshire, IL,

USA)^[31]. 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, analytical accuracy, and precision assurance. When $[Cd]_u$ and $[Cd]_b$ were less than their detection limit of 0.1 $\mu\text{g/L}$, the concentration assigned was the detection limit value divided by the square root of 2^[32].

Normalization of cadmium and $\beta_2\text{M}$ excretion rates

Excretion of substance x (E_x) was normalized to creatinine clearance (C_{cr}) with the formula $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where $x = \text{Cd}$ or $\beta_2\text{M}$, $[x]_u$ = 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} expresses the amount of x excreted per volume of glomerular filtrate^[8]. Normalization to C_{cr} simultaneously corrects for urine dilution and functional nephron mass. E_x/C_{cr} is not affected by muscle mass.

Estimated glomerular filtration rate

Estimated GFR (eGFR) was ascertained with Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations published in 2009^[33]. 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.

Derivation of a conceptual framework for analysis of $\beta_2\text{M}$ homeostasis

Assume that:

- (a) $I_{\beta_2\text{M}}$ = influx of $\beta_2\text{M}$ into plasma from nucleated cells;
- (b) $\text{GSC}_{\beta_2\text{M}}$ = glomerular sieving coefficient of $\beta_2\text{M} = 1$ (*i.e.*, all $\beta_2\text{M}$ in filtered plasma enters filtrate)^[11,12];
- (c) $F_{\beta_2\text{M}}$ = rate of filtration (and subsequent renal disposition) of $\beta_2\text{M}$;
- (d) Plasma is in equilibrium with respect to $\beta_2\text{M}$ ($I_{\beta_2\text{M}} = F_{\beta_2\text{M}}$).

It follows that:

- (1) $I_{\beta_2\text{M}} = F_{\beta_2\text{M}} = \text{GFR}[\beta_2\text{M}]_p$, where $[\beta_2\text{M}]_p$ is the plasma concentration of the protein.

Dividing by GFR and rearranging,

- (2) $[\beta_2\text{M}]_p = I_{\beta_2\text{M}}/\text{GFR} = F_{\beta_2\text{M}}/\text{GFR}$.

Since filtered $\beta_2\text{M}$ is either excreted or reabsorbed and degraded,

- (3) $F_{\beta_2\text{M}} = \text{GFR}[\beta_2\text{M}]_p = E_{\beta_2\text{M}} + \text{TD}_{\beta_2\text{M}}$,

where $E_{\beta_2\text{M}}$ and $\text{TD}_{\beta_2\text{M}}$ are rates of excretion and tubular degradation of $\beta_2\text{M}$, respectively. Dividing by GFR,

- (4) $[\beta_2\text{M}]_p$ (mass/vol of plasma) = $E_{\beta_2\text{M}}/\text{GFR} + \text{TD}_{\beta_2\text{M}}/\text{GFR}$ (both ratios in units of mass/vol of filtrate).

Substitution of creatinine clearance (C_{cr}) for GFR in equation 4 permits estimation of $E_{\beta_{2M}}/GFR$ and $TD_{\beta_{2M}}/GFR$ from measurements in simultaneous aliquots of serum and urine. Thus,

$$E_{\beta_{2M}}/C_{cr} = [\beta_2M]_u V_u / [cr]_u V_u / [cr]_p; \text{ after simplification,}$$

$$E_{\beta_{2M}}/C_{cr} = [\beta_2M]_u [cr]_p / [cr]_u. \text{ It follows that}$$

$$(5) [\beta_2M]_p = E_{\beta_{2M}}/C_{cr} + TD_{\beta_{2M}}/C_{cr} = [\beta_2M]_u [cr]_p / [cr]_u + TD_{\beta_{2M}}/C_{cr}, \text{ and}$$

$$(6) TD_{\beta_{2M}}/C_{cr} = [\beta_2M]_p - E_{\beta_{2M}}/C_{cr} = [\beta_2M]_p - [\beta_2M]_u [cr]_p / [cr]_u.$$

As functions of both $I_{\beta_{2M}}$ and GFR (equation 2), $[\beta_2M]_p$ and $TD_{\beta_{2M}}/C_{cr}$ are quite variable, and a less confounded parameter of tubular β_2M handling is needed. $FrTD_{\beta_{2M}}$, the *fraction* of filtered β_2M that is degraded by tubules, is calculated as follows:

$$(7) FrTD_{\beta_{2M}} = TD_{\beta_{2M}}/F_{\beta_{2M}} = TD_{\beta_{2M}}/[\beta_2M]_p C_{cr} = (TD_{\beta_{2M}}/C_{cr})/[\beta_2M]_p.$$

In subjects with severe Cd intoxication, $FrTD_{\beta_{2M}}$ may be substantially reduced^[34].

The conventional parameter for assessing degradation of β_2M is $[\beta_2M]_u/[cr]_u$, which is a function of $E_{\beta_{2M}}$. However, because $TD_{\beta_{2M}}/C_{cr}$ and $[\beta_2M]_p$ are highly variable, relationships between $FrTD_{\beta_{2M}}$ and $E_{\beta_{2M}}$ or $E_{\beta_{2M}}/C_{cr}$ cannot be precise. Since filtered β_2M is either excreted or reabsorbed and degraded,

$$F_{\beta_{2M}} = E_{\beta_{2M}} + TD_{\beta_{2M}}, \text{ and}$$

$$(8) E_{\beta_{2M}} = F_{\beta_{2M}} - TD_{\beta_{2M}}$$

$$= F_{\beta_{2M}} - (FrTD_{\beta_{2M}})(F_{\beta_{2M}})$$

$$= (1 - FrTD_{\beta_{2M}})(F_{\beta_{2M}}).$$

$E_{\beta_{2M}}$ is ultimately a function of both $FrTD_{\beta_{2M}}$ and $F_{\beta_{2M}}$ ($I_{\beta_{2M}}$), and a single value of $E_{\beta_{2M}}$ is compatible with multiple combinations of these parameters. The argument does not change if equations (8) above are divided by C_{cr} . Since $F_{\beta_{2M}}/C_{cr} = [\beta_2M]_s$ (equation 2),

$$(9) E_{\beta_{2M}}/C_{cr} = [\beta_2M]_s - TD_{\beta_{2M}}/C_{cr}$$

$$= [\beta_2M]_s - (FrTD_{\beta_{2M}})[\beta_2M]_s$$

$$= (1 - FrTD_{\beta_{2M}})[\beta_2M]_s.$$

$E_{\beta_{2M}}/C_{cr}$ is therefore a function of $FrTD_{\beta_{2M}}$ and $[\beta_2M]_s$, and a single value of $E_{\beta_{2M}}/C_{cr}$ is compatible with multiple combinations of $FrTD_{\beta_{2M}}$ and $[\beta_2M]_s$.

Calculations

E_{Cd}/C_{cr} , $F_{\beta_{2M}}$, $E_{\beta_{2M}}/C_{cr}$, $TD_{\beta_{2M}}/C_{cr}$, and $FrTD_{\beta_{2M}}$ were calculated in accordance with equations 1-9. For calculation of $F_{\beta_{2M}}$ (equation 1), eGFR in mL/min/1.73m² was multiplied by 1.44 to yield eGFR in L/d. For calculation of $TD_{\beta_{2M}}/C_{cr}$ (equation 5), $E_{\beta_{2M}}/C_{cr}$ in g/L of filtrate was multiplied by 10⁻³ to yield $E_{\beta_{2M}}/C_{cr}$ in mg/L of filtrate, the unit of $TD_{\beta_{2M}}/C_{cr}$.

Statistical analyses

Data in Table 2 (characteristics of diabetics and controls) were analyzed with IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). Conformity of continuous variables to a normal distribution was assessed by the one-sample Kolmogorov-Smirnov test. Logarithmic transformation was applied to E_{Cd}/E_{cr} and E_{Cd}/C_{cr} , which showed right-skewed distributions. Estimated GFR values, which showed a left-skewed distribution, were analyzed without transformation. The Mann-Whitney *U* test was used to assess differences between DM and CTRL. Pearson's chi-squared test was used to determine differences in percentages and prevalences of female gender, smoking, and eGFR < 60 mL/min/1.73 m². A multiple linear regression model was used to assess contributions of demographic parameters to variability of $FrTD_{\beta_{2M}}$. Correlation matrices were generated from regression analyses of all homeostatic relationships examined in the present study. Correlations involving $F_{\beta_{2M}}$, $FrTD_{\beta_{2M}}$, and $E_{\beta_{2M}}/C_{cr}$ were organized by gender and presence or absence of diabetes. The resulting table is presented in the [Supplementary Materials](#). Statistical significance was indicated by *P*-values ≤ 0.05.

We used Microsoft Excel to examine a simple linear regression (SLR) of eGFR on E_{Cd}/C_{cr} and SLRs of $F_{\beta_{2M}}$, $[\beta_{2M}]_p$, $FrTD_{\beta_{2M}}$, $TD_{\beta_{2M}}/C_{cr}$, and $E_{\beta_{2M}}/C_{cr}$ on determinants suggested by equations 1-9. When SLRs revealed associations of a parameter with multiple potential determinants, we performed multilinear regressions (MLRs) to identify confounders and mutually independent relationships.

RESULTS AND DISCUSSION

Participants

Characteristics of cases (DM) and controls (CTRL) are compared in Table 2. Values of E_{Cd}/E_{cr} and E_{Cd}/C_{cr} were generally lower than in our previous assessments of other populations^[7,35], and values of E_{Cd}/E_{cr} were substantially lower than in Japanese subjects from polluted and non-polluted areas who were studied in the 1990s^[34]. Mean age was slightly higher in CTRL, and body mass index (BMI) and fasting plasma glucose were higher in DM. Differences between groups in the following parameters were not statistically significant: $[Cr]_s$, $[Cr]_u$, eGFR, % eGFR < 60 mL/min/1.73m², $[Cd]_u$, $[Cd]_b$, and E_{Cd}/C_{cr} . $[\beta_{2M}]_s$, $F_{\beta_{2M}}$, $E_{\beta_{2M}}/C_{cr}$, and $TD_{\beta_{2M}}/C_{cr}$ were higher in DM, and $FrTD_{\beta_{2M}}$ was slightly higher in CTRL. All differences in homeostatic parameters were significant at *P* < 0.001.

Regression of eGFR on E_{Cd}/C_{cr}

Figure 1 depicts SLRs of eGFR on E_{Cd}/C_{cr} in DM and CTRL. The figure shows that eGFR was weakly and inversely related to E_{Cd}/C_{cr} in CTRL and unrelated to E_{Cd}/C_{cr} in DM. In both groups, the entire range of eGFR was evident at minimal values of E_{Cd}/C_{cr} . In six subjects with E_{Cd}/C_{cr} > 0.05 µg/L of filtrate, all values of eGFR were ≤ 80 mL/min/1.73m². In CTRL, the modest inverse relationship of eGFR to E_{Cd}/C_{cr} was created by points from relatively few subjects. We do not know why DM was not similarly affected.

Regressions of $F_{\beta_{2M}}$ on its determinants

Equation 1 states in part that $I_{\beta_{2M}}$ equals $F_{\beta_{2M}}$. The implied assumption is that in free-living subjects, plasma is in equilibrium with respect to β_{2M} , as it is for other substances^[36,37]. Stated differently, $I_{\beta_{2M}}$, the rate at which β_{2M} enters plasma, equals $F_{\beta_{2M}}$, the rate at which kidneys dispose of the protein. The remainder of equation 1 states that $F_{\beta_{2M}}$ ($I_{\beta_{2M}}$) equals $eGFR[\beta_{2M}]_s$. If $I_{\beta_{2M}}$ varies during the day, we infer that $[\beta_{2M}]_s$ varies

Table 2. Characteristics of cases and age-, sex- and locality-matched controls

Variables	All, n = 137	Diabetics, n = 65	Controls, n = 72	P
Females, %	78.1	76.9	79.2	0.751
Smoking, %	10.2	9.2	11.1	0.717
Age, years	59.7 (9.1)	58.0 (9.4)	61.2 (8.6)	0.048
Age range, years	41-80	41-78	43-80	-
DM duration				
< 10 years, %	n/a	59.7	n/a	-
≥ 10 years, %	n/a	40.3	n/a	-
BMI, kg/m ²	25.6 (4.8)	26.7 (4.8)	24.6 (4.6)	0.003
Fasting plasma glucose, mg/dL	129 (61)	169 (68)	94 (11)	< 0.001
eGFR, mL/min/1.73 m ²	79.3 (16.0)	80.2 (18.5)	78.5 (13.2)	0.345
eGFR < 60 mL/min/1.73 m ² , %	12.4	15.4	9.7	0.315
[Cr] _s , mg/dL	0.87 (0.19)	0.88 (0.22)	0.86 (0.16)	0.750
[Cr] _u , mg/dL	92.2 (55.1)	88.8 (57.7)	95.4 (52.9)	0.303
[β ₂ M] _s , mg/L	5.98 (3.29)	7.05 (3.88)	5.02 (2.28)	0.004
[β ₂ M] _u , μg/L	68 (58)	96 (64)	42 (37)	< 0.001
Exposure indicators				
[Cd] _b , μg/L	0.57 (0.45, 0.69)	0.50 (0.35, 0.64)	0.63 (0.44, 0.82)	0.607
[Cd] _u , μg/L	0.65 (1.11)	0.65 (1.16)	0.66 (1.07)	0.838
E _{Cd} /E _{cr} , μg/g creatinine	0.98 (0.66, 1.29)	0.96 (0.52, 1.40)	0.99 (0.53, 1.45)	0.609
E _{Cd} /C _{cr} (μg/L of filtrate) × 100	0.86 (0.57, 1.14)	0.85 (0.43, 1.27)	0.86 (0.46, 1.26)	0.543
Effects indicators (outcomes)				
E _{β₂M} /E _{cr} , μg/g creatinine	108 (88, 128)	158 (124, 191)	63 (45, 81)	< 0.001
E _{β₂M} /C _{cr} , μg/L of filtrate	0.99 (0.79, 1.18)	1.48 (1.14, 1.82)	0.54 (0.40, 0.69)	< 0.001
F _{β₂M} , mg/d	671.9 (395.1)	791.3 (469.2)	564.1 (275.3)	< 0.001
FrTD _{β₂M}	0.9983 (0.0020)	0.998 (0.003)	0.999 (0.001)	< 0.001
TD _{β₂M} /C _{cr} , mg/L of filtrate	5.97 (3.29)	7.04 (3.88)	5.04 (2.28)	< 0.001

Values of E_{Cd}/E_{cr}, E_{Cd}/C_{cr}, E_{β₂M}/E_{cr}, E_{β₂M}/C_{cr}, and [Cd]_b are mean (95% CI). All other values for continuous variables are mean (SD). Cd: Cadmium; cr: creatinine; DM: group with diabetes mellitus; BMI: body mass index; eGFR: estimated glomerular filtration rate; [Cr]_s: serum creatinine concentration; [Cr]_u: urine creatinine concentration; β₂M: β₂-microglobulin; [β₂M]_s: serum concentration of β₂M; [β₂M]_u: urine concentration of β₂M; [Cd]_b: blood Cd concentration; [Cd]_u: urinary Cd concentration; E_{Cd}/E_{cr}: Cd excreted per gram of creatinine; E_{Cd}/C_{cr}: amount of Cd excreted per volume of glomerular filtrate; E_{β₂M}/E_{cr}: β₂M excreted per gram of creatinine; E_{β₂M}/C_{cr}: amount of β₂M excreted per volume of glomerular filtrate; F_{β₂M}: rate of glomerular filtration of β₂M; FrTD_{β₂M}: fractional tubular degradation of filtered β₂M; TD_{β₂M}/C_{cr}: amount of β₂M undergoing tubular degradation per volume of glomerular filtrate; CI: confidence interval.

promptly in response because eGFR is stable. Consequently, F_{β₂M} approximates I_{β₂M} from moment to moment and can therefore be employed as an ascertainable surrogate for I_{β₂M} in linear regressions.

The graphs in Figure 2 show that in our study sample, F_{β₂M} varied over its range, 100 to 2,000 mg/d, by a factor of 20. At the same time, except for a few low outliers, eGFR varied over its range, 55 to 110 mL/min/1.73m², by a factor of 2 [Figure 2C]. Because Cd inflicts injury on multiple cell types^[38], we speculated that I_{β₂M} might correlate with E_{Cd}/C_{cr}, a crude measure of the body burden of Cd, but the SLR of F_{β₂M} on E_{Cd}/C_{cr} was not significant in DM or CTRL [Figure 2A]. F_{β₂M}, the product of eGFR and [β₂M]_s (equation 1), was closely related to [β₂M]_s in both groups, directly but marginally related to eGFR in CTRL, and unrelated to eGFR in DM [Figure 2B and C, Table 3]. In CTRL, an MLR showed that significant associations persisted between F_{β₂M} and both [β₂M]_s and eGFR [Table 4]. Differences between groups in relationships of F_{β₂M} to eGFR may have resulted from the greater range of and wider variation around the mean of F_{β₂M} in DM. The key takeaway from Figure 2 is that [β₂M]_s, a by-product of I_{β₂M} (equation 2), was the principal determinant of F_{β₂M} in both groups.

Table 3. Results of simple linear regressions

Figure	Regression	DM			CTRL		
		Relationship	R ²	P	Relationship	R ²	P
1	eGFR on E _{Cd} /C _{cr}	None	0.006	0.54	Inverse	0.07	0.025
2A	F _{β_{2M}} on E _{Cd} /C _{cr}	None	0.025	0.21	None	0.011	0.39
2B	F _{β_{2M}} on [β _{2M}] _s	Direct	0.88	< 0.001	Direct	0.88	< 0.001
2C	F _{β_{2M}} on eGFR	None	0.008	0.47	Direct	0.082	0.019
3A	[β _{2M}] _s on E _{Cd} /C _{cr}	None	0.02	0.25	None	0.0006	0.83
3B	[β _{2M}] _s on F _{β_{2M}}	Direct	0.88	< 0.001	Direct	0.86	< 0.001
3C	[β _{2M}] _s on eGFR	None	0.05	0.06	None	0.004	0.60
4A	FrTD _{β_{2M}} on E _{Cd} /C _{cr}	Inverse	0.11	0.006	Inverse	0.058	0.04
4B	FrTD _{β_{2M}} on F _{β_{2M}}	Direct	0.13	0.04	None	0.03	0.13
4C	FrTD _{β_{2M}} on [β _{2M}] _s	Direct	0.06	0.045	None	0.003	0.64
4D	FrTD _{β_{2M}} on eGFR	Direct	0.19	< 0.001	Direct	0.17	< 0.001
5A	TD _{β_{2M}} /C _{cr} on E _{Cd} /C _{cr}	None	0.02	0.24	None	0.0007	0.83
5B	TD _{β_{2M}} /C _{cr} on F _{β_{2M}}	Direct	0.88	< 0.001	Direct	0.86	< 0.001
5C	TD _{β_{2M}} /C _{cr} on [β _{2M}] _s	Direct	0.999	< 0.001	Direct	0.999	< 0.001
5D	TD _{β_{2M}} /C _{cr} on FrTD _{β_{2M}}	Direct	0.064	0.042	None	0.003	0.62
5E	TD _{β_{2M}} /C _{cr} on eGFR	None	0.05	0.07	None	0.004	0.61
6A	E _{β_{2M}} /C _{cr} on E _{Cd} /C _{cr}	Direct	0.092	0.014	None	0.014	0.33
6B	E _{β_{2M}} /C _{cr} on F _{β_{2M}}	None	< 0.001	0.98	Direct	0.11	0.005
6C	E _{β_{2M}} /C _{cr} on [β _{2M}] _s	None	0.02	0.23	Direct	0.11	< 0.001
6D	E _{β_{2M}} /C _{cr} on eGFR	Inverse	0.32	< 0.001	Inverse	0.10	0.008
6E	E _{β_{2M}} /C _{cr} on TD _{β_{2M}} /C _{cr}	None	0.02	0.24	Direct	0.21	< 0.001
6F	E _{β_{2M}} /C _{cr} on FrTD _{β_{2M}}	Inverse	0.68	< 0.001	Inverse	0.59	< 0.001

Cd: Cadmium; cr: creatinine; DM: group with diabetes mellitus; CTRL: control group without diabetes; eGFR: estimated glomerular filtration rate; β_{2M}: β₂-microglobulin; F_{β_{2M}}: rate of glomerular filtration of β_{2M}; [β_{2M}]_s: serum concentration of β_{2M}; E_{Cd}/C_{cr}: amount of Cd excreted per volume of glomerular filtrate; FrTD_{β_{2M}}: fractional tubular degradation of filtered β_{2M}; E_{Cd}/C_{cr}: amount of Cd excreted per volume of glomerular filtrate; TD_{β_{2M}}/C_{cr}: amount of β_{2M} undergoing tubular degradation per volume of glomerular filtrate.

Regressions of [β_{2M}]_s on its determinants

If $I_{\beta_{2M}} = F_{\beta_{2M}} = eGFR[\beta_{2M}]_p$ (equation 1), then $[\beta_{2M}]_p = I_{\beta_{2M}}/eGFR = F_{\beta_{2M}}/eGFR$ (equation 2). In the present study, $[\beta_{2M}]_s$, like $F_{\beta_{2M}}$, was not related to E_{Cd}/C_{cr} in DM and CTRL [Figure 3A], but it was closely and directly related to $F_{\beta_{2M}}$ in both groups [Figure 3B]. This observation is expected because an SLR of $F_{\beta_{2M}}$ on $[\beta_{2M}]_s$ was highly significant [Figure 2B]. We include Figure 3B because $F_{\beta_{2M}} = I_{\beta_{2M}}$, and the high R² values indicate that $I_{\beta_{2M}}$ was the principal determinant of $[\beta_{2M}]_s$ in our subjects. $[\beta_{2M}]_s$ was inversely but marginally related to eGFR in DM and unrelated to eGFR in CTRL. In both groups, the disproportionate influence of $I_{\beta_{2M}}$ on $[\beta_{2M}]_s$ probably resulted from the fact that $F_{\beta_{2M}}$ ($I_{\beta_{2M}}$) varied over its range by a factor of approximately 20; in contrast, eGFR varied by a factor of 2, with a few outliers at the low end of the range [Figure 3B and C].

Increased $[\beta_{2M}]_s$ in diabetics may be of more than academic interest. In an analysis of data from the Third National Health and Nutrition Examination Survey (NHANES III), Cheung et al found in diabetics > 20 years old that $[\beta_{2M}]_s$ was independently predictive of all-cause and diabetes-related mortality^[39]. Survival curves of subjects in the highest, intermediate, and lowest tertiles of $[\beta_{2M}]_s$ showed markedly different life expectancies over almost 20 years. The authors argued that these differences might have resulted from pro-inflammatory and amyloidogenic properties of β_{2M} and from cytotoxicity of advanced glycation end products of the protein. In addition to mortality, the severity of diabetes-related morbidities has also been associated with increased $[\beta_{2M}]_s$ ^[40].

Table 4. Results of multilinear regressions

Variable analyzed	Significantly associated parameter(s) in SLR	Relationship direct or inverse	R ²	P-value	Persistently associated parameter(s) in MLR	P-value	R ² for multilinear regression
F _{β₂M} , CTRL	[β ₂ M] _p	Direct	0.86	< 0.001	[β ₂ M] _p	< 0.001	0.97
	eGFR	Direct	0.082	0.019	eGFR	< 0.001	-
[β ₂ M] _p , DM	F _{β₂M}	Direct	0.88	< 0.001	F _{β₂M}	< 0.001	0.98
	eGFR	Direct	0.05	0.06	eGFR	< 0.001	-
FrTD _{β₂M} , DM	E _{Cd} /C _{cr}	Inverse	0.11	0.006	E _{Cd} /C _{cr}	0.02	0.43
	F _{β₂M}	Direct	0.13	0.04	F _{β₂M}	0.04	-
	[β ₂ M] _p	Direct	0.064	0.04	[β ₂ M] _p	0.01	-
	eGFR	Direct	0.19	< 0.001	eGFR	< 0.001	-
FrTD _{β₂M} , CTRL	E _{Cd} /C _{cr}	Inverse	0.058	0.04	eGFR	0.001	0.19
	eGFR	Direct	0.17	< 0.001	-	-	-
TD _{β₂M} /C _{cr} , DM	F _{β₂M}	Direct	0.88	< 0.001	[β ₂ M] _p	< 0.001	1
	[β ₂ M] _p	Direct	0.999	< 0.001	FrTD _{β₂M}	< 0.001	-
	FrTD _{β₂M}	Direct	0.064	0.042	-	-	-
TD _{β₂M} /C _{cr} , CTRL	F _{β₂M}	Direct	0.86	< 0.001	F _{β₂M}	0.016	1
	[β ₂ M] _p	Direct	0.999	< 0.001	[β ₂ M] _p	< 0.001	-
E _{β₂M} /C _{cr} , DM	E _{Cd} /C _{cr}	Direct	0.092	0.014	eGFR	0.001	0.73
	eGFR	Inverse	0.32	< 0.001	FrTD _{β₂M}	< 0.001	-
	FrTD _{β₂M}	Inverse	0.68	< 0.001	-	-	-
E _{β₂M} /C _{cr} , CTRL	F _{β₂M}	Direct	0.11	0.005	eGFR	0.06	0.85
	[β ₂ M] _p	Direct	0.11	< 0.001	TD _{β₂M} /C _{cr}	0.002	-
	eGFR	Inverse	0.10	0.008	FrTD _{β₂M}	< 0.001	-
	TD _{β₂M} /C _{cr}	Direct	0.21	< 0.001	-	-	-
	FrTD _{β₂M}	Inverse	0.59	< 0.001	-	-	-

Cd: Cadmium; cr: creatinine; SLR: simple linear regression; MLR: multiple linear regression; β₂M: β₂-microglobulin; F_{β₂M}: rate of glomerular filtration of β₂M; CTRL: control group without diabetes; [β₂M]_p: plasma concentration of β₂M; eGFR: estimated glomerular filtration rate; DM: group with diabetes mellitus; FrTD_{β₂M}: fractional tubular degradation of filtered β₂M; E_{Cd}/C_{cr}: amount of Cd excreted per volume of glomerular filtrate; TD_{β₂M}/C_{cr}: amount of β₂M undergoing tubular degradation per volume of glomerular filtrate.

In the present study, one easily overlooked finding emerges from [Figures 2 and 3](#). In CTRL, [β₂M]_s remained stable as F_{β₂M} rose proportionately with eGFR [[Figure 3C](#)]. We therefore infer that high GFR increased and low GFR decreased I_{β₂M}, and [Figure 2C](#) supports that inference. In DM, the slope of the line relating F_{β₂M} to eGFR was positive, but the regression was not significant [[Figure 2C](#)]; [β₂M]_s fell as eGFR rose, and the SLR just missed statistical significance [[Figure 3C](#)].

Regressions of FrTD_{β₂M} on its determinants

FrTD_{β₂M} is the ratio of the rate of degradation to the rate of filtration of β₂M; thus, FrTD_{β₂M} = TD_{β₂M}/F_{β₂M}. If the numerator and denominator of this ratio are divided by C_{cr}, FrTD_{β₂M} = (TD_{β₂M}/C_{cr})/[β₂M]_s (equation 7).

In [Figure 4](#), all graphs show that the range of FrTD_{β₂M} was 0.991 to 1 in DM and 0.996 to 1 in CTRL. These observations are consistent with the slightly lower mean FrTD_{β₂M} in DM [[Table 2](#)]. FrTD_{β₂M} was inversely but weakly related to E_{Cd}/C_{cr} in both groups [[Figure 4A](#)]. R² values indicate that in DM and CTRL, variation in E_{Cd}/C_{cr} accounted for 11% and 5.8% of variation in FrTD_{β₂M}; in both groups, high and low values of FrTD_{β₂M} were found in subjects with negligible Cd excretion, and statistically significant associations were created by relatively few points. [Figure 4A](#) suggests that in a minority of both groups, Cd impaired proximal tubular degradation of β₂M. However, it is also true that the 13 lowest values of FrTD_{β₂M} were seen in diabetics, and only four of them exhibited exceptional Cd excretion. We infer that an unidentified effect of

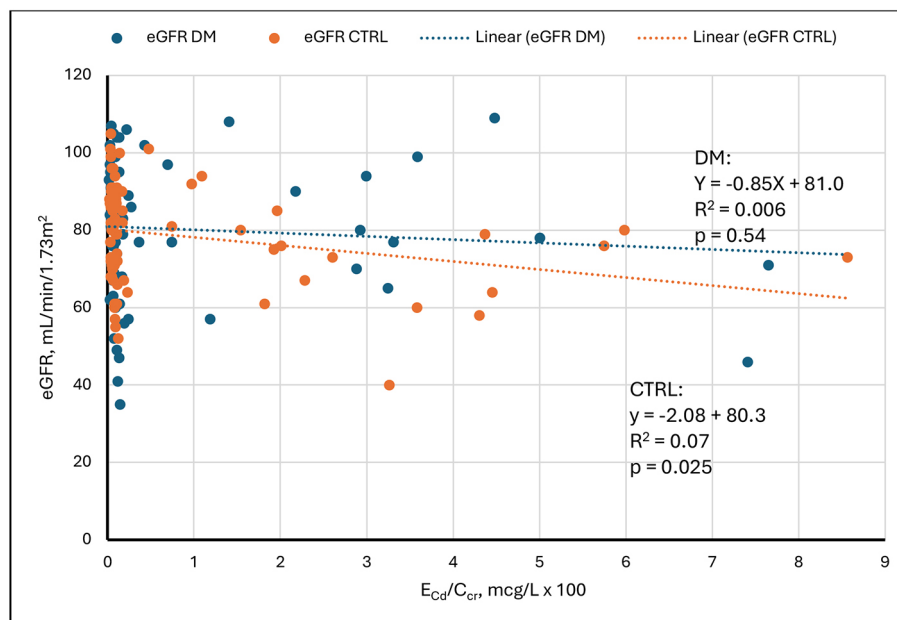


Figure 1. Linear regressions of eGFR on E_{Cd}/C_{cr} . Scatter diagrams present data from diabetics (DM, blue) and non-diabetic controls (CTRL, orange). An inverse relationship is evident in CTRL. No relationship is evident in DM. Cd: Cadmium; cr: creatinine; eGFR: estimated glomerular filtration rate; E_{Cd}/C_{cr} : amount of Cd excreted per volume of glomerular filtrate; DM: group with diabetes mellitus; CTRL: control group without diabetes.

diabetes compromised tubular processing of β_2M in some subjects.

$FrTD_{\beta_2M}$ varied directly with F_{β_2M} , $[\beta_2M]_s$, and TD_{β_2M}/C_{cr} in DM but was not significantly related to these parameters in CTRL [Figure 4B-D]. The SLRs in CTRL make mathematical sense because the numerator and denominator of $FrTD_{\beta_2M}$, TD_{β_2M}/C_{cr} , and $[\beta_2M]_s$ were virtually identical throughout their ranges, and F_{β_2M} was primarily a function of $[\beta_2M]_s$. A small cluster of diabetics with simultaneous reductions of $FrTD_{\beta_2M}$, TD_{β_2M}/C_{cr} , and $[\beta_2M]_s$ accounted for the inter-group differences in Figs 4B-D, but we have no explanation for the cluster. Again, we infer that an unidentified effect of diabetes compromised tubular processing of β_2M in some subjects.

$FrTD_{\beta_2M}$ varied directly and significantly with eGFR in DM and CTRL [Figure 4E]; in other words, $FrTD_{\beta_2M}$ was lowest at low eGFR and highest at high eGFR in both groups. In SLRs, high E_{Cd}/C_{cr} , low eGFR, and DM appeared to reduce $FrTD_{\beta_2M}$ [Figure 4]; the effects of Cd and DM were sporadic, and the effect of eGFR was somewhat more uniform [Figure 4A and E]. In DM, an MLR showed persistent associations of $FrTD_{\beta_2M}$ with E_{Cd}/C_{cr} , F_{β_2M} , $[\beta_2M]_s$, and eGFR; in CTRL, an MLR showed continued association of $FrTD_{\beta_2M}$ with eGFR but not with E_{Cd}/C_{cr} [Table 4]. Thus, in CTRL, a relationship between eGFR and E_{Cd}/C_{cr} [Figure 1] explained the significant regression of $FrTD_{\beta_2M}$ on E_{Cd}/C_{cr} . In DM, E_{Cd}/C_{cr} persisted as an independent determinant of $FrTD_{\beta_2M}$ because Cd caused a miniscule but statistically significant reduction in $FrTD_{\beta_2M}$ in a few subjects [Figure 4A]. $[\beta_2M]_s$ and its two determinants, I_{β_2M} (F_{β_2M}) and eGFR, retained independent effects on $FrTD_{\beta_2M}$ in DM [Table 4].

The relationships between $FrTD_{\beta_2M}$ and eGFR in the two groups were unexpected. Because $TD_{\beta_2M}/C_{cr} = [\beta_2M]_p - E_{\beta_2M}/C_{cr}$, and $FrTD_{\beta_2M} = ([\beta_2M]_p - E_{\beta_2M}/C_{cr})/[\beta_2M]_p$, $[\beta_2M]_p$ exerts almost identical influence on the numerator and denominator of the ratio. Since $[\beta_2M]_p = I_{\beta_2M}/GFR$, the effect of GFR on $FrTD_{\beta_2M}$ is also equally distributed. We must therefore conclude that rising eGFR increased and falling eGFR reduced

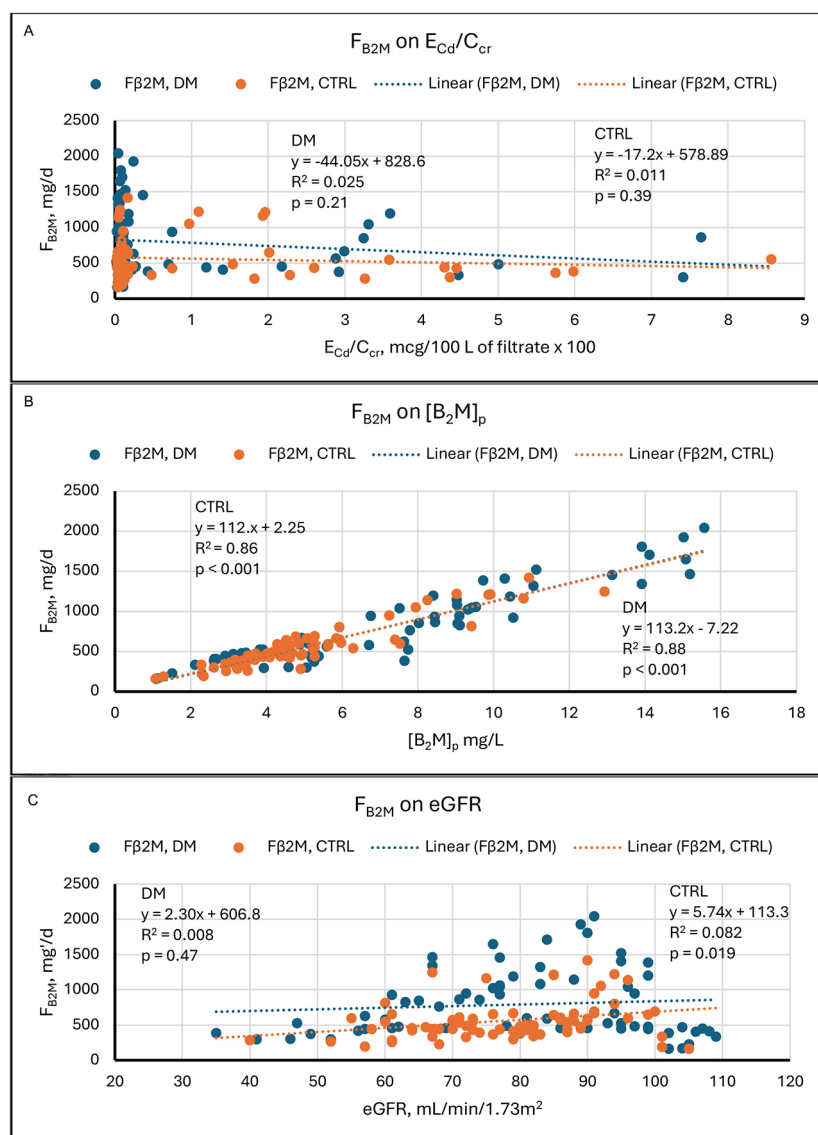


Figure 2. Linear regressions of $F_{\beta 2M}$ on potential determinants. Scatter diagrams present data from diabetics (DM, blue) and non-diabetic controls (CTRL, orange). The graphs plot $F_{\beta 2M}$ against (A) E_{Cd}/C_{Cr} , (B) $[B_2M]_p$, and (C) eGFR. No relationships are evident in (A). Strong direct relationships are evident in both groups in (B). In (C), a direct relationship is evident in CTRL, but no relationship is evident in DM. Cd: Cadmium; cr: creatinine; β_2M : β_2 -microglobulin; $F_{\beta 2M}$: rate of glomerular filtration of β_2M ; DM: group with diabetes mellitus; CTRL: control group without diabetes; E_{Cd}/C_{Cr} : amount of Cd excreted per volume of glomerular filtrate; $[B_2M]_p$: serum concentration of β_2M ; eGFR: estimated glomerular filtration rate.

$FrTD_{\beta 2M}$ for reasons that were not revealed by the present analysis.

The relatively low values of R^2 for MLRs in Figure 4 suggest that in both DM and CTRL, most of the variation of $FrTD_{\beta 2M}$ was driven by unidentified factors [Table 4]. Although this variation was discernible, we reiterate that it occurred within tenths of a percentage point in our sample. A multiple regression model in which age, body mass index, smoking, and gender were included provided no additional insight into determinants of $FrTD_{\beta 2M}$ (data not shown).

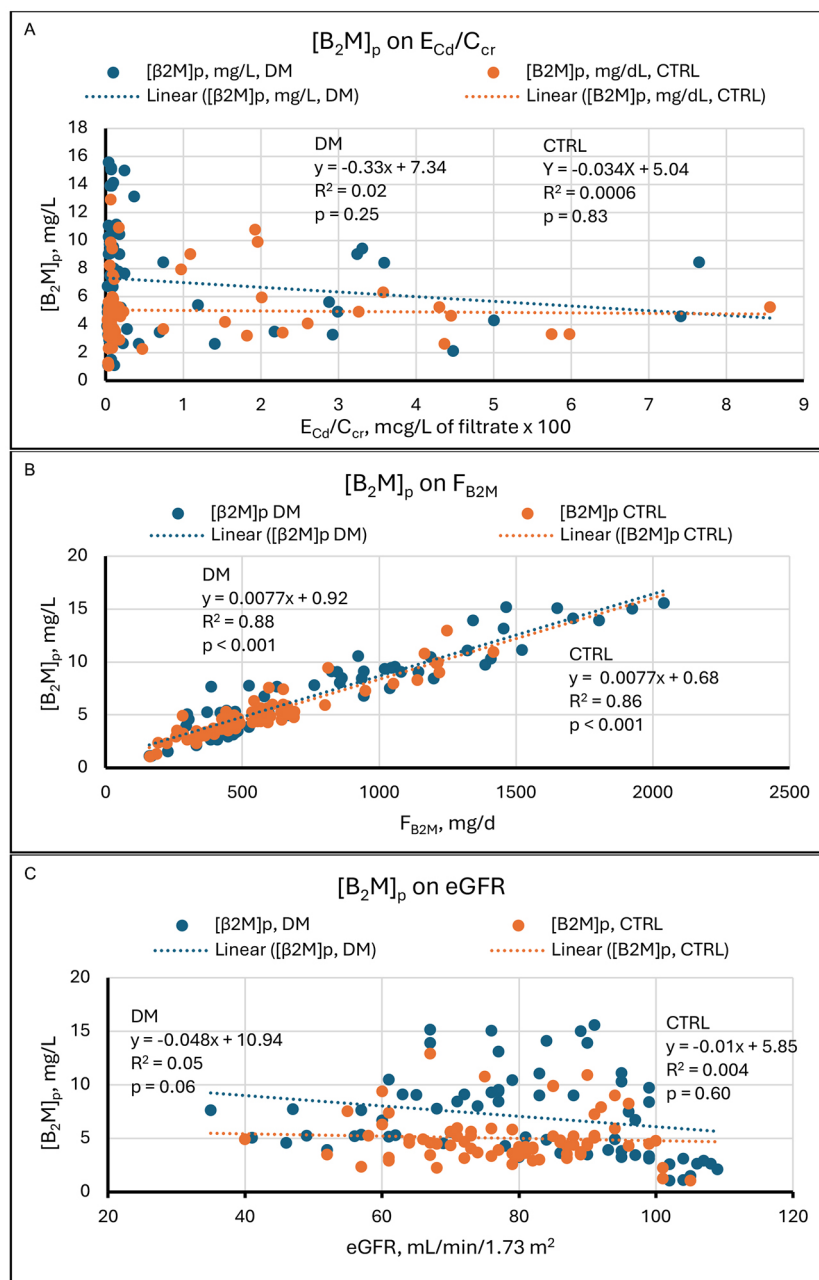


Figure 3. Linear regressions of $[\beta_2M]_s$ on potential determinants. Scatter diagrams present data from diabetics (DM, blue) and non-diabetic controls (CTRL, orange). The graphs plot $[\beta_2M]_s$ against (A) E_{Cd}/C_{cr} , (B) F_{β_2M} , and (C) eGFR. No relationships are evident in (A). Strong direct relationships are evident in both groups in (B). In (C), a weak inverse relationship is evident in DM, and no relationship is evident in CTRL. Cd: Cadmium; cr: creatinine; β_2M : β_2 -microglobulin; $[\beta_2M]_s$: serum concentration of β_2M ; DM: group with diabetes mellitus; CTRL: control group without diabetes; E_{Cd}/C_{cr} : amount of Cd excreted per volume of glomerular filtrate; F_{β_2M} : rate of glomerular filtration of β_2M ; eGFR: estimated glomerular filtration rate.

Regressions of TD_{β_2M}/C_{cr} on its determinants

TD_{β_2M}/C_{cr} quantifies the amount of β_2M reabsorbed and degraded by proximal tubules per volume of filtrate. In theory, if a maximum capacity for β_2M reabsorption exists, TD_{β_2M}/C_{cr} reaches a plateau at sufficiently increased F_{β_2M} (I_{β_2M}), and E_{β_2M}/C_{cr} rises thereafter in parallel with F_{β_2M} . If a tubular maximum does not exist, or if it exceeds all F_{β_2M} , then TD_{β_2M}/C_{cr} consistently rises in parallel with F_{β_2M} .

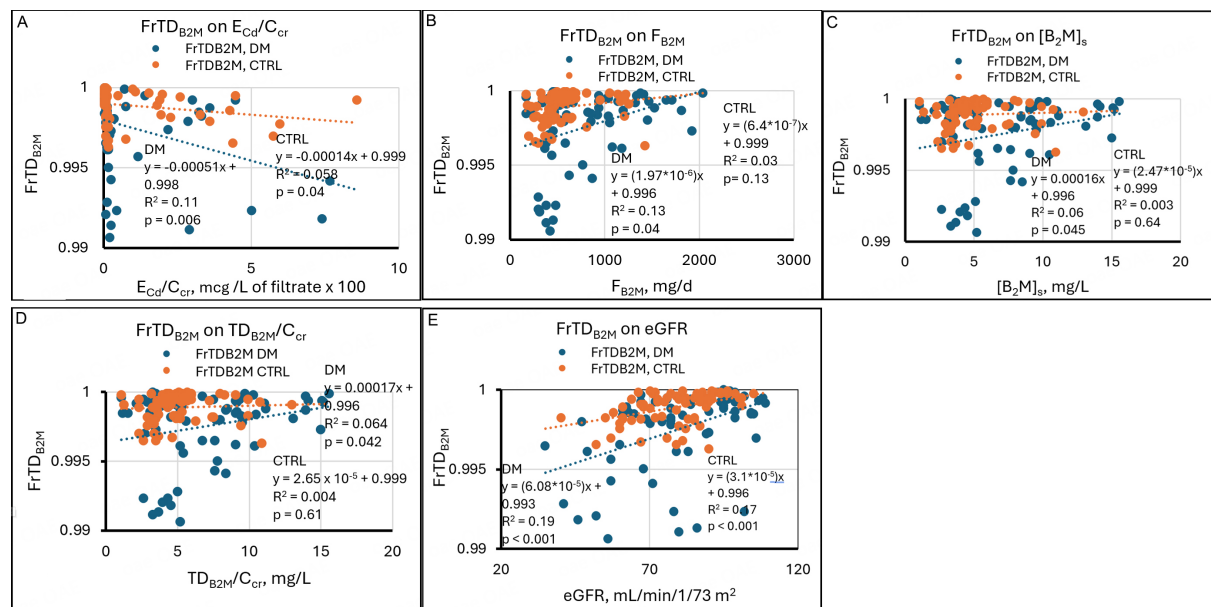


Figure 4. Linear regressions of $FrTD_{\beta_{2M}}$ on potential determinants. Scatter diagrams present data from diabetics (DM, blue) and non-diabetic controls (CTRL, orange). The graphs plot $FrTD_{\beta_{2M}}$ against (A) E_{Cd}/C_{Cr} , (B) $F_{\beta_{2M}}$, (C) $[\beta_2M]_s$, (D) $TD_{\beta_{2M}}/C_{Cr}$ and (E) eGFR. Inverse relationships are evident in both groups in (A). In (B), a weak direct relationship is evident in DM, and no relationship is evident in CTRL. That pattern is repeated in (C) and (D). Direct relationships are evident in both groups in (E). Cd: Cadmium; cr: creatinine; β_2M : β_2 -microglobulin; $FrTD_{\beta_{2M}}$: fractional tubular degradation of filtered β_2M ; DM: group with diabetes mellitus; CTRL: control group without diabetes; E_{Cd}/C_{Cr} : amount of Cd excreted per volume of glomerular filtrate; $F_{\beta_{2M}}$: rate of glomerular filtration of β_2M ; $[\beta_2M]_s$: serum concentration of β_2M ; $TD_{\beta_{2M}}/C_{Cr}$: amount of β_2M undergoing tubular degradation per volume of glomerular filtrate; eGFR: estimated glomerular filtration rate.

Because Cd injures proximal tubular cells, we speculated that $TD_{\beta_{2M}}/C_{Cr}$ might reach a plateau in sufficiently intoxicated subjects. However, Figure 5A demonstrates that $TD_{\beta_{2M}}/C_{Cr}$ was unrelated to E_{Cd}/C_{Cr} in DM and CTRL. In both groups, the highest and lowest values of $TD_{\beta_{2M}}/C_{Cr}$ were seen at the lowest values of E_{Cd}/C_{Cr} , and intermediate values of $TD_{\beta_{2M}}/C_{Cr}$ were seen at the highest values of E_{Cd}/C_{Cr} . $TD_{\beta_{2M}}/C_{Cr}$ rose linearly with $F_{\beta_{2M}}$ and did not reach a plateau [Figure 5B]. $TD_{\beta_{2M}}/C_{Cr}$ was virtually identical to $[\beta_2M]_s$ at all values of $[\beta_2M]_s$ [Figure 5C]; this finding resulted from the calculation of $TD_{\beta_{2M}}/C_{Cr}$ as $[\beta_2M]_s - E_{\beta_{2M}}/C_{Cr}$ (equation 5), and from the fact that $E_{\beta_{2M}}/C_{Cr}$ was 0.01 to 0.001 of $[\beta_2M]_s$ in all subjects.

$TD_{\beta_{2M}}/C_{Cr}$ was weakly related to $FrTD_{\beta_{2M}}$ in DM and unrelated to $FrTD_{\beta_{2M}}$ in CTRL [Figure 5D]. The relationship in DM was created by points from eight subjects with atypically low $FrTD_{\beta_{2M}}$ and $TD_{\beta_{2M}}/C_{Cr}$ at the low end of the observed range. The weakness of the relationship in DM and its absence in CTRL were due to the primacy of $I_{\beta_{2M}}$ in determining $[\beta_2M]_s$ and $TD_{\beta_{2M}}/C_{Cr}$. $TD_{\beta_{2M}}/C_{Cr}$ was weakly and inversely related to eGFR in DM and unrelated to eGFR in CTRL [Figure 5D]. We have no explanation for this difference.

In DM, SLRs showed that $TD_{\beta_{2M}}/C_{Cr}$ was directly related to $F_{\beta_{2M}}$, $[\beta_2M]_s$, and $FrTD_{\beta_{2M}}$ [Figure 5]; in an MLR, $[\beta_2M]_s$ and $FrTD_{\beta_{2M}}$ persisted as independent determinants of $TD_{\beta_{2M}}/C_{Cr}$, and $F_{\beta_{2M}}$ did not [Table 4]. In CTRL, $TD_{\beta_{2M}}/C_{Cr}$ was directly related to $F_{\beta_{2M}}$ and $[\beta_2M]_s$ but not $FrTD_{\beta_{2M}}$; an MLR showed persistence of both associations, but the p-value for the association of $TD_{\beta_{2M}}/C_{Cr}$ with $[\beta_2M]_s$ was much lower (i.e., the association was more significant). In both groups, $[\beta_2M]_s$, the sum of $E_{\beta_{2M}}/C_{Cr}$ + $TD_{\beta_{2M}}/C_{Cr}$, was the predominant determinant of $TD_{\beta_{2M}}/C_{Cr}$ because $[\beta_2M]_s$ and $TD_{\beta_{2M}}/C_{Cr}$ were virtually equal.

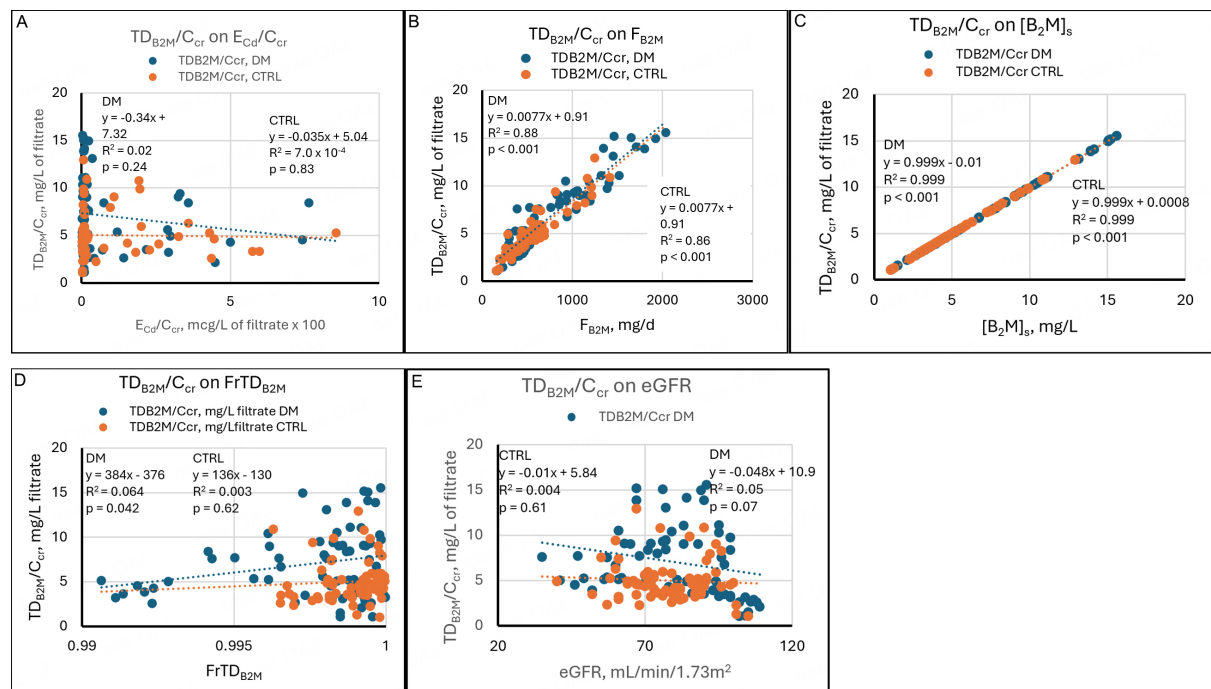


Figure 5. Linear regressions of TD_{β_2M}/C_{cr} on potential determinants. Scatter diagrams present data from diabetics (DM, blue) and non-diabetic controls (CTRL, orange). The graphs plot TD_{β_2M}/C_{cr} against (A) E_{Cd}/C_{cr} , (B) F_{β_2M} , (C) $[B_2M]_s$, (D) $FrTD_{\beta_2M}$, and (E) $eGFR$. No relationships are evident in (A). Strong direct relationships are evident in both groups in (B) and (C). In (D), a direct relationship is evident in DM, and no relationship is evident in CTRL. In (E), a marginal inverse relationship is evident in DM, and no relationship is evident in CTRL. Cd: Cadmium; cr: creatinine; β_2M : β_2 -microglobulin; TD_{β_2M}/C_{cr} : amount of β_2M undergoing tubular degradation per volume of glomerular filtrate; DM: group with diabetes mellitus; CTRL: control group without diabetes; E_{Cd}/C_{cr} : amount of Cd excreted per volume of glomerular filtrate; F_{β_2M} : rate of glomerular filtration of β_2M ; $[B_2M]_s$: serum concentration of β_2M ; $FrTD_{\beta_2M}$: fractional tubular degradation of filtered β_2M ; $eGFR$: estimated glomerular filtration rate.

Regressions of E_{β_2M}/C_{cr} on its determinants

Figure 6 depicts the relationships between E_{β_2M}/C_{cr} - the amount of β_2M excreted per volume of filtrate - and relevant variables. While E_{β_2M} was clearly related to E_{Cd} in more severely intoxicated subjects [7,35], we found in the present study that E_{β_2M}/C_{cr} was weakly and directly related to E_{Cd}/C_{cr} in DM and unrelated to E_{Cd}/C_{cr} in CTRL [Figure 6A]. E_{β_2M}/C_{cr} was equally related to F_{β_2M} and $[B_2M]_s$ in CTRL ($R^2 = 0.11$ for both SLRs), but unrelated to those variables in DM [Figure 6B and C]. E_{β_2M}/C_{cr} rose with TD_{β_2M}/C_{cr} in CTRL and was unrelated to TD_{β_2M}/C_{cr} in DM [Figure 6E].

At a constant $FrTD_{\beta_2M}$, we expect both E_{β_2M}/C_{cr} and TD_{β_2M}/C_{cr} to rise as F_{β_2M} rises, as occurred in CTRL. Under this hypothetical condition, E_{β_2M}/C_{cr} should correlate with F_{β_2M} , the mathematical determinants of F_{β_2M} ($[B_2M]_s$ and $eGFR$), and TD_{β_2M}/C_{cr} SLRs demonstrated those relationships in CTRL but not DM. In an MLR, the relationship between E_{β_2M}/C_{cr} and TD_{β_2M}/C_{cr} persisted in CTRL, while the associations with F_{β_2M} and $[B_2M]_s$ were no longer observed [Table 4].

E_{β_2M}/C_{cr} was inversely related to $eGFR$ in DM ($R^2 = 0.32$) and CTRL ($R^2 = 0.10$), and the slope of the line relating the two variables was steeper in DM [Figure 6D]. Similarly, in DM and CTRL, E_{β_2M}/C_{cr} was strongly and inversely related to $FrTD_{\beta_2M}$, which accounted respectively for 68% and 59% of variation in E_{β_2M}/C_{cr} [Figure 6F]. These observations can be understood from equation 9, which states that $E_{\beta_2M}/C_{cr} = (1 - FrTD_{\beta_2M})[B_2M]_s$. E_{β_2M}/C_{cr} falls as $FrTD_{\beta_2M}$ rises if $[B_2M]_s$ remains constant; similarly, since $[B_2M]_s = I_{\beta_2M}/GFR$ (equation 2), E_{β_2M}/C_{cr} varies inversely with GFR if $FrTD_{\beta_2M}$ and I_{β_2M} remain constant.

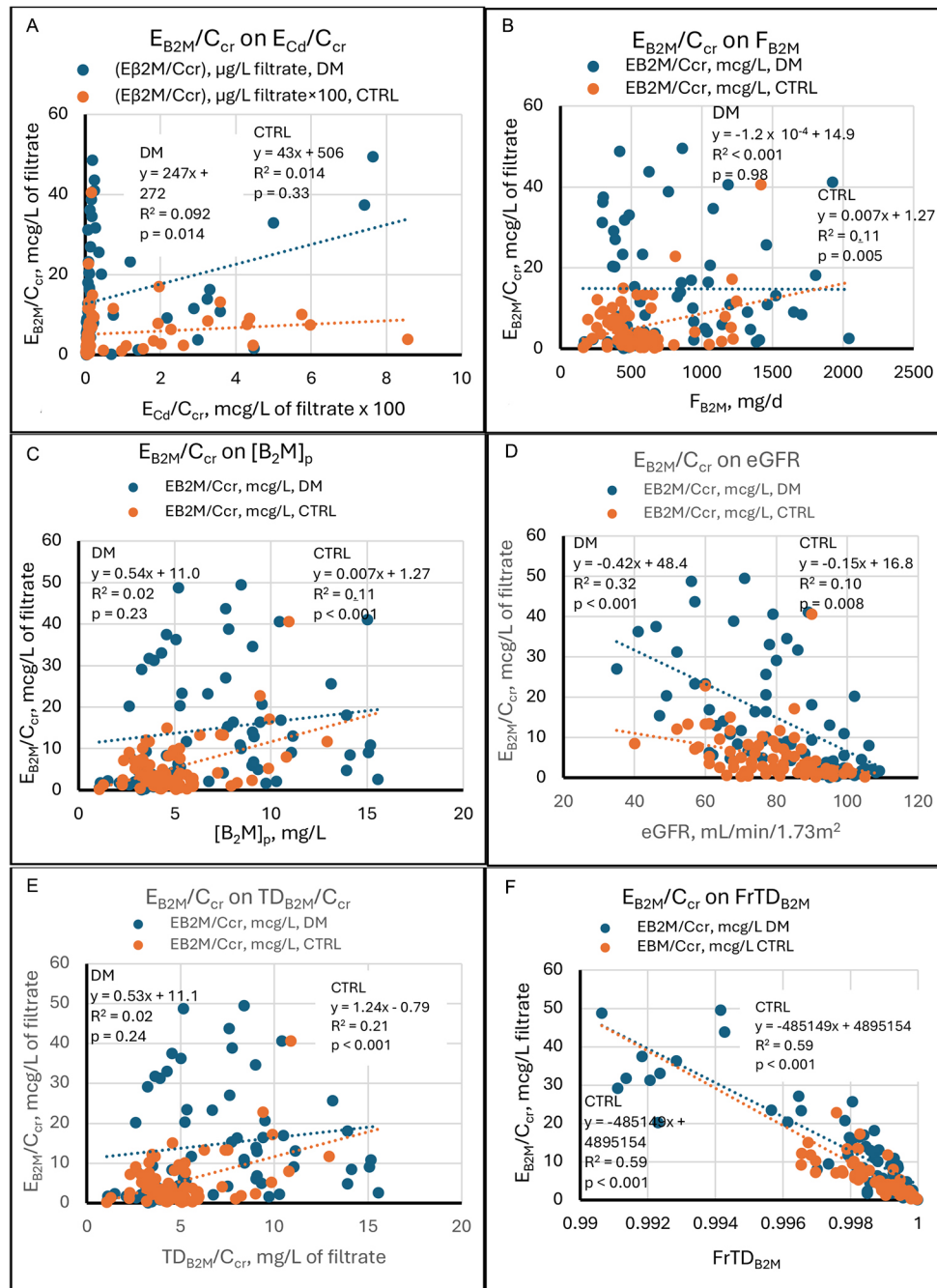


Figure 6. Linear regressions of E_{B2M}/C_{cr} on potential determinants. Scatter diagrams present data from diabetics (DM, blue) and non-diabetic controls (CTRL, orange). The graphs plot E_{B2M}/C_{cr} against (A) E_{Cd}/C_{cr} , (B) F_{B2M} , (C) $[B_2M]_p$, (D) eGFR, (E) TD_{B2M}/C_{cr} , and (F) $FrTD_{B2M}$. In (A), a positive relationship is evident in DM, and no relationship is evident in CTRL. In (B) and (C), positive relationships are evident in CTRL, and no relationships are evident in DM. Inverse relationships are evident in both groups in (D). In (E), a positive relationship is evident in CTRL, and no relationship is evident in DM. In (F), strong inverse relationships are evident in both groups. Cd: Cadmium; cr: creatinine; β_2M : β_2 -microglobulin; E_{B2M}/C_{cr} : amount of β_2M excreted per volume of glomerular filtrate; DM: group with diabetes mellitus; CTRL: control group without diabetes; E_{Cd}/C_{cr} : amount of Cd excreted per volume of glomerular filtrate; F_{B2M} : rate of glomerular filtration of β_2M ; $[B_2M]_p$: serum concentration of β_2M ; eGFR: estimated glomerular filtration rate; TD_{B2M}/C_{cr} : amount of β_2M undergoing tubular degradation per volume of glomerular filtrate; $FrTD_{B2M}$: fractional tubular degradation of filtered β_2M .

In DM, approximately 15% of subjects had $FrTD_{B2M} \leq 0.996$; in all but one of these subjects, E_{B2M}/C_{cr} was \geq

30 $\mu\text{g/L}$ of filtrate. The remainder of DM showed $\text{FrTD}_{\beta_2\text{M}} \geq 0.996$; in this subset, no subject had $E_{\beta_2\text{M}}/C_{\text{cr}} > 30 \mu\text{g/L}$ of filtrate. From our data, it appears that $E_{\beta_2\text{M}}/C_{\text{cr}} > 30 \mu\text{g/L}$ coincided with $\text{FrTD}_{\beta_2\text{M}} < 0.996$, but $E_{\beta_2\text{M}}/C_{\text{cr}}$ was nevertheless quite variable at a given $\text{FrTD}_{\beta_2\text{M}}$; for example, at $\text{FrTD}_{\beta_2\text{M}}$ of 0.998, $E_{\beta_2\text{M}}/C_{\text{cr}}$ varied between 1 and 20 $\mu\text{g/L}$ of filtrate [Figure 6F]. Equation 9 indicates that this observation resulted from the variability of $[\beta_2\text{M}]_s$.

MLRs revised some relationships suggested by SLRs [Table 4]. In DM, the regression of $E_{\beta_2\text{M}}/C_{\text{cr}}$ on $E_{\text{Cd}}/C_{\text{cr}}$ disappeared, while the regression of $E_{\beta_2\text{M}}/C_{\text{cr}}$ on eGFR persisted; variation in $\text{FrTD}_{\beta_2\text{M}}$ and eGFR then accounted for 73% of the variation in $E_{\beta_2\text{M}}/C_{\text{cr}}$. In CTRL, regressions of $E_{\beta_2\text{M}}/C_{\text{cr}}$ on $F_{\beta_2\text{M}}$ and $[\beta_2\text{M}]_s$ disappeared, while the regression of $E_{\beta_2\text{M}}/C_{\text{cr}}$ on $\text{TD}_{\beta_2\text{M}}/C_{\text{cr}}$ persisted; variation in $\text{FrTD}_{\beta_2\text{M}}$, $\text{TD}_{\beta_2\text{M}}/C_{\text{cr}}$, and eGFR then accounted for 85% of the variation in $E_{\beta_2\text{M}}/C_{\text{cr}}$. Thus, in MLRs, $E_{\beta_2\text{M}}/C_{\text{cr}}$ was not related to $E_{\text{Cd}}/C_{\text{cr}}$ in either group, but it was inversely related to eGFR and especially to $\text{FrTD}_{\beta_2\text{M}}$ in both groups, as equation 9 predicts.

Comparison of regressions in DM and CTRL

Table 5 shows that several physiologically important regressions were unaffected by diabetes. SLRs that were significant in both groups included $F_{\beta_2\text{M}}$ on $[\beta_2\text{M}]_s$ (and vice versa), $\text{FrTD}_{\beta_2\text{M}}$ on $E_{\text{Cd}}/C_{\text{cr}}$, $\text{FrTD}_{\beta_2\text{M}}$ on eGFR, $\text{TD}_{\beta_2\text{M}}/C_{\text{cr}}$ on $F_{\beta_2\text{M}}$, $\text{TD}_{\beta_2\text{M}}/C_{\text{cr}}$ on $[\beta_2\text{M}]_s$, $E_{\beta_2\text{M}}/C_{\text{cr}}$ on eGFR (inverse), and $E_{\beta_2\text{M}}/C_{\text{cr}}$ on $\text{FrTD}_{\beta_2\text{M}}$ (inverse). In CTRL, $E_{\text{Cd}}/C_{\text{cr}}$ disappeared as a determinant of $\text{FrTD}_{\beta_2\text{M}}$ in an MLR, presumably because of a linkage between eGFR and $E_{\text{Cd}}/C_{\text{cr}}$ [Figure 1], but all other associations common to the two groups persisted in MLRs [Table 4].

Several notable SLRs were significant in neither group. They included regressions of $[\beta_2\text{M}]_s$ and $\text{TD}_{\beta_2\text{M}}/C_{\text{cr}}$ on $E_{\text{Cd}}/C_{\text{cr}}$ and eGFR, and the regression of $F_{\beta_2\text{M}}$ on $E_{\text{Cd}}/C_{\text{cr}}$. SLRs of $\text{FrTD}_{\beta_2\text{M}}$ on $F_{\beta_2\text{M}}$ and $[\beta_2\text{M}]_s$, $\text{TD}_{\beta_2\text{M}}/C_{\text{cr}}$ on $\text{FrTD}_{\beta_2\text{M}}$, and $E_{\beta_2\text{M}}/C_{\text{cr}}$ on $E_{\text{Cd}}/C_{\text{cr}}$ were significant in DM but not CTRL. SLRs of eGFR on $E_{\text{Cd}}/C_{\text{cr}}$, $F_{\beta_2\text{M}}$ on eGFR, and $E_{\beta_2\text{M}}/C_{\text{cr}}$ on $F_{\beta_2\text{M}}$, $[\beta_2\text{M}]_s$, and $\text{TD}_{\beta_2\text{M}}/C_{\text{cr}}$ were significant in CTRL but not DM. These differences probably resulted from wider ranges and greater variability of $I_{\beta_2\text{M}}$ and $\text{FrTD}_{\beta_2\text{M}}$ in DM.

Diabetes appears to have depressed $\text{FrTD}_{\beta_2\text{M}}$ in a significant minority of subjects [Figure 4A-E]. The 13 lowest values of $\text{FrTD}_{\beta_2\text{M}}$ were seen in DM, and they were not uniformly attributable to high $E_{\text{Cd}}/C_{\text{cr}}$ or low eGFR. $E_{\beta_2\text{M}}/C_{\text{cr}}$ rose accordingly in the same subjects [Figure 6F]; a tentative inference from these data is that in some patients, diabetes imposed a small but easily demonstrable limitation on tubular degradation of $\beta_2\text{M}$. In an individual diabetic with moderate Cd exposure, reduced reabsorption of $\beta_2\text{M}$ may not be due to Cd toxicity or nephron loss.

Arguments as to whether diabetes enhances the nephrotoxicity of Cd, or vice versa, may be moot. Because $\text{GSC}_{\beta_2\text{M}}$ approaches 1^[11,12], increased $E_{\beta_2\text{M}}$ at a given $F_{\beta_2\text{M}}$ is necessarily a consequence of proximal tubular dysfunction. Glomerular disease does not increase $E_{\beta_2\text{M}}$. However, diabetes, which indisputably affects glomeruli, may also impair tubular handling of $\beta_2\text{M}$, and may do so in advance of any increase in albuminuria^[22,41]. In non-diabetic subjects, glycosuria promoted $\beta_2\text{M}$ excretion acutely, and in diabetics, improvement of glycemic control (and therefore of glycosuria) gradually reduced $E_{\beta_2\text{M}}$ ^[23].

Cd toxicity almost certainly causes albuminuria by interfering with the function of megalin, the brush border protein that mediates proximal tubular reabsorption of $\beta_2\text{M}$ ^[14]. A large body of research suggests that GSC_{alb} is high enough, in the range of 10^{-2} , to permit daily filtration of many grams of albumin. All but a few grams are retrieved by the process of fluid phase endocytosis, which is separate from megalin-mediated endocytosis, and returned to the circulation by transcytosis^[42-45]. If a large amount of albumin is normally

Table 5. Comparison of simple linear regressions in DM and CTRL

Regression	Relationship	Regression significant in			
		DM, not CTRL	CTRL, not DM	neither group	both groups
eGFR on E_{Cd}/C_{cr}	Inverse		x		
F_{β_2M} on E_{Cd}/C_{cr}	None			x	
F_{β_2M} on $[\beta_2M]_s$	Direct				x
F_{β_2M} on eGFR	None		x		
$[\beta_2M]_s$ on E_{Cd}/C_{cr}	None			x	
$[\beta_2M]_s$ on F_{β_2M}	Direct				x
$[\beta_2M]_s$ on eGFR	None			x	
$FrTD_{\beta_2M}$ on E_{Cd}/C_{cr}	Inverse				x
$FrTD_{\beta_2M}$ on F_{β_2M}	Direct	x			
$FrTD_{\beta_2M}$ on $[\beta_2M]_s$	Direct	x			
$FrTD_{\beta_2M}$ on eGFR	Direct				x
TD_{β_2M}/C_{cr} on E_{Cd}/C_{cr}	None			x	
TD_{β_2M}/C_{cr} on F_{β_2M}	Direct				x
TD_{β_2M}/C_{cr} on $[\beta_2M]_s$	Direct				x
TD_{β_2M}/C_{cr} on $FrTD_{\beta_2M}$	Direct	x			
TD_{β_2M}/C_{cr} on eGFR	None			x	
E_{β_2M}/C_{cr} on E_{Cd}/C_{cr}	Direct	x			
E_{β_2M}/C_{cr} on F_{β_2M}	Direct		x		
E_{β_2M}/C_{cr} on $[\beta_2M]_s$	Direct		x		
E_{β_2M}/C_{cr} on eGFR	Inverse				x
E_{β_2M}/C_{cr} on TD_{β_2M}/C_{cr}	Direct		x		
E_{β_2M}/C_{cr} on $FrTD_{\beta_2M}$	Inverse				x

Cd: Cadmium; cr: creatinine; DM: group with diabetes mellitus; CTRL: control group without diabetes; eGFR: estimated glomerular filtration rate; E_{Cd}/C_{cr} : amount of Cd excreted per volume of glomerular filtrate; β_2M : β_2 -microglobulin; F_{β_2M} : rate of glomerular filtration of β_2M ; $[\beta_2M]_s$: serum concentration of β_2M ; $FrTD_{\beta_2M}$: fractional tubular degradation of filtered β_2M ; TD_{β_2M}/C_{cr} : amount of β_2M undergoing tubular degradation per volume of glomerular filtrate; E_{β_2M}/C_{cr} : amount of β_2M excreted per volume of glomerular filtrate.

filtered each day, it seems unlikely that microalbuminuria in DM is due to increased permeability of the glomerular barrier^[27]. A more logical inference is that β_2 -microglobulinuria and albuminuria are tubular phenomena in both Cd nephropathy and early diabetic nephropathy. In the present study, the 13 lowest values of $FrTD_{\beta_2M}$ were seen in DM, and most of the affected subjects exhibited minimal Cd excretion [Figure 4].

Comparison of regressions in women and men

Supplementary Table 1 presents correlations of F_{β_2M} , $FrTD_{\beta_2M}$, and E_{β_2M}/C_{cr} with possible determinants in diabetic and control women and men. F_{β_2M} correlated more closely with eGFR in diabetic and control women than in their male counterparts. $FrTD_{\beta_2M}$ was less closely associated with E_{Cd}/C_{cr} and eGFR in control men than in diabetic women and men and control women. E_{β_2M}/C_{cr} correlated less strongly with eGFR in control men than in diabetic women and men and control women. E_{β_2M}/C_{cr} correlated with F_{β_2M} and TD_{β_2M}/C_{cr} in control men only. We have no explanation for these gender-related differences.

Strengths and weaknesses

In the present study, we analyzed β_2M homeostasis in diabetics and non-diabetics with modest Cd intoxication. To perform the analysis, we employed equations developed from the premise that plasma is normally in equilibrium with respect to β_2M . In other words, we assumed that the entry of β_2M into plasma is balanced by renal disposition of the protein through tubular degradation and urinary excretion.

Equations that followed from this assumption provided guidance in the selection of regressions for examination, and the significant associations that we document confirmed the validity of the framework. To the best of our knowledge, our method for estimating I_{β_2M} has not been previously employed. We are providing new information concerning the effects of diabetes on β_2M homeostasis, the apparent effect of eGFR on I_{β_2M} in CTRL, the superiority of $FrTD_{\beta_2M}$ as an indicator of β_2M degradation, the clear effect of GFR on $FrTD_{\beta_2M}$, the strong inverse relationships of E_{β_2M}/C_{cr} to eGFR and $FrTD_{\beta_2M}$, and the inevitable inadequacy of excretion-based parameters for precise quantification of tubular β_2M handling.

Attributes of the study sample limit the general applicability of our data. Most subjects were not seriously intoxicated with Cd, and documentation of $FrTD_{\beta_2M} > 99\%$ in all participants suggests that tubular effects of Cd and GFR were modest. In contrast, a mean $FrTD_{\beta_2M}$ of 82.7% was documented in severely intoxicated women in Japan^[34]. Although conclusions drawn from the present study may not be applicable to more seriously afflicted individuals, our methods are suitable for the investigation of such subjects.

CONCLUSIONS

Our data support the following conclusions. Flux of β_2M from cells into plasma (I_{β_2M}) was highly variable in DM and CTRL, and the mean I_{β_2M} was higher in DM. Because I_{β_2M} strongly influenced other homeostatic parameters, F_{β_2M} , $[\beta_2M]_s$, TD_{β_2M}/C_{cr} , and E_{β_2M}/C_{cr} were also variable in both groups and higher in DM. At our subjects' level of Cd intoxication, $FrTD_{\beta_2M}$ was consistently $> 99\%$ and typically $> 99.5\%$. Consequently, $[\beta_2M]_s$ and TD_{β_2M}/C_{cr} were virtually equal, and a maximum TD_{β_2M}/C_{cr} was not discernible. Because the range of I_{β_2M} ensured a similar range of TD_{β_2M}/C_{cr} , the latter parameter obscured subtler influences on β_2M degradation that were independent of I_{β_2M} .

We circumvented the limitations of TD_{β_2M}/C_{cr} by determining $FrTD_{\beta_2M}$ (*i.e.*, $TD_{\beta_2M}/F_{\beta_2M}$, calculated as $TD_{\beta_2M}/C_{cr}/[\beta_2M]_s$). This step cancelled the effects of I_{β_2M} on the numerator and denominator of the ratio and thereby quantified phenomena specific to the proximal tubule. $FrTD_{\beta_2M}$ was inversely but weakly related to E_{Cd}/C_{cr} and directly and more strongly related to eGFR. In some subjects, diabetes appeared to reduce $FrTD_{\beta_2M}$ independently of Cd and GFR.

In MLRs, E_{β_2M}/C_{cr} was unrelated to E_{Cd}/C_{cr} . E_{β_2M}/C_{cr} was inversely related to eGFR and especially to $FrTD_{\beta_2M}$, but E_{β_2M}/C_{cr} varied substantially at a given $FrTD_{\beta_2M}$. In an individual subject, E_{β_2M}/C_{cr} had no discernible quantitative relationship with $FrTD_{\beta_2M}$, which was the least confounded and therefore the most useful descriptor of tubular β_2M handling. For quantification of β_2M degradation, we recommend $FrTD_{\beta_2M}$ rather than $[\beta_2M]_u/[cr]_u$ or E_{β_2M}/C_{cr} .

DECLARATIONS

Acknowledgments

The authors thank Dr. Aleksandar Cirovic for his assistance in creating the Graphical Abstract and [Diagram 1](#). The work was supported with resources of Centre for Kidney Disease Research, Translational Research Institute, and Department of Kidney and Transplant Services, Princess Alexandra Hospital, QLD, Australia. It was also supported with resources of the Stratton Veterans Affairs Medical Center, Albany, NY, USA. Opinions expressed herein are those of the authors and do not represent the official position of the United States Department of Veterans Affairs.

Authors' contributions

Conception and design of the study: Phelps, K. R.; Satarug, S.

Data acquisition: Yimthiang, S.; Pouyfung, P.; Khamphaya, T.

Data curation, analysis, visualization, and interpretation: Phelps, K. R.; Satarug, S.

Writing of the initial and subsequent drafts: Phelps, K. R.; Satarug, S.

Administrative, technical, and material support: Yimthiang, S.; Vesey, D. A.

Review and approval of the final draft: All authors.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Financial support and sponsorship

None.

Conflicts of interest

Soisungwan Satarug is a Guest Editor for the Special Issue *The Health Risks of Heavy Metal Exposure*. Soisungwan Satarug was not involved in any steps of editorial processing, notably including reviewer selection, manuscript handling, or decision making. The other authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The present study was conducted following the principles outlined in the Declaration of Helsinki. Written informed consent to participate in the study was obtained from participants. The Human Research Ethics Committee of Walailak University approved the study protocol (Approval number WUEC-20-132-01, 28 May 2020).

Consent for publication

Not applicable.

Copyright

© The Author(s) 2025.

REFERENCES

1. Watanabe, T.; Kataoka, Y.; Hayashi, K.; Matsuda, R.; Uneyama, C. Dietary exposure of the Japanese general population to elements: total diet study 2013-2018. *Food. Saf.* **2022**, *10*, 83-101. [DOI](#) [PubMed](#) [PMC](#)
2. Pokharel, A.; Wu, F. Dietary exposure to cadmium from six common foods in the United States. *Food. Chem. Toxicol.* **2023**, *178*, 113873. [DOI](#) [PubMed](#)
3. Egger, A. E.; Grabmann, G.; Gollmann-Tepeköylü, C.; et al. Chemical imaging and assessment of cadmium distribution in the human body. *Metallomics* **2019**, *11*, 2010-9. [DOI](#) [PubMed](#)
4. Almerud, P.; Zamaratskaia, G.; Lindroos, A. K.; et al. Cadmium, total mercury, and lead in blood and associations with diet, sociodemographic factors, and smoking in Swedish adolescents. *Environ. Res.* **2021**, *197*, 110991. [DOI](#) [PubMed](#)
5. Hill, D. T.; Jandev, V.; Petroni, M.; et al. Airborne levels of cadmium are correlated with urinary cadmium concentrations among young children living in the New York state city of Syracuse, USA. *Environ. Res.* **2023**, *223*, 115450. [DOI](#) [PubMed](#) [PMC](#)
6. Johri, N.; Jacquillet, G.; Unwin, R. Heavy metal poisoning: the effects of cadmium on the kidney. *Biometals* **2010**, *23*, 783-92. [DOI](#) [PubMed](#)
7. Satarug, S.; Vesey, D. A.; Ruangyuttikarn, W.; Nishijo, M.; Gobe, G. C.; Phelps, K. R. The source and pathophysiologic significance of excreted cadmium. *Toxics* **2019**, *7*, 55. [DOI](#) [PubMed](#) [PMC](#)
8. Phelps, K. R.; Gosmanova, E. O. A generic method for analysis of plasma concentrations. *Clin. Nephrol.* **2020**, *94*, 43-9. [DOI](#) [PubMed](#)
9. Heymsfield, S. B.; Arteaga, C.; McManus, C.; Smith, J.; Moffitt, S. Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am. J. Clin. Nutr.* **1983**, *37*, 478-94. [DOI](#) [PubMed](#)
10. Argyropoulos, C. P.; Chen, S. S.; Ng, Y. H.; et al. Rediscovering beta-2 microglobulin as a biomarker across the spectrum of kidney diseases. *Front. Med.* **2017**, *4*, 73. [DOI](#) [PubMed](#) [PMC](#)

11. Gauthier, C.; Nguyen-Simonnet, H.; Vincent, C.; Revillard, J. P.; Pellet, M. V. Renal tubular absorption of beta 2 microglobulin. *Kidney. Int.* **1984**, *26*, 170-5. DOI PubMed
12. Norden, A. G.; Lapsley, M.; Lee, P. J.; et al. Glomerular protein sieving and implications for renal failure in Fanconi syndrome. *Kidney. Int.* **2001**, *60*, 1885-92. DOI PubMed
13. Polesel, M.; Kaminska, M.; Haenni, D.; et al. Spatiotemporal organisation of protein processing in the kidney. *Nat. Commun.* **2022**, *13*, 5732. DOI PubMed PMC
14. Satarug, S.; Vesey, D. A.; Gobe, G. C.; Phelps, K. R. The pathogenesis of albuminuria in cadmium nephropathy. *Curr. Res. Toxicol.* **2024**, *6*, 100140. DOI PubMed PMC
15. Zavala-Guevara, I. P.; Ortega-Romero, M. S.; Narváez-Morales, J.; et al. Increased endocytosis of cadmium-metallothionein through the 24p3 receptor in an *in vivo* model with reduced proximal tubular activity. *Int. J. Mol. Sci.* **2021**, *22*, 7262. DOI PubMed PMC
16. Thévenod, F.; Herbrechter, R.; Schlabs, C.; et al. Role of the SLC22A17/lipocalin-2 receptor in renal endocytosis of proteins/metalloproteins: a focus on iron- and cadmium-binding proteins. *Am. J. Physiol. Renal. Physiol.* **2023**, *325*, F564-77. DOI PubMed
17. Bernier, G. M.; Conrad, M. E. Catabolism of human beta-2-microglobulin by the rat kidney. *Am. J. Physiol.* **1969**, *217*, 1359-62. DOI PubMed
18. Karlsson, F. A.; Groth, T.; Sege, K.; Wibell, L.; Peterson, P. A. Turnover in humans of beta 2-microglobulin: the constant chain of HLA-antigens. *Eur. J. Clin. Invest.* **1980**, *10*, 293-300. DOI PubMed
19. Sumpio, B. E.; Maack, T. Kinetics, competition, and selectivity of tubular absorption of proteins. *Am. J. Physiol.* **1982**, *243*, F379-92. DOI PubMed
20. Peterson, P. A.; Evrin, P. E.; Berggård, I. Differentiation of glomerular, tubular, and normal proteinuria: determinations of urinary excretion of beta-2-macroglobulin, albumin, and total protein. *J. Clin. Invest.* **1969**, *48*, 1189-98. DOI PubMed PMC
21. Viberti, G. C.; Pickup, J. C.; Jarrett, R. J.; Keen, H. Effect of control of blood glucose on urinary excretion of albumin and beta2 microglobulin in insulin-dependent diabetes. *N. Engl. J. Med.* **1979**, *300*, 638-41. DOI PubMed
22. Watts, G. F.; Powell, M.; Rowe, D. J.; Shaw, K. M. Low-molecular-weight proteinuria in insulin-dependent diabetes mellitus: a study of the urinary excretion of beta 2-microglobulin and retinol-binding protein in alkalinized patients with and without microalbuminuria. *Diabetes. Res.* **1989**, *12*, 31-6. PubMed
23. Groop, L.; Mäkipernaa, A.; Stenman, S.; DeFronzo, R. A.; Teppo, A. M. Urinary excretion of kappa light chains in patients with diabetes mellitus. *Kidney. Int.* **1990**, *37*, 1120-5. DOI PubMed
24. Satarug, S.; Yimthiang, S.; Pouyfung, P.; Khamphaya, T.; Vesey, D. A. Cadmium-induced tubular dysfunction in type 2 diabetes: a population-based cross-sectional study. *Toxics* **2023**, *11*, 390. DOI PubMed PMC
25. Yimthiang, S.; Vesey, D. A.; Pouyfung, P.; Khamphaya, T.; Gobe, G. C.; Satarug, S. Chronic kidney disease induced by cadmium and diabetes: a quantitative case-control study. *Int. J. Mol. Sci.* **2023**, *24*, 9050. DOI PubMed PMC
26. Han, E.; Kim, M. K.; Lee, Y. H.; Kim, H. S.; Lee, B. W. Association between nonalbumin proteinuria and renal tubular damage of N-acetyl-β-d-glucosaminidase and its clinical relevance in patients with type 2 diabetes without albuminuria. *J. Diabetes. Complications.* **2019**, *33*, 255-60. DOI PubMed
27. Thethi, T. K.; Batuman, V. Challenging the conventional wisdom on diabetic nephropathy: is microalbuminuria the earliest event? *J. Diabetes. Complications.* **2019**, *33*, 191-2. DOI PubMed
28. Adokwe, J. B.; Waeyeng, D.; Suwan, K.; et al. Plant-based diet and glycemic control in type 2 diabetes: evidence from a Thai Health-Promoting Hospital. *Nutrients* **2024**, *16*, 619. DOI PubMed PMC
29. Xu, R.; Tan, X.; Li, T.; Liu, S.; Li, Y.; Li, H. Norepinephrine-induced AuPd aerogels with peroxidase- and glucose oxidase-like activity for colorimetric determination of glucose. *Mikrochim. Acta.* **2021**, *188*, 362. DOI PubMed
30. Apple, F.; Bandt, C.; Prosch, A.; Erlandson, G.; Holmstrom, V.; Scholen, J.; Googins, M. Creatinine clearance: enzymatic vs Jaffé determinations of creatinine in plasma and urine. *Clin. Chem.* **2022**, *32*, 388-90. PubMed
31. Trzcinka-Ochocka, M.; Brodzka, R.; Janasik, B. Useful and fast method for blood lead and cadmium determination using ICP-MS and GF-AAS; validation parameters. *J. Clin. Lab. Anal.* **2016**, *30*, 130-9. DOI PubMed PMC
32. Hornung, R. W.; Reed, L. D. Estimation of average concentration in the presence of nondetectable values. *Appl. Occup. Environ. Hyg.* **1990**, *5*, 46-51. DOI
33. Levey, A. S.; Stevens, L. A.; Schmid, C. H.; et al; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) A new equation to estimate glomerular filtration rate. *Ann. Intern. Med.* **2009**, *150*, 604-12. DOI PubMed PMC
34. Hayashi, T.; Nogawa, K.; Watanabe, Y.; et al. Benchmark dose of urinary cadmium for assessing renal tubular and glomerular function in a cadmium-polluted area of Japan. *Toxics* **2024**, *12*, 836. DOI PubMed PMC
35. Satarug, S.; Vesey, D. A.; Nishijo, M.; Ruangyuttikarn, W.; Gobe, G. C.; Phelps, K. R. The effect of cadmium on GFR is clarified by normalization of excretion rates to creatinine clearance. *Int. J. Mol. Sci.* **2021**, *22*, 1762. DOI PubMed PMC
36. Portale, A. A.; Halloran, B. P.; Morris, R. C. J. Dietary intake of phosphorus modulates the circadian rhythm in serum concentration of phosphorus. Implications for the renal production of 1,25-dihydroxyvitamin D. *J. Clin. Invest.* **1987**, *80*, 1147-54. DOI PubMed PMC
37. Cappuccio, F. P.; Buchanan, L. A.; Ji, C.; Siani, A.; Miller, M. A. Systematic review and meta-analysis of randomised controlled trials on the effects of potassium supplements on serum potassium and creatinine. *BMJ. Open.* **2016**, *6*, e011716. DOI PubMed PMC
38. Satarug, S.; Phelps, K. R. Cadmium exposure and toxicity. *Metal. Toxicology. Handbook.* CRC Press, 2020; pp 219-72. DOI
39. Cheung, C. L.; Lam, K. S.; Cheung, B. M. Serum β-2 microglobulin predicts mortality in people with diabetes. *Eur. J. Endocrinol.*

- 2013**, *169*, 1-7. DOI PubMed
40. Kim, M. K.; Yun, K. J.; Chun, H. J.; et al. Clinical utility of serum beta-2-microglobulin as a predictor of diabetic complications in patients with type 2 diabetes without renal impairment. *Diabetes. Metab.* **2014**, *40*, 459-65. DOI PubMed
 41. Poortmans, J.; Dorchy, H.; Toussaint, D. Urinary excretion of total proteins, albumin, and beta 2-microglobulin during rest and exercise in diabetic adolescents with and without retinopathy. *Diabetes. Care.* **1982**, *5*, 617-23. DOI PubMed
 42. Russo, L. M.; Sandoval, R. M.; McKee, M.; et al. The normal kidney filters nephrotic levels of albumin retrieved by proximal tubule cells: retrieval is disrupted in nephrotic states. *Kidney. Int.* **2007**, *71*, 504-13. DOI PubMed
 43. Dickson, L. E.; Wagner, M. C.; Sandoval, R. M.; Molitoris, B. A. The proximal tubule and albuminuria: really! *J. Am. Soc. Nephrol.* **2014**, *25*, 443-53. DOI PubMed PMC
 44. Comper, W. D.; Vuchkova, J.; McCarthy, K. J. New insights into proteinuria/albuminuria. *Front. Physiol.* **2022**, *13*, 991756. DOI PubMed PMC
 45. Molitoris, B. A.; Sandoval, R. M.; Yadav, S. P. S.; Wagner, M. C. Albumin uptake and processing by the proximal tubule: physiological, pathological, and therapeutic implications. *Physiol. Rev.* **2022**, *102*, 1625-67. DOI PubMed PMC