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Urinary Calcium Excretion and Risk of Chronic Kidney Disease in the General Population

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Introduction: High urinary calcium excretion (UCaE) has been shown to lead to accelerated renal function decline in individuals with renal tubular diseases. It is not known whether this association also exists in the general population. Therefore, we investigated whether high UCaE is associated with risk of developing chronic kidney disease (CKD) in community-dwelling subjects.

Methods: Urine samples of 5491 subjects who were free of CKD at baseline and participated in the Prevention of Renal and Vascular End-Stage Disease study (a prospective, observational, general population-based cohort of Dutch men and women aged 28–75 years) were examined for UCaE. UCa concentration was measured in two 24-hour urine samples at baseline (1997–1998) by indirect potentiometry. UCaE was treated as a continuous variable and a categorical variable grouped according to sex-specific quintiles for UCaE. UCaE was compared with *de novo* development of estimated glomerular filtration rate $<$60 ml/min per 1.73 m^2 and/or albuminuria $>$30 mg/24 h.

Results: Baseline median UCaE was 4.13 mmol/24 h for men and 3.52 mmol/24 h for women. During a median follow-up of 10.3 years, 899 subjects developed CKD. After multivariable adjustment, every 1 mmol/24 h higher baseline UCaE was associated with a 6% lower risk for incident CKD during follow-up (hazard ratio: 0.94 [0.88–0.99], $P = 0.02$). The association was shown to be significantly nonlinear, with highest risk of CKD in the lowest quintile for UCaE (hazard ratio: 1.28 [0.97–1.68], $P = 0.09$). There was no association between UCaE and mortality or cardiovascular health during follow-up, suggesting that this association was not a reflection of poor nutritional intake due to bad health.

Discussion: These findings indicate that high UCaE does not increase risk of CKD, but rather that low UCaE may be harmful.

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Calcium and vitamin D are essential nutrients for human health, playing a pivotal role in normal bone mineralization. Yet, data from Dutch and American cohorts show that less than 50% of adults meet their daily recommended intake for calcium and are assumed to be at risk for bone fractures.[1–3] To meet these recommendations, a substantial portion of the population must increase calcium intake through dietary interventions, such as by increasing the intake of dairy products, or by being prescribed daily calcium supplements. Recently, however, investigators have questioned whether increasing the intake of calcium is beneficial, because higher intakes of calcium were not associated with a reduction in bone fractures.[4] The promotion of higher intakes of calcium has also come under scrutiny because this will likely lead to higher high urinary calcium excretion (UCaE) and kidneys may be susceptible to damage from high UCaE.

It has been shown that specific renal tubular diseases that result in hypercalciuria lead to nephrocalcinosis and accelerated renal function loss. For instance, 30% to 80% of males suffering from Dent's disease develop chronic kidney disease (CKD) between 30 and 50 years of age, and familial hypomagnesemia with hypercalciuria results in CKD by age 30 in 50% to 73% of cases.[5–8] These findings suggest that elevated levels of

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UCaE may have deleterious renal effects. Cohort studies that investigated the impact of UCaE, such as the Nurses' Health Study, have focused on the association between UCaE and kidney stones, leaving unknown whether high UCaE affects long-term kidney function in community-dwelling subjects.[9] Therefore, we investigated the association between high UCaE and risk of developing CKD in a general population cohort.

MATERIALS AND METHODS

Study Design and Population

The Prevention of Renal and Vascular End-Stage Disease (PREVEND) study is designed to prospectively investigate the natural course of increased levels of urinary albumin excretion (UAE) and its relation with renal and cardiovascular outcome in a large cohort drawn from the general population. Details of this study have been described elsewhere.[10] In brief, from 1997 to 1998, all inhabitants of Groningen (the Netherlands), aged 28 to 75 years (n = 85,421), were sent a short questionnaire on demographics and renal and cardiovascular morbidity and a vial to collect a first morning void urine sample. Altogether, 40,856 people (48%) responded and their urinary albumin concentration was assessed. Of these people, 9966 had a urinary albumin concentration of ≥10 mg/l. After exclusion of pregnant women and subjects with type 1 diabetes mellitus, 7768 subjects were invited to participate, of whom 6000 consented and were enrolled. In addition, of the 40,856 responders, there were 30,890 people with a urinary albumin concentration <10 mg/l. After exclusion of pregnant women and subjects with type 1 diabetes mellitus, a randomly selected group with a urinary albumin concentration of <10 mg/l (n = 3394) was invited to participate in the cohort, of whom 2592 consented and were enrolled. These 8592 individuals form the PREVEND cohort and completed an extensive examination in 1997 and 1998 (baseline). A total of 6894 participants completed a second examination between 2001 and 2003, 5863 a third examination between 2003 and 2006, 5078 a fourth examination between 2006 and 2008, and 4600 a fifth examination between 2008 and 2011.

For the present analyses, we excluded subjects with CKD (n = 1397), unknown CKD status (n = 501), renal disease requiring dialysis (n = 11), and missing values of UCaE (n = 5) at baseline. In addition, those with no follow-up data available for assessment of CKD status (n = 1187) were also excluded. This left 5491 participants to be included in the analysis (Figure 1). The PREVEND study was approved by the medical ethics committee of the University Medical Center Groningen. Written informed consent was obtained from all participants.

Data Collection

The procedures at each examination in the PREVEND study have been described in detail previously.[11] In brief, each examination included 2 visits to an outpatient clinic separated by a maximum of 3 weeks. Before the first visit, all participants completed a self-administered questionnaire regarding demographics, cardiovascular and renal disease history, smoking habits, alcohol consumption, and medication use. Information on medication use was combined with information from community pharmacies. During the first visit, participants' height and weight were assessed. During each examination and during each visit, blood pressure was measured on the right arm, every minute for 10 and 8 minutes, respectively, by an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical, Tampa, FL). The mean of the last 2 recordings from each of the 2 visits was used. In the week before the second visit, subjects collected 2 consecutive 24-hour specimens after thorough oral and written instruction. The participants were asked to avoid heavy exercise as much as possible during the urine collection and to postpone the urine collection in case of urinary tract infection, menstruation, or fever. The collected urine was stored in a cold environment (4°C) for a maximum of 4 days before handing in the urine collections. Aliquots of these urine specimens were then stored at −20°C. Furthermore, fasting blood samples were obtained at the second visit and were stored at −80°C.

Assessment of Urinary Calcium Excretion

Urinary calcium concentration was determined by indirect potentiometry with a MEGA clinical chemistry analyzer (Merck, Darmstadt, Germany).[12] Urinary calcium concentration was multiplied by urine volume to obtain UCaE (in mmol per 24 hours). The average value of the paired 24-hour collections was calculated and used for analysis.

Ascertainment of Incident CKD, Cardiovascular Events, and Mortality

The primary outcome of incident CKD was defined as reaching an estimated glomerular filtration rate (eGFR) of <60 ml/min per 1.73 m^2 and/or a UAE of >30 mg/24 h *de novo*. GFR was estimated with the combined creatinine cystatin C-based Chronic Kidney Disease Epidemiology (CKD-EPI) Collaboration equation from 2012.[13]

Serum creatinine concentration was measured by an isotope dilution mass spectrometry traceable enzymatic method on a Roche Modular analyzer (Roche Diagnostics, Mannheim, Germany). Serum cystatin C concentration was measured by an immunoassay

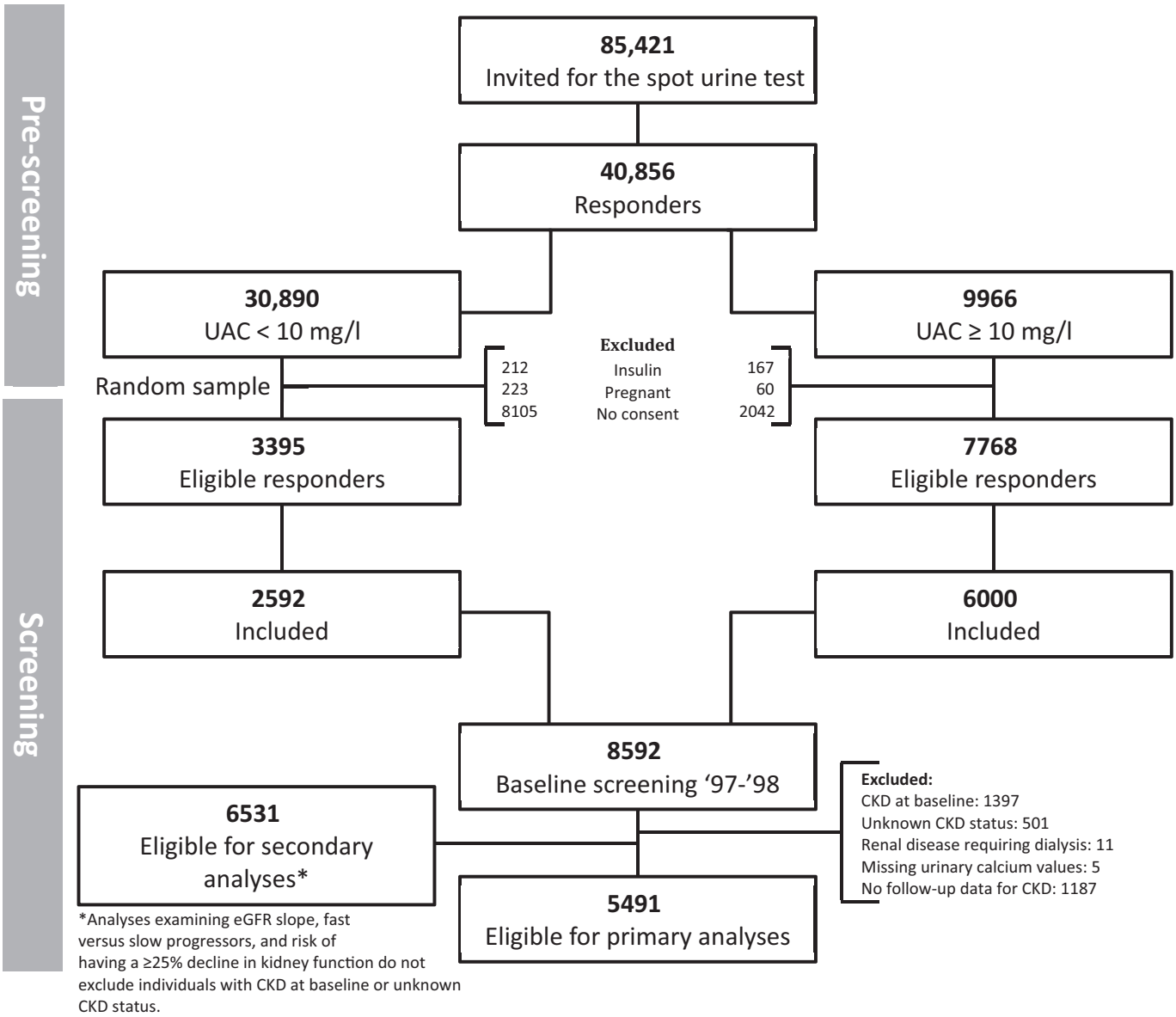

Figure 1. Flow chart of study design and exclusion criteria for analyses. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; UAC, urinary albumin concentration.

(Gentian AS, Moss, Norway) on a Modular analyzer (Roche Diagnostics) calibrated directly using the standard supplied by the manufacturer (traceable to the International Federation of Clinical Chemistry Working Group for Standardization of Serum Cystatin C).[14] Urinary albumin concentration was measured in aliquots from the 24-hour urine collections by nephelometry (Dade Behring Diagnostic, Marburg, Germany) with a threshold of 2.3 mg/l. Urinary albumin concentration was multiplied by 24-hour urine volume to obtain UAE (in mg/24 h). The two 24-hour UAE values of each subject per examination were averaged and used in the analysis.

Data on mortality and incident fatal and nonfatal cardiovascular events during follow-up were examined to investigate whether low UCaE may reflect poor dietary intake due to impaired general health. Data on mortality and fatal cardiovascular events were received through the municipal register, whereas data on nonfatal cardiovascular events were obtained from Prismant, the Dutch national registry of hospital discharge diagnoses. Cardiovascular events were coded according to the *International Classification of Diseases, Ninth Revision*, and consisted of cardiac events (code 410, 411, 414, 36.0), cerebrovascular events (code 430–434), and vascular interventions, including percutaneous transluminal angioplasty and bypass grafting of aorta and peripheral vessels.

Assessment of Covariates

Smoking status was self-reported as never smoker, former smoker, or current smoker. Alcohol intake was

self-reported as no/rarely, 1–4 drinks/mo, 2–6 drinks/wk, 1–3 drinks/d, or ≥4 drinks/d. Parental history of CKD was defined as having a first-degree relative who had a renal disease requiring dialysis for >6 weeks. Hypertension was defined as systolic blood pressure of ≥140 mm Hg, a diastolic blood pressure of ≥90 mm Hg, or the use of antihypertensive agents as previously described.[15,16] Urinary sodium, potassium, magnesium, urea and circulating levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, calcium, phosphorus, parathyroid hormone (PTH), magnesium, total cholesterol, high-density lipoprotein cholesterol, triglycerides, and glucose were determined as previously described.[12,17–20] Hypercholesterolemia was defined as having a total cholesterol >6.2 mmol/l without a history of myocardial infarction, a total cholesterol >5.2 mmol/l with a history of myocardial infarction, or use of a lipid lowering drug. Diabetes was defined as a fasting plasma glucose ≥7.0 mmol/l (>126 mg/dl) or the use of glucose-lowering drugs.[21] Information on calcium and vitamin D supplements, thiazide and loop diuretics, and bisphosphonates was obtained from the IADB.nl database, a database that contains pharmacy-dispensing data from community pharmacies in the northern part of the Netherlands.[22]

Statistical Analyses

Baseline characteristics are presented according to sex-specific quintiles of UCaE. Ranges for the quintiles remained the same during sensitivity analyses to allow for ease comparing results. Continuous data are presented as mean ± SD or as median (interquartile range [IQR]) in the case of a skewed distribution. Categorical data are presented as percentages. Linear-by-linear associations were determined by the χ^2 test for categorical variables and linear regression for continuous variables.

UCaE was analyzed as a continuous variable and in sex-specific quintiles. To study the prospective association between UCaE and risk of developing CKD, Cox proportional hazards regression analyses were used to calculate hazard ratios (HRs) and 95% confidence intervals. Nonlinearity was tested by using the likelihood ratio test, comparing nested models with linear and cubic spline terms.

We first calculated HRs (95% confidence intervals) for the crude model. Second, we adjusted for body size and nonmodifiable factors associated with risk of kidney dysfunction, including age, sex, height, weight, race, and baseline eGFR and lnUAE. Third, we adjusted for renal disease risk factors, including smoking, alcohol, hypertension, diabetes, parental history of CKD, and hypercholesterolemia. Fourth, we adjusted for plasma markers and supplement/medication usage that may affect calcium uptake and excretion, including plasma magnesium, calcium, phosphorus, PTH, 1,25 dihydroxyvitamin D, albumin, use of loop diuretics, thiazide diuretics, calcium supplements, vitamin D supplements, and bisphosphonates. Finally, we additionally adjusted for dietary factors potentially associated with risk of CKD and UCaE, including urinary sodium, potassium, urea, and magnesium excretion. In secondary analyses, CKD incidence was defined by either impaired eGFR or albuminuria alone. We also evaluated potential effect modification by sex, plasma calcium, PTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D, by fitting models containing both the main effects and their cross-product terms.

Several sensitivity analyses were performed to examine the robustness of the associations between UCaE and risk of CKD. First, we excluded subjects at baseline with an eGFR <66 ml/min per 1.73 m^2 (instead of <60) and/or a UAE >25 mg/24 h (instead of >30) to assure a more pronounced decline in kidney function to define the primary outcome of incident CKD. Second, we analyzed the data excluding subjects with potential inadequate 24-hour urine collections. We defined potential inadequate 24-hour urine collections (i.e., over or under collection) as the upper and lower 2.5% of the difference between the estimated and measured volume of a subject's 24-hour urine sample. The estimated 24-hour urine volume was derived from the formula: Creatinine clearance = ([urine creatinine] × 24-hour urine volume)/[serum creatinine]), where creatinine clearance was estimated using the Cockcroft-Gault formula.[23] Third, we addressed the oversampling of subjects with higher urinary albumin concentrations by analyzing the data in a subset of subjects from the cohort that are representative of the Netherlands population. This subset included all subjects with a urinary albumin concentration of <10 mg/l who completed the first screening (n = 2592) with the addition of the "oversampled" subjects whose urinary albumin concentration was ≥10 mg/l by proportionally taking a computer-generated random subset (n = 840). After applying the same exclusion criteria as in the primary analysis, 929 subjects were excluded leaving a cohort of 2503 subjects to be analyzed as an unbiased sample. Fourth, the association between UCaE and decline in eGFR per year was also examined to identify CKD risk. Linear mixed model analysis was performed with the repeated measures of eGFR as the outcome and a random effect for time, to allow for individual deviations to the overall population slope. Individuals with at least 2 observations were included in the analysis (without excluding individuals with CKD at baseline). Betas are expressed per 1 SD increase for

continuous variables and versus the reference category for dichotomous variables. Fifth, the same group was divided into fast progressors and slow progressors to CKD, and the association between UCaE and risk of being a fast progressor was examined using a logistic regression analysis. Fast progressors were defined as the 20% of individuals with the steepest decline in eGFR per year in the cohort. Finally, in this group, we also analyzed the association between UCaE and risk of having an eGFR decline ≥25% during follow-up.

In addition, to study the prospective association between UCaE and risk of all-cause mortality and fatal and nonfatal cardiovascular events, Cox proportional hazards regression analyses were used to calculate HRs and 95% confidence intervals. Nonlinearity was tested by using the likelihood ratio test, comparing nested models with linear and cubic spline terms. The same covariates were adjusted for in the models as was done when examining risk of developing CKD, with the addition of history of cardiovascular events that was added to models 2–4.

All P values are 2-tailed. A P value <0.05 was considered statistically significant. All analyses were conducted with the use of the statistical package IBM SPSS (version 22; SPSS, Chicago, IL) and Rstudio (version 0.99.491; Rstudio, Boston, MA).

RESULTS

Baseline Demographic, Clinical, and Laboratory Characteristics

Median UCaE at baseline was 3.82 mmol/24 h (IQR: 2.64–5.12 mmol/24 h), with a higher excretion in men (median: 4.13 mmol/24 h; IQR: 2.93–5.49 mmol/24 h) than in women (median: 3.52 mmol/24 h; IQR: 2.40–4.81 mmol/24 h). Baseline characteristics are therefore shown according to sex-specific quintiles of UCaE (Table 1). Subjects with a higher UCaE at baseline were older, more likely to be hypertensive, hypercholesterolemic, diabetic, and have a higher weight, height, and body mass index. These same individuals were also less likely to be current smokers, consume no alcohol, and be on thiazide diuretics. In addition, subjects with higher UCaE had higher plasma calcium, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, a lower plasma phosphorus, and higher urinary potassium, sodium, magnesium, urea, creatinine, and albumin excretion.

Risk of CKD During Follow-up

During a median follow-up of 10.3 years (IQR: 6.2–11.4 years), a total of 899 incident CKD events (defined as an eGFR of <60 ml/min per 1.73 m^2 and/or a UAE >30 mg/24 h) were identified. Examination of linear trends showed that, after adjustment for age, sex, height, weight, race, and baseline eGFR and lnUAE, every 1 mmol/24 h higher baseline UCaE was associated with an 8% lower risk for incident CKD during follow-up (HR: 0.92 [0.89–0.95], $P < 0.001$) (Table 2). These findings persisted after additional adjustment for renal risk factors (model 2), plasma markers, and supplement/medication usage that may affect calcium uptake and excretion (model 3) and dietary factors potentially associated with risk of CKD (model 4) (HR: 0.94 [0.88–0.99], $P = 0.02$) (Table 2).

The association between UCaE and CKD was significantly nonlinear ($P \leq 0.01$ in all models), and this was confirmed visually by the multivariable-adjusted spline curve (Table 2, Figure 2). Thus, we conducted further analyses using sex-specific quintiles for UCaE to allow investigation of nonlinear associations. In these analyses, no increased risk of CKD was observed in the higher UCaE range. However, surprisingly, the highest risk was observed in the lowest quintile for UCaE (Table 2). After adjustment for age, sex, height, weight, race, and baseline eGFR and lnUAE, individuals in the lowest quintile for UCaE had a significantly increased risk of developing CKD (HR: 1.41 [1.15–1.73], $P = 0.001$). This increased risk remained in the lowest quintile, even after extensive adjustment (model 4, HR: 1.28 [0.97–1.68], $P = 0.09$). No effect modification by sex, plasma calcium, PTH, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D was detected in the association between UCaE and CKD ($P > 0.05$).

Secondary Analyses of CKD Using Only eGFR or UAE to Define Endpoint

In a secondary analysis, incident CKD was defined as reaching an eGFR <60 ml/min per 1.73 m^2. Again, a significant association between UCaE and risk of incident CKD during follow-up was observed (model 1, HR: 0.89 [0.83–0.95], $P = 0.001$). This association remained significant after adjustment for all other variables in the final model (HR: 0.89 [0.80–0.99], $P = 0.03$) (Table 2). In a separate secondary analysis, incident CKD was defined as reaching a UAE >30 mg/24 h. The association between UCaE and CKD was significantly nonlinear, and was confirmed visually by the multivariable-adjusted spline curve ($P < 0.05$ in all models) (Table 2, Figure 2). After adjustment for age, sex, height, weight, race, and baseline eGFR and lnUAE, individuals in the lowest quintile for UCaE had a significantly increased risk of developing CKD (HR: 1.40 [1.11–1.78], $P = 0.01$), a trend that remained in the lowest quintile through the rest of the models (model 4, HR: 1.39 [0.99–1.93], $P = 0.05$).

Table 1. Baseline characteristics according to quintiles of urinary calcium excretion of 5491 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study

			Sex-specific quintiles of urinary calcium excretion, mmol/24 h					
			♂ <2.65	2.65–3.67	3.68–4.58	4.59–5.80	>5.80	
Variables	N	Overall	♀ <2.16	2.16–3.08	3.09–4.00	4.01–5.14	>5.14	P_{trend} linear[a]
Participants, N	5491	–	1098	1098	1101	1097	1097	
Women, %	5491	52.6	52.6	52.6	52.7	52.6	52.6	0.98
Age, yr	5491	48.3 ± 11.7	47.9 ± 12.7	47.9 ± 12.2	48.2 ± 11.7	48.2 ± 11.2	49.4 ± 10.8	0.001
Race, whites, %	5454	95.9	91.8	95.4	96.7	97.3	98.3	<0.001
Parental history of CKD, %	5491	1.5	1.7	1.4	1.1	1.7	1.5	1.00
Smoking status, current, %	5491	31.8	34.7	33.1	34.1	29.9	27.3	<0.001
Alcohol consumption, none, %	5491	26.7	27.4	25.5	21.3	22.3	20.2	<0.001
Weight, kg	5434	77.1 ± 13.5	75.5 ± 13.3	75.7 ± 13.0	75.8 ± 12.6	78.1 ± 13.7	80.5 ± 14.4	<0.001
Length, cm	5436	173.1 ± 9.5	172.4 ± 9.6	172.7 ± 9.5	173.0 ± 9.0	173.5 ± 9.7	174.1 ± 9.4	<0.001
BMI, kg/m^2	5434	25.7 ± 4.0	25.4 ± 4.1	25.4 ± 3.8	25.3 ±3.7	25.9 ± 4.0	26.5 ± 4.1	<0.001
Systolic blood pressure, mm Hg	5490	126 ± 18	124 ± 18	125 ± 19	126 ± 18	126 ± 17	128 ± 17	<0.001
Diastolic blood pressure, mm Hg	5490	73 ± 9	72 ± 9	72 ± 9	73 ± 9	73 ± 9	74 ± 9	<0.001
Antihypertensive use, %	5491	11.6	13.9	11.2	10.7	11.0	11.1	0.06
Hypertension, %	5412	26.7	25.8	24.1	26.2	27.7	29.6	0.008
Total cholesterol, mmol/l	5469	5.58 ± 1.11	5.49 ± 1.11	5.55 ± 1.12	5.58 ± 1.06	5.58 ± 1.17	5.73 ± 1.08	<0.001
HDL cholesterol, mmol/l	5436	1.35 ± 0.40	1.32 ± 0.40	1.35 ± 0.39	1.36 ± 0.40	1.35 ± 0.39	1.37 ± 0.41	0.01
Triglycerides, mmol/l	5436	1.11 (0.81–1.60)	1.10 (0.81–1.58)	1.10 (0.80–1.55)	1.10 (0.81–1.58)	1.08 (0.80–1.54)	1.17 (0.85–1.69)	0.01
Lipid lowering drug use, %	5491	5.1	5.4	5.2	5.0	4.9	5.1	0.70
Hypercholesterolemia, %	5470	29.8	27.9	29.0	29.8	29.1	33.2	0.01
Glucose, mmol/l	5476	4.7 ± 0.9	4.7 ± 0.8	4.7 ± 0.7	4.7 ± 0.8	4.8 ± 0.8	4.9 ± 1.3	<0.001
Antidiabetic drug use, %	5473	1.0	0.6	0.6	1.4	1.1	1.5	0.03
Diabetes, %	5456	2.0	1.3	1.2	1.9	2.2	3.3	<0.001
eGFR, ml/min per 1.73 m^2	5491	97 ± 15	96 ± 16	97 ± 15	98 ± 15	98 ± 14	98 ± 14	<0.001
Plasma calcium, mmol/l	5130	2.28 ± 0.08	2.27 ± 0.08	2.27 ± 0.08	2.28 ± 0.08	2.28 ± 0.08	2.28 ± 0.09	0.02
Plasma magnesium, mmol/l	5136	0.81 ± 0.06	0.81 ± 0.06	0.81 ± 0.07	0.81 ± 0.06	0.81 ± 0.05	0.81 ± 0.06	0.08
Plasma phosphorus, mmol/l	5136	1.1 ± 0.16	1.01 ± 0.16	1.02 ± 0.16	1.02 ± 0.16	1.01 ± 0.15	1.00 ± 0.16	0.02
PTH, pmol/l	5301	3.62 (2.92–4.49)	3.84 (3.10–4.74)	3.62 (2.90–4.43)	3.52 (2.89–4.36)	3.49 (2.85–4.38)	3.67 (2.92–4.56)	<0.001
25-Hydroxyvitamin D, nmol/l	5329	58 ± 23	53 ± 24	57 ± 23	57 ± 23	60 ± 22	61 ± 23	<0.001
1,25-Dihydroxyvitamin D, pmol/l	5324	145 ± 47	136 ± 45	141 ± 43	143 ± 45	150 ± 47	157 ± 52	<0.001
Plasma albumin, g/l	5136	45.9 ± 2.6	45.7 ± 2.6	45.7 ± 2.6	46.0 ± 2.4	45.9 ± 2.5	45.9 ± 2.6	0.01
Urinary excretion:								
Calcium, mmol/24 h	5491	3.82 (2.64–5.12)	1.70 (1.27–2.06)	2.88 (2.64–3.13)	3.83 (3.50–4.11)	4.80 (4.45–5.12)	6.51 (5.92–7.51)	<0.001
Potassium, mmol/24 h	5491	70.4 (57.3–84.9)	64.2 (51.1–78.6)	67.2 (53.5–81.4)	71.7 (58.2–85.3)	73.4 (60.8–87.0)	75.3 (62.4–89.7)	<0.001
Sodium, mmol/24 h	5491	135 (105–169)	115 (88–146)	123 (96–156)	133 (107–164)	144 (117–177)	160 (131–197)	<0.001
Magnesium, mmol/24 h	5463	3.80 (2.93–4.78)	2.78 (1.81–3.71)	3.37 (2.58–4.17)	3.83 (3.11–4.70)	4.19 (3.39–5.13)	4.69 (3.88–5.72)	<0.001
Urea, mmol/24 h	5491	346 (283–418)	294 (237–358)	317 (263–392)	341 (290–407)	367 (311–439)	407 (341–475)	<0.001
Creatinine, mmol/24 h	5471	11.8 (9.6–14.5)	10.7 (9.0–13.2)	11.4 (9.1–13.9)	11.7 (9.6–14.3)	12.3 (10.1–15.0)	12.8 (10.5–16.1)	<0.001
Albumin, mg/24 h	5491	8.1 (5.9–12.1)	7.0 (5.2–11.0)	7.5 (5.6–11.1)	8.1 (6.0–12.3)	8.5 (6.2–12.0)	9.5 (7.0–14.1)	<0.001

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Table 1. (Continued) Baseline characteristics according to quintiles of urinary calcium excretion of 5491 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study

			Sex-specific quintiles of urinary calcium excretion, mmol/24 h					
			♂ <2.65	2.65–3.67	3.68–4.58	4.59–5.80	>5.80	
Variables	N	Overall	♀ <2.16	2.16–3.08	3.09–4.00	4.01–5.14	>5.14	P_{trend} linear[a]
Supplementation:								
Calcium supplement use, %	4671	0.7	0.8	0.5	0.7	0.8	0.4	0.46
Vitamin D supplement use, %	4671	0.3	0.3	0.3	0.4	0.4	0.2	0.87
Bisphosphonate use, %	5348	0.3	0.3	0.7	0.4	0.3	0.1	0.18
Thiazide diuretic use, %	4672	1.8	3.2	2.0	1.3	1.2	1.3	0.001
Loop diuretic use, %	4671	0.5	0.3	0.4	0.4	0.5	0.7	0.18

Continuous variables are reported as mean ± SD or median (interquartile range), and categorical variables are reported as percentage.
BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; PTH, parathyroid hormone.
[a]Determined by the χ^2 test—linear-by-linear association (categorical variables) and linear regression (continuous variables).

Sensitivity Analyses and Alternate Endpoints to Define CKD

We also conducted several sensitivity analyses. First, we removed individuals with an eGFR 60–66 ml/min per 1.73 m^2 and UAE 25–30 mg/24 h at baseline to assure that the endpoint of incident CKD reflects a more prominent decrease in eGFR and increase in albuminuria. Compared with the initial findings, we found similar HRs for CKD in the lowest quintiles of UCaE in the final models when defined by the combined eGFR and UAE endpoint and when assessing CKD only by UAE (HR: 1.28 [0.94, 1.74], $P = 0.12$ and HR: 1.46 [1.03, 2.07], $P = 0.03$, respectively). When CKD was defined only by eGFR, a similar linear association with the initial findings was observed in the final model (HR: 0.88 [0.79, 1.00], $P = 0.04$) (Supplementary Table S1). Second, when we accounted for potential over- and undercollection of 24-hour urine samples in the cohort, results were in accord with the original findings (Supplementary Table S2). Third, after accounting for the oversampling of individuals with elevated UAE (>10 mg/l) due to the study design, significant nonlinear associations were also observed between UCaE and risk of the combined endpoint ($P = 0.03$) and UAE endpoint ($P = 0.03$). In agreement with the initial findings, an increased risk of CKD was observed in the lowest UCaE quintile for the combined and UAE endpoint, but did not reach statistical significance potentially due to loss of power (n = 2503 instead of n = 5491). The association between UCaE and eGFR was consistent with the original findings (HR: 0.83 [0.69–1.00], $P = 0.05$) in this sensitivity analysis (Supplementary Table S3). Fourth, when examining the association between UCaE and decrease in eGFR per year (without excluding individuals with CKD at baseline [n = 6531]), after adjustment for all covariates, having a higher UCaE was associated with a steeper decline in eGFR during follow-up (model 4, $P < 0.001$) (Table 3) (baseline characteristics in Supplementary Table S4). We also examined the association between UCaE and the risk of being a fast progressor to CKD in the same group, defined as belonging to the 20% of individuals with the steepest decline in eGFR per year during follow-up. This sensitivity analysis demonstrated that for every 1 mmol/24 h increase in UCaE, the risk of being a fast progressor was reduced by 8% (odds ratio: 0.92 [0.87–0.97], $P = 0.003$), further confirming the initial findings (Table 4). In a final sensitivity analysis, we analyzed the association between UCaE and risk having an eGFR decline ≥25% during follow-up. Again, there was an inverse relationship between UCaE and risk of kidney function decline (HR: 0.91 [0.84–0.99], $P = 0.02$) (Table 5).

Table 2. Association between urinary calcium excretion and risk of chronic kidney disease in 5491 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study

			Sex-specific quintiles of urinary calcium excretion, mmol/24 h					
			♂ <2.65	2.65–3.67	3.68–4.58	4.59–5.80	>5.80	
CKD definition	Continuous urinary calcium excretion, per 1 mmol/24 h increment	P_{trend} linear[a]	♀ <2.16	2.16–3.08	3.09–4.00	4.01–5.14	>5.14	P_{trend} nonlinear[b]
eGFR <60 ml/min per 1.73 m^2 or UAE >30 mg/24 h								
Person-years	48,648		9371	9609	9752	9969	9947	
Number of events	899		213	180	166	155	185	
Crude model	0.98 (0.95, 1.02)	0.28	1.34 (1.09, 1.64)	1.10 (0.89, 1.36)	1.00 (ref)	0.91 (0.73, 1.13)	1.09 (0.88, 1.34)	0.001
Model 1[c]	0.92 (0.89, 0.95)	<0.001	1.41 (1.15, 1.73)	1.17 (0.95, 1.45)	1.00 (ref)	0.88 (0.70, 1.09)	0.86 (0.70, 1.07)	0.01
Model 2[d]	0.91 (0.88, 0.94)	<0.001	1.45 (1.18, 1.79)	1.18 (0.95, 1.46)	1.00 (ref)	0.84 (0.67, 1.05)	0.85 (0.68, 1.05)	0.002
Model 3[e]	0.90 (0.86, 0.95)	<0.001	1.38 (1.06, 1.80)	1.21 (0.93, 1.58)	1.00 (ref)	0.73 (0.55, 0.98)	0.80 (0.61, 1.06)	0.002
Model 4[f]	0.94 (0.88, 0.99)	0.02	1.28 (0.97, 1.68)	1.19 (0.91, 1.55)	1.00 (ref)	0.77 (0.57, 1.03)	0.88 (0.66, 1.17)	0.004
eGFR <60 ml/min per 1.73 m^2								
Person-years	51,938		10,003	10,191	10,381	10,595	10,768	
Number of events	299		95	76	53	36	39	
Crude model	0.79 (0.74, 0.85)	<0.001	1.87 (1.34, 2.62)	1.47 (1.03, 2.08)	1.00 (ref)	0.66 (0.43, 1.01)	0.70 (0.47, 1.06)	0.34
Model 1[c]	0.89 (0.83, 0.95)	0.001	1.36 (0.97, 1.90)	1.37 (0.96, 1.95)	1.00 (ref)	0.82 (0.54, 1.26)	0.79 (0.52, 1.20)	0.85
Model 2[d]	0.89 (0.83, 0.96)	0.001	1.35 (0.96, 1.90)	1.31 (0.92, 1.88)	1.00 (ref)	0.76 (0.50, 1.17)	0.81 (0.53, 1.24)	0.72
Model 3[e]	0.83 (0.76, 0.91)	<0.001	1.62 (1.06, 2.48)	1.54 (1.01, 2.36)	1.00 (ref)	0.89 (0.53, 1.48)	0.67 (0.39, 1.16)	0.48
Model 4[f]	0.89 (0.80, 0.99)	0.03	1.30 (0.82, 2.05)	1.46 (0.95, 2.24)	1.00 (ref)	0.95 (0.57, 1.59)	0.73 (0.42, 1.27)	0.90
UAE >30 mg/24 h								
Person-years	52,209		10,169	10,386	10,523	10,588	10,543	
Number of events	692		153	116	128	132	163	
Crude model	1.05 (1.01, 1.09)	0.02	1.23 (0.97, 1.56)	0.92 (0.71, 1.18)	1.00 (ref)	1.02 (0.80, 1.30)	1.27 (1.00, 1.60)	0.02
Model 1[c]	0.94 (0.91, 0.98)	0.002	1.40 (1.11, 1.78)	1.03 (0.80, 1.32)	1.00 (ref)	0.96 (0.75, 1.23)	0.93 (0.74, 1.18)	0.02
Model 2[d]	0.93 (0.90, 0.97)	0.001	1.43 (1.12, 1.82)	1.03 (0.80, 1.33)	1.00 (ref)	0.92 (0.71, 1.18)	0.90 (0.71, 1.14)	0.01
Model 3[e]	0.94 (0.89, 0.99)	0.02	1.39 (1.02, 1.89)	0.97 (0.70, 1.36)	1.00 (ref)	0.78 (0.56, 1.10)	0.91 (0.66, 1.25)	0.004
Model 4[f]	0.96 (0.90, 1.02)	0.20	1.39 (0.99, 1.93)	0.98 (0.70, 1.37)	1.00 (ref)	0.82 (0.59, 1.15)	0.99 (0.71, 1.38)	0.003

Hazard ratios (HR) and 95% confidence intervals were derived from Cox proportional hazards regression models.
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; UAE, urinary albumin excretion.
[a]Derived from a Cox proportional hazards model by using urinary calcium excretion as a continuous linear term.
[b]Derived by using the likelihood ratio test, comparing nested Cox proportional hazards regression models with a linear or linear and cubic spline terms.
[c]Model 1: Adjusted for age, sex, height, weight, race, baseline eGFR, and baseline lnUAE.
[d]Model 2: Model 1 + smoking, alcohol, hypertension, diabetes, parental history of CKD, and hypercholesterolemia.
[e]Model 3: Model 2 + plasma magnesium, calcium, phosphorus, PTH and 1,25-dihydroxyvitamin D, albumin, and use of loop diuretics, thiazide diuretics, calcium supplements, vitamin D supplements, and bisphosphonates.
[f]Model 4: Model 3 + urinary sodium, potassium, urea, and magnesium excretion.

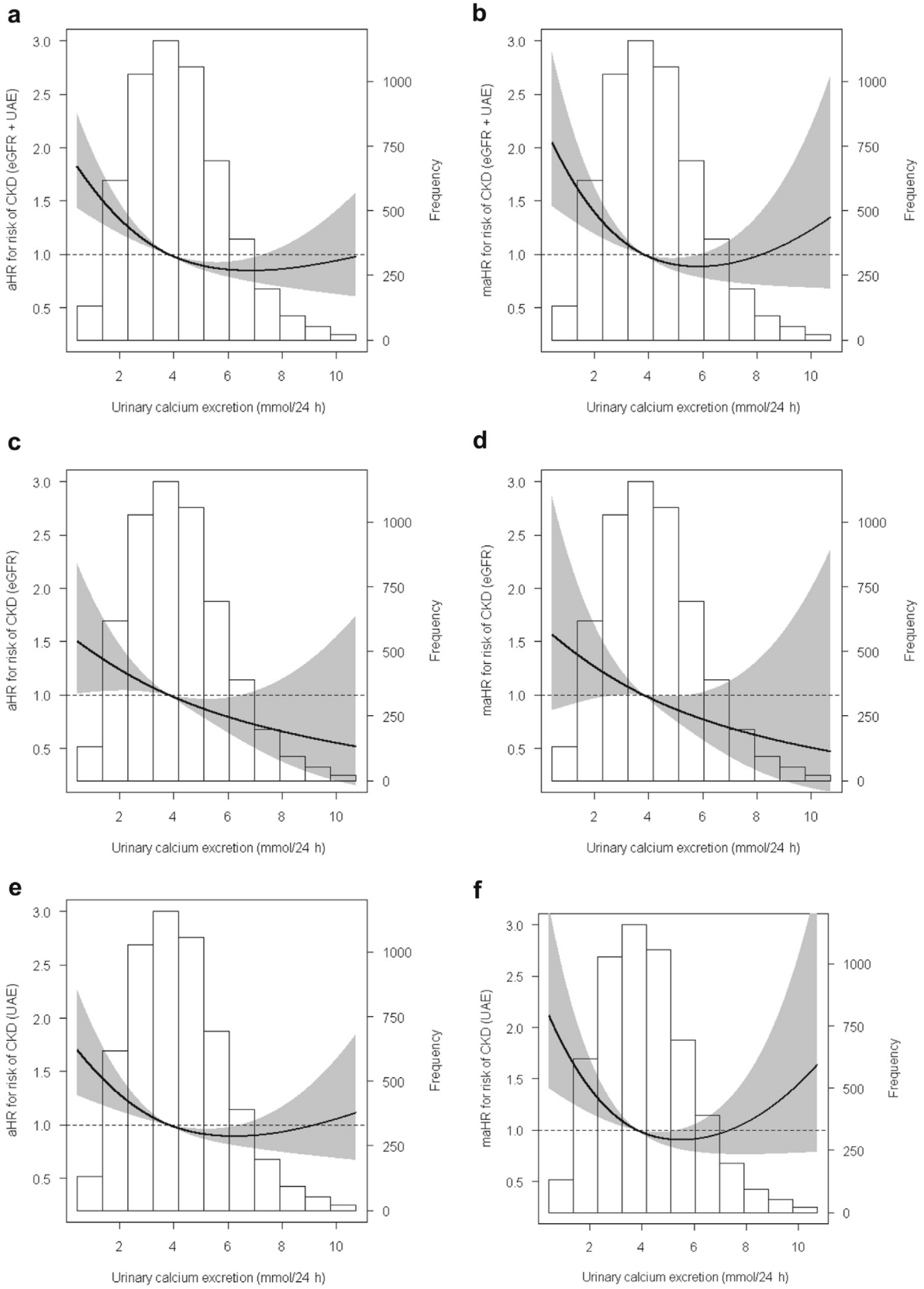

Figure 2. Associations between UCaE and risk of chronic kidney disease in 5491 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study. Data were fit by time-dependent Cox proportional hazards regression models based on restricted cubic splines with 3 knots and adjusted for the covariates in model 1 (a, c, e) and model 4 (b, d, f). The 2 upper panels represent the associations between UCaE and risk of CKD defined as $eGFR_{creatinine\text{-}cystatin\ C} < 60$ ml/min per 1.73 m^2 and/or UAE > 30 mg/24 h (a, b). The 2 middle panels represent the associations between UCaE and risk of CKD defined as $eGFR_{creatinine\text{-}cystatin\ C} < 60$ ml/min per 1.73 m^2 alone (c, d). The 2 lower (continued)

Table 3. Association of urinary calcium excretion and decline in eGFR (ml/min/1.73m^2) per year in 6531 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study

	Unadjusted		Multivariable adjustment							
	Crude model		Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
Variable	β	*P* value	β	*P* value	β	*P* value	β	*P* value	β	*P* value
UCaE	0.035 (0.011, 0.059)	0.01	0.075 (0.052, 0.099)	<0.001	0.084 (0.057, 0.111)	<0.001	0.069 (0.035, 0.101)	<0.001	0.072 (0.031, 0.112)	<0.001

Beta is expressed per 1 SD increase for continuous variables and versus the reference category for dichotomous variables. 95% CI are in parentheses.
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; UAE, urinary albumin excretion; UCa, urinary calcium excretion.
[a]Model 1: Adjusted for age, sex, height, weight, race, baseline eGFR, and baseline lnUAE.
[b]Model 2: Model 1 + smoking, alcohol, hypertension, diabetes, parental history of CKD, and hypercholesterolemia.
[c]Model 3: Model 2 + plasma magnesium, calcium, phosphorus, PTH and 1,25-dihydroxyvitamin D, albumin, and use of loop diuretics, thiazide diuretics, calcium supplements, vitamin D supplements, and bisphosphonates.
[d]Model 4: Model 3 + urinary sodium, potassium, urea, and magnesium excretion.

Association Between UCaE and Mortality and Cardiovascular Events

Lastly, because low UCaE may reflect poor dietary intake associated with impaired general health, we also studied the association between UCaE and all-cause mortality and cardiovascular events. No such associations were observed (Table 6). In particular, those in the lowest quintile of UCaE did not have a significantly increased risk of all-cause mortality (HR: 1.17 [0.68–2.01], $P = 0.57$) or cardiovascular events (HR: 0.98 [0.66–1.45], $P = 0.92$) in the final (or any) model.

DISCUSSION

The findings from this study indicate that, in a population-based cohort, having a higher UCaE was not associated with an increased risk of CKD. Surprisingly, lower UCaE was associated with a higher risk of incident CKD. When CKD was defined only by eGFR, a significant linear trend was present, with every 1 mmol/24 h increase in UCaE being associated with an 11% lower risk of incident CKD during follow-up. When CKD was defined by both eGFR and UAE, or only by UAE, significant nonlinear trends were observed, with the lowest quintiles of UCaE having the highest risk of CKD during follow-up. Alternative endpoint analyses and sensitivity analyses confirmed the robustness of these findings.

It has been shown that specific renal tubular diseases that result in hypercalciuria lead to nephrocalcinosis and accelerated renal function loss.[5–8] These findings suggest that high UCaE may have deleterious renal effects. Although many regulatory factors may affect calcium uptake and excretion, increasing dietary intake will give rise to UCaE, which has the potential to be harmful to kidney tissue. Because less than 50% of the population meet their recommended intake for calcium and are at risk for fractures, supplementation to increase calcium intake for the benefits of improving bone health seems logical.[1–3,24] However, a recent systematic review and meta-analysis concluded that, although increasing calcium intake may result in small increases in bone mineral density, it is unlikely to be clinically meaningful for the reduction of bone fractures.[4] Given that no benefit was observed in reducing bone fractures, consuming high amounts of calcium may ultimately pose more of a risk than a benefit. Surprisingly, studies examining the effects of UCaE on the kidneys have focused on the risk for kidney stones, and did not report on kidney function outcome. This makes our study the first to examine the effects of UCaE on long-term kidney function in the general population.

Interestingly, in this cohort of community-dwelling adults, we found that high UCaE did not result in an increased risk of developing CKD. It should be noted that the studies demonstrating the effects of calcium intake on the development of kidney stones have primarily included individuals taking calcium and/or vitamin D supplements.[9,25] In our cohort, only 0.6% of individuals (31/4671) were reported to be on calcium supplementation, and even fewer were on vitamin D. We also observed lower mean UCaE than other epidemiological studies that have shown adverse renal events in individuals with high UCaE (Nurses' Health Study I, Nurses' Health Study II, and the Health Professionals Follow-up Study, 4.02 mmol/24 h compared with 4.90, 5.30, 4.96 mmol/24 h, respectively).[9] Both the low rate of individuals on supplements and lower UCaE compared with previous epidemiologic cohorts may have hindered the ability to find an association between increasing risk of CKD and higher UCaE. However, it may also be that increasing intake of

Figure 2. (continued) panels represent the associations between UCaE and risk of CKD defined as UAE > 30 mg/24 h (e, f). The gray areas indicate the 95% confidence intervals (CIs). The spline curve is truncated at the 0.5th and 99.5th percentile of the distribution curve. Reference standard for UCaE was 3.82 mmol/24 h. *P* values for nonlinear association are $P = 0.01$ for (a), $P = 0.004$ for (b), $P = 0.85$ for (c), $P = 0.90$ for (d), $P = 0.02$ for (e), and $P = 0.003$ for (f). CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; maHR, multivariable adjusted hazard ratio; UAE, urinary albumin excretion; UCaE, urinary calcium excretion.

Table 4. Association between urinary calcium excretion and risk of being a fast progressor in 6531 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study

Outcome	Continuous urinary calcium excretion, per 1 mmol/24 h increment	P_{trend} linear[a]	Sex-specific quintiles of urinary calcium excretion, mmol/24 h				
			♂ <2.57 / ♀ <2.12	2.57–3.60 / 2.12–3.05	3.61–4.58 / 3.05–3.99	4.59–5.84 / 3.99–5.17	>5.84 / >5.17
N	6531		1304	1309	1307	1305	1306
Risk of being a fast progressor							
Number of fast progressors	1306		307	281	228	251	239
Crude model	0.95 (0.92, 0.98)	0.001	1.46 (1.20, 1.77)	1.29 (1.07, 1.57)	1.00 (ref)	1.13 (0.92, 1.37)	1.06 (0.87, 1.30)
Model 1[b]	0.92 (0.89, 0.96)	<0.001	1.42 (1.15, 1.75)	1.37 (1.11, 1.69)	1.00 (ref)	1.12 (0.90, 1.38)	0.89 (0.72, 1.11)
Model 2[c]	0.92 (0.89, 0.96)	<0.001	1.38 (1.11, 1.71)	1.38 (1.11, 1.70)	1.00 (ref)	1.11 (0.89, 1.38)	0.87 (0.70, 1.09)
Model 3[d]	0.94 (0.89, 0.98)	0.01	1.39 (1.05, 1.83)	1.40 (1.07, 1.83)	1.00 (ref)	1.14 (0.86, 1.51)	0.92 (0.69, 1.22)
Model 4[e]	0.92 (0.87, 0.97)	0.003	1.43 (1.07, 1.91)	1.42 (1.08, 1.86)	1.00 (ref)	1.12 (0.84, 1.48)	0.86 (0.64, 1.16)

Odds ratios (OR) and 95% confidence intervals were derived from logistic regression models.
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; UAE, urinary albumin excretion.
[a]Derived from a logistic regression model by using urinary calcium excretion as a continuous term.
[b]Model 1: Adjusted for age, sex, height, weight, race, baseline eGFR, and baseline lnUAE.
[c]Model 2: Model 1 + smoking, alcohol, hypertension, diabetes, parental history of CKD, and hypercholesterolemia.
[d]Model 3: Model 2 + plasma magnesium, calcium, phosphorus, PTH and 1,25-dihydroxyvitamin D, albumin, and use of loop diuretics, thiazide diuretics, calcium supplements, vitamin D supplements, and bisphosphonates.
[e]Model 4: Model 3 + urinary sodium, potassium, urea, and magnesium excretion.

calcium through supplementation poses more of a risk to the kidney than if done so through dietary sources. This may be because some calcium supplements have been shown to also include contaminants (such as lead) that may be damaging the kidney, or it may be due to the fact that calcium-rich foods also contain additional nutrients and bioactive food components that may alter the absorption or utilization of calcium, making the risk associated with these two sources of calcium potentially different from one another.[26]

Unexpectedly, low UCaE resulted in an increased risk of developing CKD. It has been suggested that low excretion of nutrients (i.e., poor nutritional status) may be a reflection of individuals in poor health. We therefore examined the association between UCaE and risk of all-cause mortality and cardiovascular events. We found no association with all-cause mortality or cardiovascular events, including no association in the low UCaE range. This suggests that these findings are not related to the fact that those subjects in the lowest quintile of UCaE are in poor overall health. Unfortunately, given the observational nature of this study, it is not possible to determine the mechanism(s) by which low UCaE leads to CKD. Hypothetically, it may be that increases in parathyroid hormone (resulting from low plasma calcium) have deleterious renal effects, for instance, by the PTH-induced epithelial-mesenchymal transition leading to fibrosis.[27,28] However, even after adjusting for PTH, UCaE was still associated with the development of CKD in our analyses. The finding that

Table 5. Association between urinary calcium excretion and risk of having a ≥25% decline in kidney function in 6531 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study

Outcome	Continuous urinary calcium excretion, per 1 mmol/24 h increment	P_{trend} linear[a]	Sex-specific quintiles of urinary calcium excretion, mmol/24 h					P_{trend} nonlinear[b]
			♂ <2.57 / ♀ <2.12	2.57–3.60 / 2.12–3.05	3.61–4.58 / 3.05–3.99	4.59–5.84 / 3.99–5.17	>5.84 / >5.17	
N	6531		1304	1309	1307	1305	1306	
Risk of ≥25% decline in kidney function								
Person-years	59,406		11,275	11,646	11,947	12,113	12,425	
Number of events	549		145	119	90	85	110	
Crude model	0.92 (0.88, 0.97)	<0.001	1.77 (1.36, 2.30)	1.40 (1.06, 1.84)	1.00 (ref)	0.92 (0.68, 1.24)	1.14 (0.86, 1.50)	<0.001
Model 1[c]	0.92 (0.88, 0.96)	<0.001	1.63 (1.24, 2.14)	1.45 (1.10, 1.92)	1.00 (ref)	1.02 (0.76, 1.38)	1.00 (0.75, 1.32)	0.004
Model 2[d]	0.92 (0.87, 0.96)	<0.001	1.64 (1.24, 2.17)	1.48 (1.11, 1.97)	1.00 (ref)	1.00 (0.74, 1.35)	0.99 (0.74, 1.33)	0.01
Model 3[e]	0.91 (0.86, 0.97)	0.01	1.65 (1.13, 2.42)	1.68 (1.14, 2.47)	1.00 (ref)	1.05 (0.69, 1.59)	0.91 (0.61, 1.36)	0.01
Model 4[f]	0.91 (0.84, 0.99)	0.02	1.71 (1.14, 2.56)	1.72 (1.17, 2.54)	1.00 (ref)	1.09 (0.71, 1.66)	0.90 (0.59, 1.38)	0.01

Hazard ratios (HR) and 95% confidence intervals were derived from Cox proportional hazards regression models.
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; UAE, urinary albumin excretion.
[a]Derived from a Cox proportional hazards model by using urinary calcium excretion as a continuous linear term.
[b]Derived by using the likelihood ratio test, comparing nested Cox proportional hazards regression models with a linear or linear and cubic spline terms.
[c]Model 1: Adjusted for age, sex, height, weight, race, baseline eGFR, and baseline lnUAE.
[d]Model 2: Model 1 + smoking, alcohol, hypertension, diabetes, parental history of CKD, hypercholesterolemia, and history of cardiovascular events.
[e]Model 3: Model 2 + plasma magnesium, calcium, phosphorus, PTH and 1,25-dihydroxyvitamin D, albumin, and use of loop diuretics, thiazide diuretics, calcium supplements, vitamin D supplements, and bisphosphonates.
[f]Model 4: Model 3 + urinary sodium, potassium, urea, and magnesium excretion.

Table 6. Association between urinary calcium excretion and risk of mortality in 5491 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study

Outcome	Continuous urinary calcium excretion, per 1 mmol/24 h increment	P_{trend} linear[a]	Sex-specific quintiles of urinary calcium excretion, mmol/24 h					P_{trend} nonlinear[b]
			♂ <2.65	2.65–3.67	3.68–4.58	4.59–5.80	>5.80	
			♀ <2.16	2.16–3.08	3.09–4.00	4.01–5.14	>5.14	
Mortality								
Person-years	66,843		13,334	13,315	13,417	13,344	13,433	
Number of events	267		63	62	45	52	45	
Crude model	0.96 (0.90, 1.02)	0.19	1.41 (0.97, 2.07)	1.40 (0.95, 2.05)	1.00 (ref)	1.17 (0.78, 1.74)	1.00 (0.66, 1.51)	0.98
Model 1[c]	0.94 (0.88, 1.01)	0.09	1.36 (0.92, 2.01)	1.49 (1.01, 2.20)	1.00 (ref)	1.29 (0.86, 1.94)	0.99 (0.65, 1.50)	0.66
Model 2[d]	0.96 (0.89, 1.03)	0.28	1.42 (0.93, 2.17)	1.53 (1.00, 2.32)	1.00 (ref)	1.38 (0.89, 2.13)	1.15 (0.74, 1.80)	0.75
Model 3[e]	0.95 (0.87, 1.03)	0.21	1.26 (0.75, 2.11)	1.76 (1.08, 2.86)	1.00 (ref)	1.27 (0.75, 2.14)	1.06 (0.62, 1.82)	0.54
Model 4[f]	0.97 (0.87, 1.07)	0.52	1.17 (0.68, 2.01)	1.72 (1.05, 2.81)	1.00 (ref)	1.31 (0.78, 2.22)	1.14 (0.65, 2.00)	0.44
Cardiovascular event								
Person-years	64,272		12,809	12,760	12,930	12,839	12,936	
Number of events	448		98	93	92	84	81	
Crude model	1.01 (0.96, 1.06)	0.72	1.08 (0.81, 1.43)	1.03 (0.77, 1.37)	1.00 (ref)	0.92 (0.68, 1.24)	0.88 (0.65, 1.18)	0.34
Model 1[c]	0.98 (0.93, 1.02)	0.32	0.98 (0.73, 1.30)	0.98 (0.73, 1.31)	1.00 (ref)	0.92 (0.68, 1.24)	0.80 (0.59, 1.09)	0.68
Model 2[d]	0.99 (0.94, 1.04)	0.69	1.05 (0.77, 1.43)	0.94 (0.69, 1.27)	1.00 (ref)	0.91 (0.67, 1.25)	0.91 (0.66, 1.25)	0.37
Model 3[e]	1.01 (0.95, 1.08)	0.69	1.01 (0.70, 1.47)	0.99 (0.69, 1.43)	1.00 (ref)	0.88 (0.60, 1.29)	0.98 (0.67, 1.42)	0.29
Model 4[f]	1.04 (0.96, 1.12)	0.31	0.98 (0.66, 1.45)	0.96 (0.67, 1.39)	1.00 (ref)	0.91 (0.62, 1.34)	1.04 (0.70, 1.54)	0.28

Hazard ratios (HR) and 95% confidence intervals were derived from Cox proportional hazards regression models.
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; UAE, urinary albumin excretion.
[a]Derived from a Cox proportional hazards model by using urinary calcium excretion as a continuous linear term.
[b]Derived by using the likelihood ratio test, comparing nested Cox proportional hazards regression models with a linear or linear and cubic spline terms.
[c]Model 1: Adjusted for age, sex, height, weight, race, baseline eGFR, and baseline lnUAE.
[d]Model 2: Model 1 + smoking, alcohol, hypertension, diabetes, parental history of CKD, hypercholesterolemia, and history of cardiovascular events.
[e]Model 3: Model 2 + plasma magnesium, calcium, phosphorus, PTH and 1,25-dihydroxyvitamin D, albumin, and use of loop diuretics, thiazide diuretics, calcium supplements, vitamin D supplements, and bisphosphonates.
[f]Model 4: Model 3 + urinary sodium, potassium, urea, and magnesium excretion.

low UCaE increases risk of developing CKD needs confirmation and further study.

Importantly, our findings were confirmed by various sensitivity analyses. First, we found similar outcomes when using a buffer by removing individuals with the lowest baseline eGFR (60–66 ml/min per 1.73 m^2) and highest baseline UAE (25–30 mg/24 h). Second, we also found similar trends when adjusting for the oversampling of individuals with UAE ≥10 mg/l due to the PREVEND study design. Third, an effect between UCaE and decline in eGFR per year was observed. Fourth, we found an inverse association between UCaE and risk of being a fast progressor to CKD, defined as the 20% with the steepest decline in eGFR per year. Finally, when CKD was defined by having a ≥25% decline in eGFR, we found that those in the lowest quintile of UCaE had the highest risk of developing CKD during follow-up. These findings support the notion that low UCaE is associated with risk of CKD.

Limitations of this study should be considered. First, while we controlled for many factors in the analyses, residual confounding may still be possible as in all epidemiological studies. Second, only 8.3% of the population was classified as hypercalciuric (men: UCaE >7.5 mmol/24 h, women: UCaE >6.2 mmol/24 h).[29] This limits the ability to determine whether hypercalciuria may lead to an increased risk for CKD in the future. However, even in this subgroup, no increased risk for incident CKD was found. Third, these results may not be generalizable to all races because >95% of the PREVEND cohort is Caucasian. Lastly, the PREVEND cohort oversampled individuals with elevated UAE. However, subjects with elevated albuminuria at baseline were excluded from these analyses, and when we additionally adjusted for sample design in one of the sensitivity analyses this rendered similar trends.

Strengths of this study include that it is the first study to examine the associations of UCaE with risk of CKD in a population-based cohort, along with this study being conducted prospectively, collecting multiple 24-hour urine samples, and having a relatively large sample size. We also used a CKD endpoint that consisted of a creatinine-cystatin C-based eGFR and 2 consecutive 24-hour UAE at each time point to determine CKD events. Lastly, several sensitivity analyses (some using alternative endpoints, such as decline in eGFR per year, risk of being a fast progressor to CKD, and risk of having a ≥25% decline in kidney function during follow-up) were performed to confirm our findings.

In conclusion, low UCaE, rather than high UCaE, was associated with an increased risk of developing CKD in this prospective, population-based cohort study. This effect remained even after adjustment for various confounders. Although the effect of increasing calcium intake on bone health may be in question, one

modifiable factor that may be renoprotective is the avoidance of low UCaE.

DISCLOSURE

All the authors declared no competing interests.

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SJLB and RTG created the concept and design of the study; SJLB, RTG, STV, and IPK acquired the data; JMT and LMK performed the statistical analyses and wrote the first draft of the manuscript; and MHB, SJLB, and RTG provided critical review, advice, and consultation throughout.

SUPPLEMENTARY MATERIAL

Table S1. Association between urinary calcium excretion and risk of chronic kidney disease after removing subjects with eGFR $<$66 ml/min per 1.73 m^2 and/or UAE $>$25 mg/24 h in 5237 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study.

Table S2. Association between urinary calcium excretion and risk of chronic kidney disease in 5223 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study when accounting for over- and under-collection of urine.

Table S3. Association between urinary calcium excretion and risk of chronic kidney disease after adjusting for oversampling of individuals with $\geq$10 mg/l of UAE taken into account in 2503 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study.

Table S4. Baseline characteristics according to quintiles of urinary calcium excretion of all 6531 subjects of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study.

Supplementary material is linked to the online version of the paper at www.kireports.org.

REFERENCES

1. van den Berg P, van Haard PM, van den Bergh JP, et al. First quantification of calcium intake from calcium-dense dairy products in Dutch fracture patients (the Delft cohort study). *Nutrients*. 2014;6:2404–2418.
2. National Institute for Public Health and the Environment. Dutch National Food Consumption Survey 2007–2010: Diet of Children and Adults Aged 7 to 69 Years; 2011.
3. Lee AW, Cho SS. Association between phosphorus intake and bone health in the NHANES population. *Nutr J*. 2015;14:28.
4. Tai V, Leung W, Grey A, et al. et al. Calcium intake and bone mineral density: systematic review and meta-analysis. *BMJ*. 2015;351:h4183.
5. Wrong OM, Norden AG, Feest TG. Dent's disease; a familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure and a marked male predominance. *QJM*. 1994;87:473–493.
6. Lloyd SE, Gunther W, Pearce SH, et al. Characterization of renal chloride channel, CLCN5, mutations in hypercalciuric nephrolithiasis (kidney stones) disorders. *Human Mol Genet*. 1997;6:1233–1239.
7. Thakker RV. Pathogenesis of Dent's disease and related syndromes of X-linked nephrolithiasis. *Kidney Int*. 2000;57:787–793.
8. Godron A, Harambat J, Boccio V, et al. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis: phenotype-genotype correlation and outcome in 32 patients with CLDN16 or CLDN19 mutations. *Clin J Am Soc Nephrol*. 2012;7:801–809.
9. Curhan GC, Taylor EN. 24-h uric acid excretion and the risk of kidney stones. *Kidney Int*. 2008;73:489–496.
10. Hillege HL, Janssen WM, Bak AA, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med*. 2001;249:519–526.
11. Halbesma N, Brantsma AH, Bakker SJ, et al. Gender differences in predictors of the decline of renal function in the general population. *Kidney Int*. 2008;74:505–512.
12. Verhave JC, Hillege HL, Burgerhof JG, et al. Sodium intake affects urinary albumin excretion especially in overweight subjects. *J Intern Med*. 2004;256:324–330.
13. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367:20–29.
14. Grubb A, Blirup-Jensen S, Lindstrom V, et al. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. *Clin Chem Lab Med*. 2010;48:1619–1621.
15. Kieneker LM, Gansevoort RT, Mukamal KJ, et al. Urinary potassium excretion and risk of developing hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension*. 2014;64:769–776.
16. Joosten MM, Gansevoort RT, Mukamal KJ, et al. Urinary magnesium excretion and risk of hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension*. 2013;61:1161–1167.
17. Oterdoom LH, Gansevoort RT, Schouten JP, et al. Urinary creatinine excretion, an indirect measure of muscle mass, is an independent predictor of cardiovascular disease and mortality in the general population. *Atherosclerosis*. 2009;207:534–540.
18. Joosten MM, Gansevoort RT, Mukamal KJ, et al. Urinary and plasma magnesium and risk of ischemic heart disease. *Am J Clin Nutr*. 2013;97:1299–1306.
19. Keyzer CA, Lambers-Heerspink HJ, Joosten MM, et al. Plasma vitamin D level and change in albuminuria and eGFR according to sodium intake. *Clin J Am Soc Nephrol*. 2015;10:2119–2127.

20. van Ballegooijen AJ, Gansevoort RT, Lambers-Heerspink HJ, et al. Plasma 1,25-dihydroxyvitamin D and the risk of developing hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension*. 2015;66:563–570.

21. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 1997;20:1183–1197.

22. Visser ST, Schuiling-Veninga CC, Bos JH, et al. The population-based prescription database IADB.nl: its development, usefulness in outcomes research and challenges. *Expert Rev Pharmacoecon Outcomes Res*. 2013;13:285–292.

23. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16:31–41.

24. Beasley JM, LaCroix AZ, Neuhouser ML, et al. Protein intake and incident frailty in the Women's Health Initiative observational study. *J Am Geriatr Soc*. 2010;58:1063–1071.

25. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med*. 2006;354:669–683.

26. Rehman S, Adnan M, Khalid N, Shaheen L. Calcium supplements: an additional source of lead contamination. *Biol Trace Elem Res*. 2011;143:178–187.

27. Guo Y, Zhang A, Ding Y, et al. Genistein ameliorates parathyroid hormone-induced epithelial-to-mesenchymal transition and inhibits expression of connective tissue growth factor in human renal proximal tubular cells. *Arch Med Sci*. 2013;9:724–730.

28. Guo Y, Yuan W, Wang L, et al. Parathyroid hormone-potentiated connective tissue growth factor expression in human renal proximal tubular cells through activating the MAPK and NF-kappaB signalling pathways. *Nephrol Dial Transplant*. 2011;26:839–847.

29. Crook MA, Renal calculi. In: *Clinical Biochemistry and Metabolic Medicine*. 8th ed. Boca Raton, FL: CRC Press; 2012:55.