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Calcitriol and FGF-23, but neither PTH nor sclerostin, are associated with calciuria in CKD

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Abstract

Purpose The recent observation that urinary calcium excretion (UCE) drops considerably with CKD and that this effect may occur beyond compensation for reduced intestinal calcium absorption suggests that CKD per se is a state of sustained positive calcium balance, a mechanism likely to contribute to vascular calcification and CVD in CKD. However, the determinants of UCE reduction in CKD are not well understood and there is a lack of clinical studies, particularly in the CKD population. Therefore, in this study, we aimed to evaluate variables associated with UCE in a CKD cohort.

Methods Baseline data on 356 participants of the Progredir Study, Sao Paulo, Brazil, essentially composed of CKD G3a–G4, were analyzed according to UCE (24 h urine collection).

Results Median 24 h UCE was 38 mg/day (IQR 21–68 mg/day) and 0.48 mg/kg/day (IQR 0.28–0.82 mg/kg/day). In univariate analysis, UCE was inversely related to age, phosphorus, 1-84 PTH, FGF-23 and sclerostin, and positively associated with eGFR, DBP, $1,25(OH)_2$-vitamin D, calcium, bicarbonate, total calorie intake and spironolactone use. After adjustments for age, sex and eGFR, only $1,25(OH)_2$-vitamin D, calcium, FGF-23, bicarbonate and total calorie intake remained associated with it, but not PTH nor sclerostin. Lastly, in a multivariable model, eGFR, serum $1,25(OH)_2$-vitamin D, calcium, and FGF-23 remained associated with UCE. Similar results were observed when calcium fractional excretion was used instead of UCE, with eGFR, 1-25-vitamin D and FGF-23 remaining as independent associations.

Conclusion Our results showed that CKD is associated with very low levels of UCE and that $1,25(OH)_2$-vitamin D, serum calcium and FGF-23 were independently associated with UCE in this population, raising the question whether these factors are modulators of the tubular handling of calcium in CKD.

Keywords Urinary calcium excretion · CKD · $1,25(OH)_2$-vitamin D · Calcium

Introduction

Chronic kidney disease (CKD) is associated with complex mineral and bone disorders (CKD–MBD), which include abnormalities in calcium homeostasis and extra-skeletal calcification [1]. There is increasing evidence showing that vascular calcification (VC) is an independent predictor of cardiovascular morbidity and mortality in CKD patients [2, 3].

VC has been recognized as a complex active process mediated by trans-differentiated calcifying cells and exacerbated by a reduction in native calcification inhibitors such as fetuin and pyrophosphate [4]. A positive calcium balance may also favor VC [5] and can occur in CKD as a result of several factors, such as increased use of calcium-containing drugs [6] increased intestinal absorption due to the use of calcitriol and vitamin D analogs and reduced urinary calcium excretion (UCE) [7–9]. Among these mechanisms, the later is still under investigation [5]. A recent study showed that CKD patients presented a decreased UCE and that this effect was occurring despite no difference in stool calcium/phosphorus ratio between CKD and controls [7]. This finding challenges the traditional concept that the reduction in calcium excretion occurs as a consequence of reduced intestinal calcium absorption [10] and suggests that CKD per

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se is a state associated with a sustained positive calcium balance.

However, if CKD is related to a non-compensatory reduction of UCE, not much is known about its determinants. Tubular calcium handling is known to be primarily mediated by the calciotropic hormones, PTH and $1,25(OH)_2D$. Recently, animal studies have suggested that FGF-23 [11] and sclerostin [12] may also regulate calcium excretion. However, the role of these factors in regulating calcium tubular reabsorption in the setting of CKD is not clear and few clinical studies have evaluated the association between CKD–MBD biomarkers and UCE in the CKD population. Therefore, the aim of this cross-sectional study was to explore factors (including CKD–MBD biomarkers) associated with UCE in a CKD cohort, primarily composed of CKD G3a–G4.

Materials and methods

Study population

PROGREDIR Study is an ongoing CKD Cohort in Sao Paulo, Brazil. Study population and methods have been described elsewhere [13]. Briefly, patients from Hospital das Clínicas Outpatient Service, São Paulo, with age ≥ 30 years and at least two measurements of serum creatinine ≥ 1.6 mg/dl for men or ≥ 1.4 mg/dl for women were considered potential candidates. Patients attending oncology, psychiatry, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), viral hepatitis and glomerulonephritis services were not included. Exclusion criteria were hospitalization or acute myocardial infarction within the last 6 months, autoimmune diseases, pregnancy, psychiatric diseases, ongoing chemotherapy or immunosuppressive therapy, ongoing RRT, glomerulonephritis, HIV/AIDS infection, hepatitis B or C and any organ transplantation. Eligible individuals were invited to participate. Recruitment took place between March 2012 and December 2013, and 454 participants were enrolled. The study was approved by two local Ethics Committees (Ethics in Research Committee—University Hospital, Sao Paulo University, no. 11,147/11; and Ethics Commission for Analysis of Research Projects, Hospital das Clínicas, Medical School, Sao Paulo University, no. 0798/11) and written informed consent was obtained from all participants.

Participants visited the research center for interviews, including medical history and food frequency questionnaire, and clinical exams according to standard protocols. Fasting blood samples, 24 h and spot urine were collected. Urine and blood aliquots were prepared and stored in liquid nitrogen. Serum and urinary creatinine concentrations were determined by the Jaffé enzymatic reaction. Estimated glomerular filtration rate (eGFR) was calculated by the CKD–EPI equation [14]. Comprehensive metabolic panels and urinary sodium, potassium, calcium and phosphate were measured using standard assays [13]. Serum 1-84 PTH (LIAISON® 1-84 PTH Assay, DiaSorin, Italy, reference range 5.7–47.8 pg/mL), serum 25-OH-vitamin D (LIAISON® 25 OH-Vitamin D Total Assay, DiaSorin, Italy, RR 30–100 ng/ml), plasma $1,25(OH)_2D$ (LIAISON® XL 1,25 Dihydroxyvitamin D Assay, DiaSorin, Italy, RR = 25.0 and 86.5 pg/mL), serum sclerostin (LIAISON® Sclerostin Assay, Diasorin, Italy, RR 0.148–0.955 ng/ml) and plasma FGF-23 (LIAISON® FGF-23 Assay, DiaSorin, Italy, RR = 22.7–93.1 pg/ml) were all determined with a chemiluminescent immunoassay at baseline. UCE was defined as amount of calcium excreted in 24 h urine collection (in mg) divided by weight (in kilograms) and calcium fractional excretion was defined as the ratio between 24-h urinary calcium clearance and 24-h creatinine clearance. After excluding those with missing values for 24-h urinary calcium, FGF-23, PTH, sclerostin and 1,25-vitamin D, 356 individuals were left for the analysis.

Statistical analysis

For descriptive analysis, variables were evaluated according to tertiles of 24 h UCE. Jonckheere–Terpstra tests for ordered alternatives were performed for comparison of continuous variables and the Chi-square test was performed for comparison of categorical variables. Univariable and multivariable linear regressions were used to explore associations with UCE (log2-transformed due to highly skewed distribution). As glomerular filtration was believed to be a major confounder, in a first multivariable model, variables were adjusted for age, sex and eGFR only. A second model included all variables independently associated with UCE. Multivariable linear regression was also performed using (log2) calcium fractional excretion as the dependent variable. Statistical analyses were conducted using SPSS 25.0 (SPSS, Inc.) and all tests were two sided.

Results

In the overall group, median 24 h UCE was 38 mg/day (IQR 21–68 mg/day) and 0.48 mg/kg/day (IQR 0.28–0.82 mg/kg/day). In Table 1, variables are shown according to tertiles of UCE. Higher tertile of UCE was related to younger age, spironolactone use, lower levels of serum phosphorus, PTH, FGF-23 and sclerostin, and higher levels of eGFR, DBP, and $1,25(OH)_2D$, calcium and bicarbonate. In addition, while micronutrient intake was generally not different among UCE tertiles, total calorie intake was significantly higher in those with higher UCE.

Table 1 Descriptive characteristics among 356 participants of the Progredir Study according to tertiles of UCE (mg/kd/day)

	Urinary calcium excretion (mg/kg/d)				
	All participants ($n=356$)	1° tertile ($n=118$)	2° tertile ($n=119$)	3° tertile ($n=119$)	p*
Urinary calcium excretion (mg/kg/d)	0.49 (0.28–0.86)	0.24 (0.18–0.28)	0.48 (0.40–0.59)	1.18 (0.85–1.54)	< 0.001
Calcium fractional excretion (%)	0.69 (0.45–1.13)	0.40 (0.29–0.58)	0.63 (0.53–0.89)	1.29 (0.97–1.83)	< 0.001
Age (years)	68 (12)	70 (10)	67 (13)	65 (12)	0.002
Sex (male)	223 (63%)	69 (59%)	76 (64%)	78 (66%)	0.5
Self-declared skin color (white)	233 (63%)	80 (67.8%)	76 (64%)	77 (65%)	0.89
Hypertension	323 (91%)	114 (97%)	103 (87%)	106 (90%)	0.02
Diabetes mellitus	207 (58%)	61 (52%)	69 (58%)	77 (65%)	0.13
BMI	29.6 (5.7)	30.1 (5.6)	30.0 (6.3)	28.6 (5.1)	0.10
Smoking (never)	150 (42%)	52 (44%)	47 (40%)	51 (43%)	0.76
eGFR (ml/min/1.73 m^2)	37 (28–46)	29 (26–33)	35 (26–43)	43 (36–51)	< 0.001
Urinary ACR (mg/g)	80 (15–615)	88 (15–675)	72 (23–626)	85 (12–401)	0.32
SBP (mmHg)	140 (24)	141 (25)	142 (25)	137 (23)	0.14
DBP (mmHg)	76 (13)	74 (13)	76 (13)	78 (13)	0.009
Thiazide diuretic	86 (24%)	36 (31)	26 (22%)	24 (20%)	0.14
Furosemide use	134 (37%)	51 (43)	39 (33%)	44 (37%)	0.25
Spironolactone use	29 (8%)	5 (4)	8 (7%)	16 (13.4%)	0.03
Serum total calcium (mg/dL)	9.6 (0.6)	9.4 (0.5)	9.6 (0.6)	9.8 (0.6)	< 0.001
Serum phosphorus (mg/dL)	3.7 (0.6)	3.7 (0.6)	3.7 (0.7)	3.5 (0.6)	0.001
1,25(OH)-vitamin D (pg/mL)	31 (24–40)	29 (22–37)	31 (24–39)	34 (27–44)	< 0.001
25(OH)-vitamin D (ng/mL)	24 (17–32)	22 (16–31)	26 (17–34)	25 (18–31)	0.24
1–84 PTH (pg/mL)	36 (27–51)	41 (33–57)	38 (28–51)	33 (21–44)	< 0.001
FGF-23 (pg/mL)	93 (69–129)	96 (75–133)	98 (66–141)	90 (67–115)	0.03
Sclerostin (pg/mL)	492 (347–652)	486 (387–674)	517 (323–664)	462 (329–607)	0.06
Bicarbonate (mmol/L)	25.6 (2.9)	25.4 (3.0)	25.3 (2.8)	26.1 (2.7)	0.04
Food frequency questionnaire					
Energy (kcal/d)	1910 (1491–2480)	1735 (1381–2271)	1860 (1509–2504)	2108 (1565–2637)	0.01
Protein (g/kg/d)	1.12 (0.92–1.37)	1.03 (0.89–1.34)	1.15 (0.93–1.36)	1.15 (0.95–1.38)	0.16
Sodium (g/d)	2.2 (1.8–2.5)	2.2 (1.8–2.5)	2.2 (1.8–2.5)	2.2 (1.8–2.5)	0.68
Calcium (mg/d)	757 (550–965)	765 (530–1027)	717 (550–893)	785 (561–996)	0.71

*Jonckheere–Terpstra or Chi square

Table 2 shows (1) the multivariable model including age, sex and eGFR only; and (2) the univariable and multivariable models for the other variables, adjusted for sex, age and eGFR. After adjustment, DBP, spironolactone use, 1–84 PTH, and sclerostin were no longer associated with UCE, whereas total serum calcium, $1,25(OH)_2D$, bicarbonate and total calorie intake remained significantly associated with it. Interestingly, FGF-23 was not associated with UCE in the univariable model, but showed a significant positive association with UCE after the adjustment for eGFR, sex and age.

In Table 3, we present a fully adjusted model, and eGFR, serum $1,25(OH)_2D$, total serum calcium, and energy consumption intake remained positively associated with UCE, with FGF-23 showing a trend for association. We repeated this model using fractional calcium excretion as the dependent variable instead of UCE, an approach that allows for comparing the tubular handling of calcium excretion among people with very different GFRs and, therefore, different filtered calcium load. In this model, eGFR was now inversely related to calcium fractional excretion, an expected effect in CKD. FGF-23 and $1,25(OH)_2D$ were significantly and positively associated with calcium fractional excretion, while energy consumption and total serum calcium were not.

Lastly, in Fig. 1, we show a bar graph of median UCE values according to quartiles of 1,25-vitamin D and eGFR.

Discussion

In this cross-sectional analysis of CKD subjects primarily presenting CKD G3 and G4, a positive independent association between UCE and glomerular filtration rate was evident and strong. First, our population presented very low levels of calcium excretion, with a median daily value of 38 mg/

Table 2 Univariable and multivariable models adjusted for sex, age and eGFR on (log2) UCE among 356 participants of the Progredir Study

	B	95% CI B		*p*
Age (years)	−0.006	−0.016	0.004	0.22
Sex (male)	0.055	−0.186	0.297	0.65
eGFR (ml/min/1.73 m^2)	0.024	0.016	0.033	< 0.0001
DBP (mmHg)				
Crude	0.008	−0.002	0.017	0.10
Adjusted	0.004	−0.005	0.013	0.43
Spironolactone use				
Crude	0.41	−0.02	0.85	0.06
Adjusted	0.24	−0.18	0.66	0.26
Serum total calcium (mg/dL)				
Crude	0.45	0.25	0.66	< 0.0001
Adjusted	0.45	0.25	0.65	< 0.0001
1,25(OH)-vitamin D (pg/mL)				
Crude	0.02	0.01	0.03	< 0.0001
Adjusted	0.02	0.01	0.03	0.001
1–84 PTH (pg/mL)				
Crude	−0.004	−0.007	−0.001	0.01
Adjusted	−0.001	−0.004	0.002	0.45
FGF-23 (per 10 pg/mL)				
Crude	0.001	−0.006	0.008	0.87
Adjusted	0.008	0.001	0.015	0.02
Sclerostin (per 100 pg/mL)				
Crude	−0.054	−0.102	−0.007	0.02
Adjusted	−0.003	−0.057	0.050	0.90
Bicarbonate (mmol/L)				
Crude	0.046	0.004	0.088	0.03
Adjusted	0.043	−0.0005	0.086	0.05
Energy (per 100 kcal/d)				
Crude	0.023	0.008	0.038	0.003
Adjusted	0.018	0.003	0.033	0.02

day (0.48 mg/kg/day). This finding is in accordance with previous studies reporting values of calcium excretion lower than 80 mg/day [6–9, 15, 16], in contrast to 120–250 mg/day observed in non-CKD populations [17]. Second, decreasing eGFR in this CKD population was associated with decreasing values of UCE. It is interesting to note that despite the positive association between eGFR and UCE shown, we observed an inverse relationship between fractional calcium excretion and eGFR. This result is compatible with the concept that as eGFR decreases there is a significant reduction in the overall amount of calcium being excreted despite increase in the fractional calcium excretion.

The factors determining the reduction of calcium excretion in CKD are not fully understood. The traditional explanation is that the decrease in UCE occurs in compensation to the reduced calcium intestinal absorption attributable to lower 1,25(OH)2D levels [10]. However, recent studies have challenged this concept. Spiegel et al. demonstrated that, on a high-calcium diet, individuals with CKD G3 and G4 have a calcium balance significantly more positive than controls with normal kidney function [7]. Moreover, despite lower UCE, there was no difference in the stool calcium/phosphorus ratio in CKD patients in comparison to controls, neither in the high- nor in the low-calcium diet groups. In another study by Hill et al. [6], CKD patients on a 957 mg calcium diet presented a lower UCE in comparison with healthy historical controls (46 vs. 121 mg/day). In the same study, addition of calcium carbonate supplement (elemental calcium 1500 mg/day) to dietary calcium led to a significant positive calcium balance due to failure to increase UCE [6]. These studies are small and were performed under restricted conditions, but their interesting provoking findings suggest a potential new mechanism operating in CKD–MBD and lead to the hypothesis that if the reduction in UCE observed in CKD is not only a compensatory response to the reduced intestinal calcium absorption, the resulting positive calcium balance can actively contribute to VC and its complications. Several problems need to be faced to advance research in this particular field, including definition and standardization

Table 3 Multivariable models on (log2)UCE and (log2) fractional calcium excretion among 356 participants of the Progredir Study

	UCE (mg/kg)				Calcium fraction excretion (%)			
	B	95% CI B		*p*	B	95% CI B		*p*
Age (years)	−0.009	−0.019	0.001	0.08	−0.008	−0.017	0.001	0.10
Sex (male)	0.10	−0.15	0.35	0.45	0.22	−0.02	0.45	0.07
eGFR (ml/min/1.73 m^2)	0.021	0.012	0.030	< 0.0001	−0.009	−0.018	−0.001	0.04
1,25(OH)-vitamin D (pg/mL)	0.015	0.006	0.024	0.002	0.012	0.003	0.021	0.01
Serum total calcium (mg/dL)	0.40	0.20	0.61	0.0001	0.03	−0.16	0.22	0.76
Bicarbonate (mmol/L)	0.03	−0.01	0.07	0.19	0.04	0.0002	0.08	0.05
FGF-23 (per 10 pg/mL)	0.006	−0.001	0.013	0.08	0.012	0.006	0.019	0.0002
Energy consumption (per 100 kcal/d)	0.02	0.005	0.03	0.01	0.01	−0.01	0.02	0.30

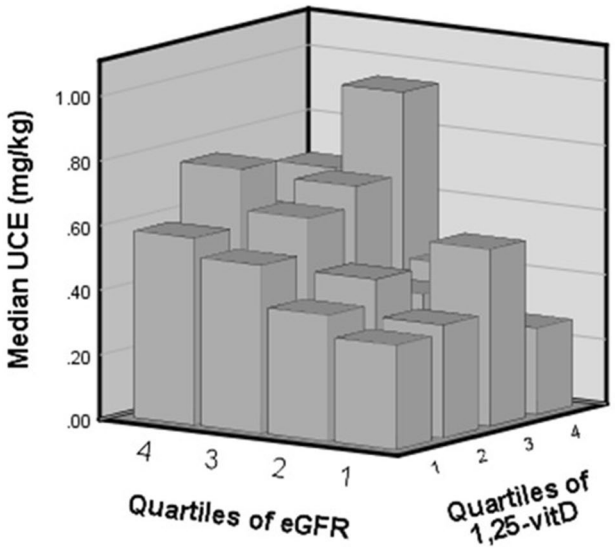

Fig. 1 Scatter plot of median UCE according to quartiles of eGFR and 1,25-vitamin D among 356 participants of the Progredir Study

of measures of calcium intake, intestinal calcium absorption and calcium balance. Unfortunately, in our study, we did not have any measure of intestinal calcium absorption and could not account for this.

If the hypothesis that the CKD-related reduction in UCE is not occurring only as a compensatory mechanism holds true, it would be important to understand what factors determine or modulate this effect. According to current knowledge, main variables determining calcium excretion are diet, bone metabolism, glomerular filtration and tubular function, all variables deeply disturbed by CKD. Under physiological conditions, most of the calcium filtered by the glomerulus is reabsorbed in the subsequent segments of the nephron, and normally only 1%–2% of filtered calcium is excreted in the urine. The reabsorption occurs through a passive paracellular mechanism in proximal tubule and thick ascending loop of Henle, therefore majorly dependent on sodium reabsorption, and through active transcellular mechanisms in the distal convoluted tubules (DCT) and connecting tubules (CT), intensely regulated by calciotropic hormones, PTH and $1,25(OH)_2D$ [18]. However, less is known about what happens to these mechanisms of reabsorption and regulation specific in the setting of CKD.

In our study, $1,25(OH)_2D$ was associated with UCE, although we could not quantify how much of this effect is compensatory for calcium intestinal absorption. The possibility that $1,25(OH)_2D$ has direct tubular effects is intriguing, but has not been extensively examined. Animal and cell studies are controversial, with some studies showing that $1,25(OH)_2D$ increases expression of calcium transport proteins and calcium reabsorption in the kidney and in cultured renal cells [19–21], while others suggest there is an increase in calcium and magnesium excretions through inhibition of claudin-16 expression in the thick ascending limb of the Henle loop by $1,25(OH)_2D$ [22].

PTH has been shown to regulate the expression of the proteins transient receptor potential vanilloid 5 (TRPV5), calbindin-D28K, Na+/Ca2+exchanger (NCX), and plasma membrane Ca2+ATPase (PMCA), responsible for the active calcium transport in the distal nephron, increasing renal calcium reabsorption [23], an effect compatible with the normocalcemic action of PTH. This contrasts, however, with the augmentation in calcium excretion observed in hyperparathyroidism, a condition that increases bone reabsorption and filtered calcium load, as well as the risk of kidney stones [24] and osteoporosis. Therefore, it was interesting to observe that despite the PTH role on tubular calcium handling, in this CKD population, we did not find an independent association between 1 and 84 PTH and UCE (nor fractional calcium excretion). This suggests that, at least once CKD is already established, mechanisms primarily related to renal failure itself (such as decreased calcium-filtered load and increased tubular reabsorption of calcium possibly independent of PTH) are the major determinants of reduced UCE, but not PTH. It is important to highlight that our cohort is composed majorly of elderly people, so our findings cannot be extrapolated to other populations, such as younger patients with more intense secondary hyperparathyroidism.

Although FGF-23 was inversely related to UCE in the univariable analysis (Table 1), it was positively associated with UCE and fractional calcium excretion after adjustment for eGFR. This result is in contrast to studies performed in individuals with preserved renal function showing that FGF-23 was inversely related to UCE [25, 26], although it is not clear how much of this effect could be mediated by decreased $1,25(OH)_2D$. In addition, in a mouse model, reduction in the fully glycosylated TRPV5 channel in the distal nephron and in calcium reabsorption was observed with deletion of FGF-23 [11]. One alternative explanation for the positive association between FGF-23 and UCE observed in our CKD population is that it is being mediated by decreased Klotho expression in the kidney [27]. Klotho expression has been demonstrated to be inversely related to calcium excretion, possibly through its effect on TRPV5 expression and activity [28].

Recent research suggested the osteocyte-specific protein sclerostin regulates UCE [12]. Deletion of the sclerostin gene in mice significantly diminished absolute urinary calcium excretion and renal fractional excretion [12]. However, it also leads to increased serum $1,25(OH)_2D$ concentrations and thus it is not established if sclerostin has an independent effect on calciuria [29]. In our sample, the association between sclerostin and UCE did not persist after adjustments for confounders.

Other factors related to UCE were identified. Serum calcium is directly related to the activation of calcium-sensing receptor, both in the kidney and in other organs [30]. Higher serum calcium leads to increased calcium offer to the tubules and increased UCE, an effect maximized in condition such as primary hyperparathyroidism. Interestingly, serum calcium was not associated with fractional calcium excretion, suggesting that the association between calcemia and UCE is mostly related to the extent of calcium-filtered load, at least in CKD.

In this study, we found no association between UCE and dietary intakes of sodium and calcium. However, a positive association between energy intake and UCE remained significant even after adjustments, although the same was not seen for fractional calcium excretion. Again, this suggests that this association is more dependent on renal insufficiency generally (a condition where many confounding variables may be operating) than on tubular function itself. One possibility is that this relationship is mediated by hormones regulating appetite and energy intake, known to be abnormal in CKD [31, 32]. It has been described that adiponectin regulates UCE, at least partly due to Klotho expression, with transgenic mice presenting increased UCE and knockout mice presenting lower UCE [33]. In addition, it has been shown that leptin exerts effects on FGF-23 [34, 35] and on bone mass [36]. Unfortunately, we did not measure leptin and adiponectin in our cohort and could not further explore the effect of these measurements on the relationship seen between energy intake and UCE.

Our study has limitations. Its design is cross-sectional and this limits our ability to fully adjust for confounding variables. This may be particularly important for mineral metabolism variables, which show complex relationships, with actual values possibly reflecting a balance state and not initial disturbances. Second, we did not have measurements on intestinal absorption of calcium, serum leptin and adiponectin, or Klotho, variables that would be important to adjust for. Lastly, in our analysis we used total serum calcium concentrations and not ionized calcium, although these two variables are highly correlated.

In conclusion, we showed that decreasing eGFR is associated with reduced levels of UCE in a CKD population and that $1,25(OH)_2D$, serum calcium, and FGF-23, but neither serum sclerostin nor PTH, were independently associated with UCE. Future studies should investigate how much reduced UCE contributes to a positive calcium balance, as well as mechanisms underlying this effect in CKD.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interests.

Ethical statement All subjects included in this study have given informed written consent, and the study protocol was approved by two local Ethics Committees (Ethics in Research Committee—University Hospital, Sao Paulo University, no. 11,147/11; and Ethics Commission for Analysis of Research Projects, Hospital das Clínicas, Medical School, Sao Paulo University, no. 0798/11).

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