

Postprandial Mineral Metabolism and Secondary Hyperparathyroidism in Early CKD

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ABSTRACT

Normophosphatemia and normocalcemia are maintained in chronic kidney disease (CKD) by increased levels of fibroblast growth factor-23 (FGF-23) and parathyroid hormone (PTH), but the stimuli for secretion of these hormones in early CKD are incompletely understood. Most human physiologic studies have focused on random or fasting measurements of phosphorus, calcium, FGF-23, and PTH, but in this study, the hypothesis was that measurements in the postprandial state may reveal intermittent stimuli that lead to increased FGF-23 and PTH levels. The 4-h postprandial response in 13 patients with CKD and fasting normophosphatemia and normocalcemia (mean GFR 41 ± 8 ml/min per m^2) was compared with 21 healthy volunteers. Compared with healthy subjects, fasting patients with CKD had significantly higher levels of FGF-23 and fractional excretion of phosphorus; lower fractional excretion of calcium; and no difference in serum calcium, phosphorus, and PTH levels. After standardized meals, urinary phosphorus excretion in both groups increased despite unchanged serum phosphorus and FGF-23 levels. Postprandial urinary calcium excretion also increased in both groups, and this was accompanied by significantly reduced serum calcium and increased PTH levels in patients with CKD only; therefore, FGF-23 does not seem to be an acute postprandial regulator of phosphaturia in CKD or in health, but inappropriate postprandial calciuria with episodic, relative hypocalcemia may represent a previously unreported mechanism of secondary hyperparathyroidism in CKD.

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Patients with chronic kidney disease (CKD) are at dramatically increased risk for death as a result of cardiovascular disease,¹ but traditional risk factors do not fully account for this risk, suggesting that additional CKD-specific factors may also contribute.^{2,3} Abnormalities in mineral metabolism are associated with mortality on hemodialysis,⁴ perhaps by promoting nonatherosclerotic calcification of the vasculature.⁵ Importantly, abnormal mineral metabolism is an early complication of CKD that begins long before patients reach dialysis, suggesting that it may also be a risk factor for the dismal cardiovascular outcomes observed in predialysis CKD.^{6,7}

In health, normophosphatemia and normocalcemia are maintained despite wide variation in dietary intake by modulation of calcitriol (1,25D),

parathyroid hormone (PTH), and fibroblast growth factor-23 (FGF-23).^{8,9} A high-phosphorus diet stimulates increased FGF-23 secretion, which induces phosphaturia by downregulating the expression of sodium-phosphate co-transporters in the proximal tubule,^{10–12} and inhibits renal 25-hydroxyvitamin D 1- α hydroxylase, leading to decreased 1,25D levels.^{8,13} The opposite occurs in response to dietary phosphorus restriction: FGF-23

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levels decrease, 1,25D increases, and the kidney reabsorbs phosphorus more avidly.^{10–12} In both cases, serum phosphorus levels remain in the normal range as a result of the metabolic adaptations. Likewise, serum calcium is tightly regulated within a narrow range by PTH, which alters bone resorption, renal tubular reabsorption, and dietary absorption of calcium (indirectly *via* 1,25D) in response to minute changes in ionized calcium levels.¹⁴

In advancing CKD, normophosphatemia is maintained by augmenting the per nephron urinary phosphorus excretion stimulated by progressive increases in FGF-23 and PTH.^{15,16} Increased PTH prevents hypocalcemia despite decreased dietary calcium absorption as a result of impaired renal 1,25D production. Together, decreased 1,25D and increased PTH lead to decreased urinary calcium excretion.¹⁴ Thus, predialysis patients with CKD demonstrate increased FGF-23, PTH, and fractional excretion of phosphorus and decreased 1,25D and fractional excretion of calcium^{16–19}; however, these physiologic alterations are detectable long before hyperphosphatemia or hypocalcemia first appears (typically once GFR decreases to <30 ml/min per 1.73 m²),^{16,17,19} and thus the early triggers that stimulate compensatory FGF-23 and PTH secretion in early CKD remain unclear. Most human physiologic studies focused on random or fasting measurements of phosphorus, calcium, FGF-23, and PTH, which is when differences between individuals with and without CKD and are likely least pronounced; few if any studies compared patients with CKD and healthy subjects in the postprandial state. We tested the hypothesis that postprandial alterations in phosphorus and calcium regulation could represent intermittent stimuli that contribute to the constitutively elevated FGF-23 and PTH levels observed in normophosphatemic, normocalcemic patients with CKD.

RESULTS

Compared with the healthy volunteers, the patients with CKD were older (63 ± 12 versus 46 ± 13 yr; $P < 0.01$), included a greater proportion of men (77 versus 33% ; $P = 0.01$), and, by

definition, had a significantly lower mean GFR (41 ± 8 versus 80 ± 12 ml/min per 1.73 m²; $P < 0.01$). Fasting laboratory values before meals 1 and 2 are presented in Table 1 according to CKD status. In the fasting state, patients with CKD demonstrated significantly increased fractional excretion of phosphorus and FGF-23 and decreased 1,25D and fractional excretion of calcium. There were no differences in serum phosphorus, calcium, 25D, or PTH between the groups. Considering that meals 1 and 2 were separated by a median of 52 d (range 14 to 111), fasting levels of all parameters were remarkably stable with no significant differences for all within-group, between-meal comparisons.

Meal 1: 500 mg of Phosphorus/390 mg of Calcium

The postprandial response in serum phosphorus levels was significantly different among the patients with CKD and healthy volunteers ($P = 0.03$). In the healthy volunteers, the maximal deviation in mean serum phosphorus levels was a modest 5% increase at 210 min compared with fasting (Figure 1A). In the patients with CKD, the overall postprandial changes in serum phosphorus levels were also modest, but the maximal deviation from baseline was a statistically significant decrease of 7% at 120 to 150 min compared with fasting (Figure 1A).

There was a significant difference over time in the postprandial fractional excretion of phosphorus between the healthy volunteers and patients with CKD ($P = 0.04$). The healthy volunteers demonstrated an immediate increase in fractional excretion of phosphorus that remained significantly above baseline at all time points ($P < 0.01$ for each), peaking at 60 min with a 75% increase from fasting (Figure 1B). In contrast, the patients with CKD demonstrated a greater fasting fractional excretion of phosphorus but after the meal did not augment it to the extent of the healthy subjects, achieving a maximal increase of only 10% over fasting at 120 min (Figure 1B). Although FGF-23 levels were significantly increased in the patients with CKD compared with the healthy volunteers at all time points, there was no interaction between group and time, indicating no significant difference in the postprandial FGF-23 response between patients with CKD and healthy subjects (Figure 1C).

Table 1. Fasting laboratory results in the healthy and CKD groups^a

Parameter	Meal 1			Meal 2		
	Healthy (n = 21)	CKD (n = 13)	P	Healthy (n = 11)	CKD (n = 10)	P
Serum creatinine (mg/dl)	0.9 ± 0.1	1.8 ± 0.3	<0.01	0.9 ± 0.1	1.8 ± 0.3	<0.01
Serum phosphorus (mg/dl)	3.1 ± 0.5	3.1 ± 0.6	NS	3.2 ± 0.6	3.0 ± 0.6	NS
Serum calcium (mg/dl)	9.2 ± 0.3	9.3 ± 0.3	NS	9.2 ± 0.4	9.2 ± 0.3	NS
Fractional excretion phosphorus (%)	16 ± 7	30 ± 9	<0.01	16 ± 8	29 ± 10	<0.01
Fractional excretion calcium (%)	0.9 (0.7 to 1.3)	0.5 (0.2 to 0.7)	<0.01	0.9 (0.8 to 1.8)	0.5 (0.2 to 0.8)	0.02
PTH (pg/ml)	30 ± 14	38 ± 19	NS	25 ± 6	29 ± 17	NS
FGF-23 (pg/ml)	52 ± 24	84 ± 24	<0.01	50 ± 21	78 ± 20	<0.01
25D (ng/ml)	39 ± 9	35 ± 8	NS	–	–	–
1,25D (pg/ml)	41 ± 11	24 ± 10	<0.01	–	–	–

^aData are means \pm SD for normally distributed variables and median (interquartile range) for variables with skewed distributions.

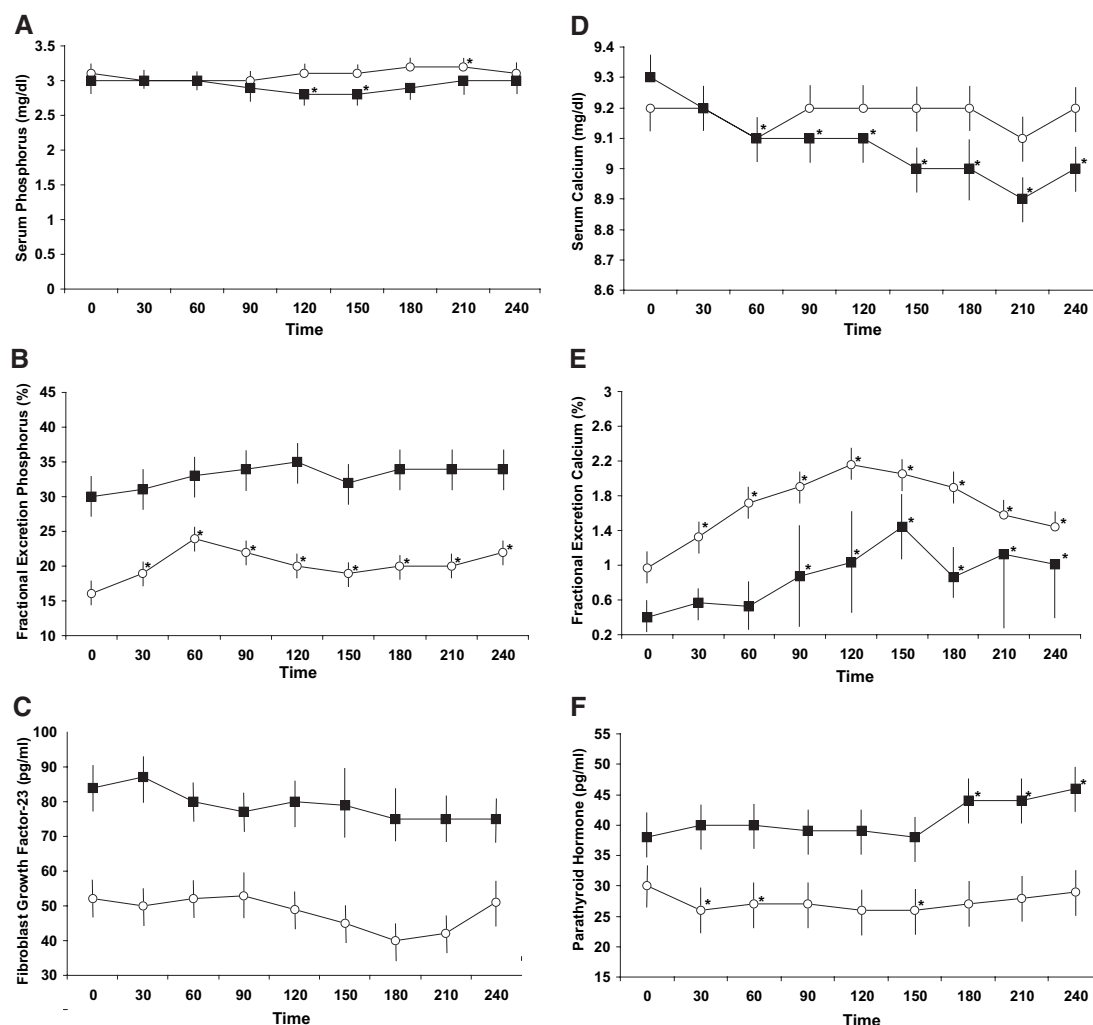


Figure 1. Fasting and postprandial measurements of serum phosphorus (A), fractional excretion of phosphorus (B), FGF-23 (C), serum calcium (D), fractional excretion of calcium (E), and PTH (F) after meal 1 in healthy volunteers (○) and patients with CKD (■). Time 0 represents the fasting measurements. Results are reported as means \pm SE. *Significant ($P < 0.05$) differences at individual time points compared with the within-group's fasting level based on repeated measures linear regression models.

The postprandial response in serum calcium levels was significantly different among the patients with CKD and healthy volunteers ($P < 0.01$). Whereas serum calcium did not significantly deviate from baseline at any time point in the healthy subjects, the patients with CKD demonstrated a significant reduction in serum calcium levels beginning at 60 min and persisting through the end of the postprandial period (Figure 1D).

There was no significant difference between the postprandial fractional excretion of calcium between the groups. Although patients with CKD began with significantly decreased fasting levels compared with the healthy volunteers, fractional excretion of calcium increased significantly in both groups in parallel after the meal (Figure 1E).

There was a significant difference in postprandial PTH secretion between the patients with CKD and healthy subjects ($P = 0.05$). Although PTH levels were greater in the patients with CKD compared with the healthy volunteers at all time

points, healthy subjects demonstrated a significant postprandial reduction in PTH levels of up to 13% at 150 min. In contrast, PTH levels increased in patients with CKD by as much as 21% at 240 min and were still rising at the conclusion of the observation period (Figure 1F).

Meal 2: 250 mg of Phosphorus/270 mg of Calcium

Although there were no significant interactions between group and time in the postprandial response curves of serum phosphorus, fractional excretion of phosphorus, or FGF-23, there were several significant within-group differences over time after meal 2. Serum phosphorus levels decreased significantly, by as much as 10%, in the patients with CKD after meal 2, which was greater than the reductions observed in the healthy group (Figure 2A) or in comparison with meal 1. Fractional excretion of phosphorus increased in the healthy group by 59% at 60 min (Figure 2B), which was statistically similar to their response

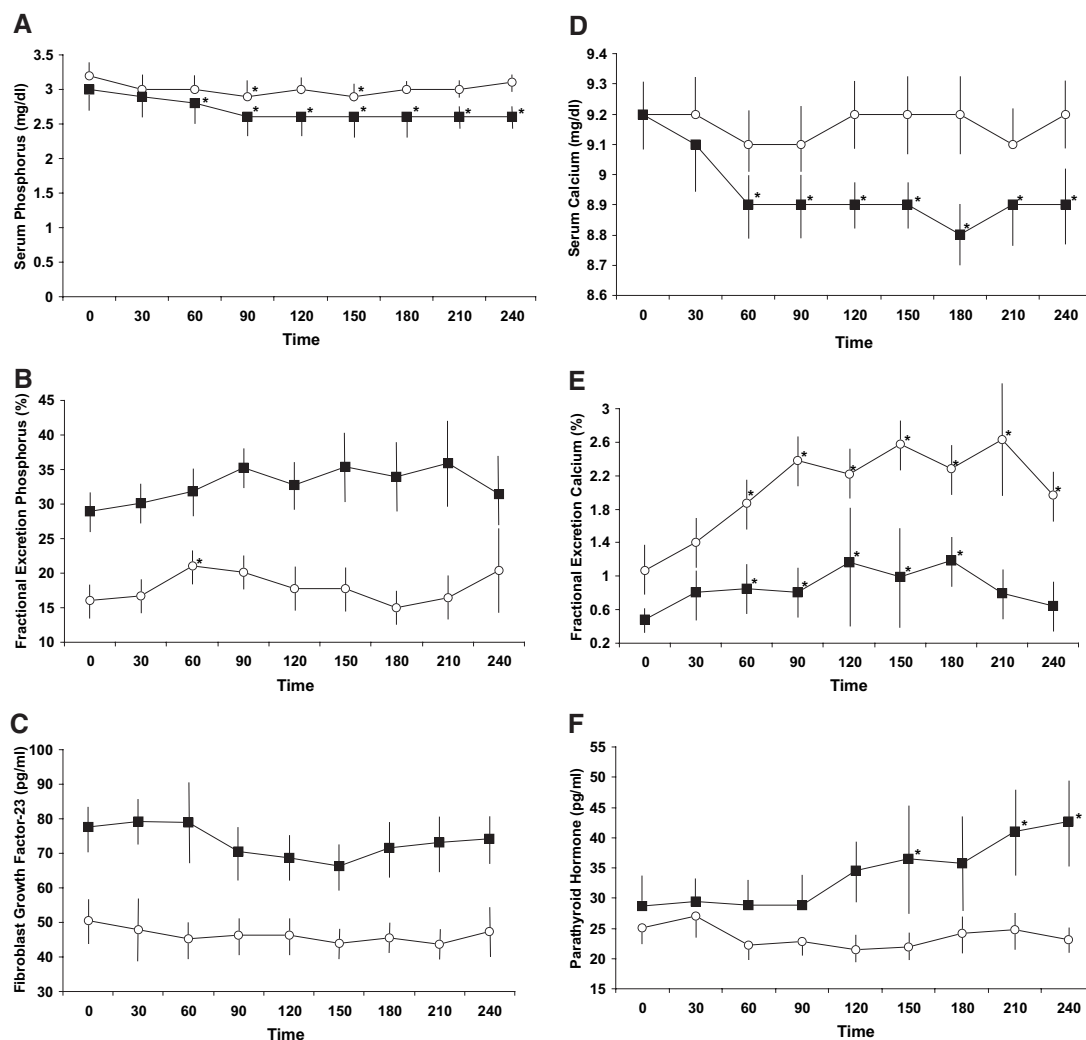


Figure 2. Fasting and postprandial measurements of serum phosphorus (A), fractional excretion of phosphorus (B), FGF-23 (C), serum calcium (D), fractional excretion of calcium (E), and PTH (F) after meal 2 in healthy volunteers (○) and patients with CKD (■). Time 0 represents the fasting measurements. Results are reported as means \pm SE. *Significant ($P < 0.05$) differences at individual time points compared with the within-group's fasting level based on repeated measures linear regression models.

after meal 1. Likewise, the postprandial fractional excretion of phosphorus curve in the patients with CKD after meal 2 mirrored that after meal 1 with no significant increases at any postprandial time point (Figure 2B). As with meal 1, FGF-23 levels did not vary with time in either group after meal 2 (Figure 2C).

Similar to meal 1, there was a significant difference in the groups' postprandial serum calcium levels ($P = 0.02$). Whereas there was no change in postprandial calcium levels in the healthy group, the patients with CKD again demonstrated a significant reduction from fasting, reaching a nadir calcium level 4.4% below baseline at 180 min (Figure 2D). Postprandial fractional excretion of calcium increased in both groups in parallel with time (Figure 2E), and the magnitude of the response was not different comparing meals 1 and 2 in either group. As in meal 1, there was a significant difference in postprandial PTH secretion comparing the patients with CKD and

healthy subjects ($P = 0.04$). Whereas healthy subjects demonstrated no change in postprandial PTH levels over time, PTH levels increased by as much as 38% at 240 min in the patients with CKD, and, just as in meal 1, levels were still rising at the conclusion of the observation period (Figure 2F).

Pooled Data

Because there were no significant differences in the response curves of any of the analytes comparing meal 1 with meal 2 in both the patients with CKD and healthy volunteers (NS for all meal \times group tests of interaction), we pooled the results within each group to compare the relative timing of changes in postprandial serum calcium, fractional excretion of calcium, and PTH (Figure 3). Calciuria increased significantly in both groups by the first postprandial measurement at 30 min. This was followed by a significant decrease in serum calcium levels beginning at 60 min in the patients with CKD alone, whose

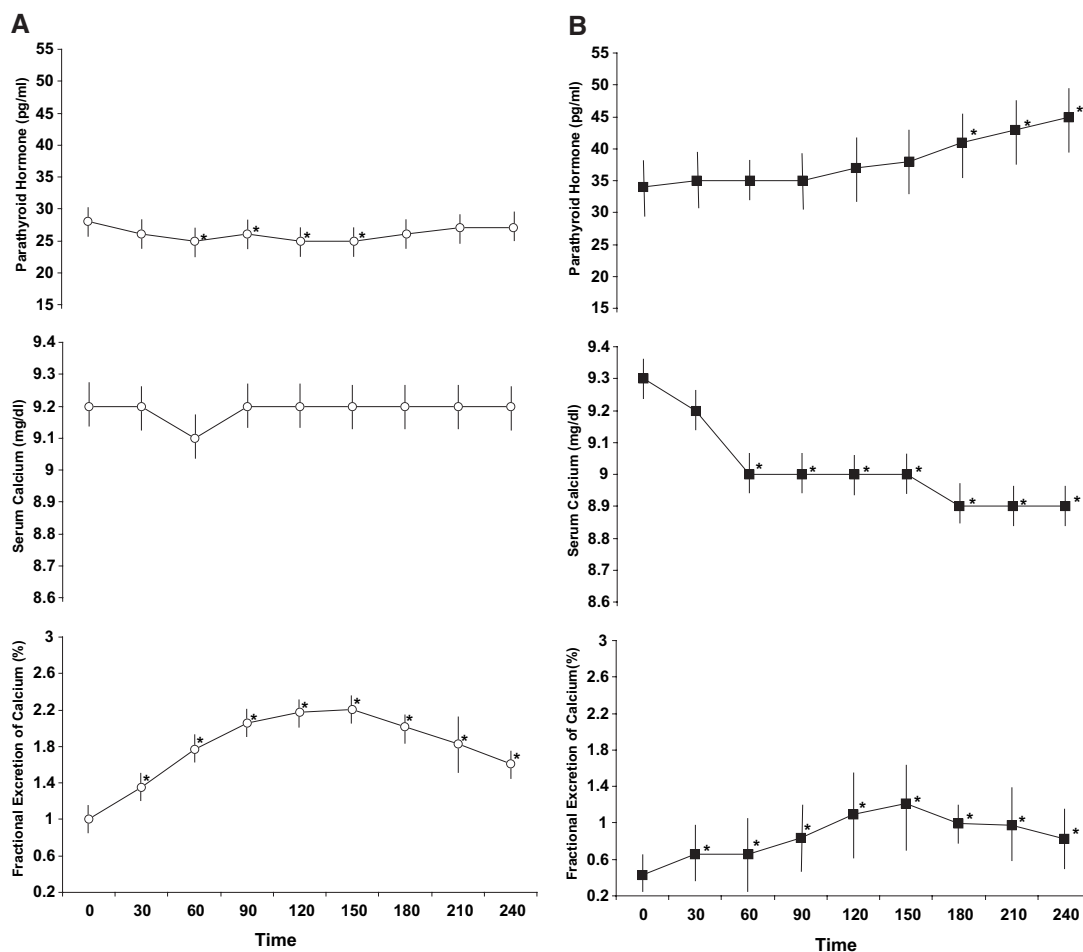


Figure 3. Pooled results from meal 1 and meal 2 for healthy volunteers (A) and patients with CKD (B) to compare the temporal relationships between changes in serum calcium, fractional excretion of calcium, and PTH. Time 0 represents the fasting measurements. Results are reported as means \pm SE. *Significant ($P < 0.05$) differences at individual time points compared with the within-group's fasting level based on repeated measures linear regression models.

serum calcium remained significantly decreased from baseline throughout the remainder of the postprandial period. After 180 min, the patients with CKD demonstrated significantly increased PTH levels, the time that coincided with the nadir in their serum calcium levels. In contrast, the only significant changes in PTH levels in the healthy group were modest reductions between 60 and 150 min after the meals.

DISCUSSION

Hypocalcemia and hyperphosphatemia are widely known stimuli for excessive PTH secretion in ESRD; however, these alterations are rare in early-stage CKD, when secondary hyperparathyroidism and increased FGF-23 secretion are often already established.^{16,17,19} Thus, the initial pathophysiologic triggers of increased PTH and FGF-23 secretion in early-stage CKD are less clear. The purpose of this study was to test whether abnormal handling of dietary phosphorus and calcium in the immediate postprandial period might represent unrecognized stimuli for PTH and FGF-23 secretion in nor-

mophosphatemic, normocalcemic patients with CKD. Both patients with CKD and healthy subjects demonstrated significant increases in postprandial urinary excretion of phosphorus and calcium. We observed minimal changes in postprandial serum phosphorus levels in both groups, and, although the patients with CKD demonstrated significantly higher FGF-23 levels at all time points compared with the healthy volunteers, FGF-23 levels did not vary postprandially in either group. In contrast, whereas postprandial serum calcium and PTH levels were mostly unchanged in the healthy volunteers, the increase in calciuria in the patients with CKD was followed by significant decreases in serum calcium and subsequent increases in PTH. These data suggest that the normal postprandial response to augment phosphaturia and calciuria becomes maladaptive in patients with CKD, in whom relative hypocalcemia and increased PTH secretion ensue. We propose that postprandial calciuria with recurrent relative hypocalcemia may represent an early mechanism of secondary hyperparathyroidism in CKD that, to our knowledge, has not been previously reported.

Urinary phosphorus and calcium excretion increased significantly in the healthy volunteers within 30 min after both meals in the absence of increases in serum levels. This suggests that the phosphaturic and calciuric response to food intake is independent of serum mineral levels and dietary intake, as was observed in previous studies of healthy volunteers.^{20,21} It is unlikely that we missed transient increases in serum mineral levels before the first postprandial time point when we sampled blood and urine, because the maximal gastrointestinal absorption of calcium and phosphorus occurs 40 to 60 min after a meal.²² Furthermore, if efficient gastrointestinal absorption occurred earlier, then we should have observed a different response between the meals. An alternative hypothesis is that neurohormonal changes associated with eating led to renal vasodilation with an increase in glomerular filtration of calcium and phosphorus and a decrease in their tubular reabsorption that was independent of serum levels and intake. Indeed, the postprandial state is characterized by decreased catecholamine and increased insulin, secretin, and cholecystokinin secretion, which increase GFR and thereby decrease sodium, calcium, and phosphorus reabsorption.^{23–25} Although further studies are needed to explore this hypothesis, we speculate that increased urinary calcium and phosphorus excretion in the early postprandial period is nonspecific and could represent an innate, loosely regulated renal adaptation that prevents rapid and potentially dangerous increases in serum calcium and phosphorus (and potassium) levels that would otherwise be induced by dietary intake.

Nonspecific postprandial phosphaturia would tend to protect against hyperphosphatemia in patients with CKD, but concomitant calciuria seems to be detrimental given the tenuous calcium balance in patients with CKD. Urinary calcium excretion decreases as kidney disease progresses because of decreased gastrointestinal calcium absorption as a result of declining renal production of 1,25D and increased tubular calcium reabsorption stimulated by increased PTH.^{14,26} As expected, we observed significantly lower urinary calcium excretion at all time points in the patients with CKD compared with the healthy volunteers, but there was a significant increase in postprandial calciuria even within the CKD group. We hypothesize that these postprandial urinary calcium losses are inappropriate in the face of limited gastrointestinal calcium absorption and led to relative hypocalcemia that stimulated increased postprandial PTH secretion. In contrast, postprandial serum calcium levels did not decrease in the healthy volunteers despite their greater urinary calcium excretion. Although we could not measure dietary calcium absorption directly, we speculate that the healthy volunteers were able to balance their urinary calcium losses with concomitant gastrointestinal absorption. We conclude that although 1,25D deficiency predisposes to negative calcium balance in CKD, postprandial calciuria may be another previously unrecognized pathophysiologic factor. Previous studies support this hypothesis. When thiazide diuretics were used to reduce calciuria in patients with hypercalciuria-induced hyperparathyroidism, PTH levels decreased.²⁷ Conversely, PTH levels increased in healthy volunteers who de-

veloped hypercalciuria induced by furosemide.²⁷ When the PTH gene was knocked out in *Hyp* mice, an animal model of human X-linked hypophosphatemia, lethal hypocalcemia resulted unless the animals were rescued with PTH injections.²⁸ This suggests that PTH is critical for overcoming hypocalcemia in states of FGF-23 excess and 1,25D depletion, which characterize the patients with early CKD whom we studied.

There are several potential explanations for why we did not detect postprandial changes in FGF-23 levels. It is possible that the amount of phosphorus in the meals did not constitute enough of a stress above the participants' usual intake to elicit further increases in FGF-23; however, we specifically designed the meals to test the effects of consuming a "usual" breakfast, and this approach enabled us to detect significant differences in the calcium-PTH axis. Alternatively, it is possible that FGF-23 regulates postprandial phosphorus homeostasis but *via* effects that are not reflected by changes in its plasma levels. For example, acute increases in membrane-bound klotho, which we were unable to measure, could have sensitized FGF receptor 1(IIIc) to increase responsiveness to static circulating levels of FGF-23.^{29,30} We also could not measure other phosphatonins, such as matrix extracellular phosphoglycoprotein, FGF-7, and frizzled related protein 4, which could have an impact on postprandial phosphorus handling.^{31,32} Further studies are needed to measure the effects of dietary intake on these factors.

Another possible explanation is that FGF-23 is not an acute regulator of postprandial phosphorus handling. Indeed, a previous study found no acute postprandial increases in FGF-23 levels even after subjects consumed 1200 mg of phosphorus and developed postprandial hyperphosphatemia; the earliest change in FGF-23 levels was a 20% increase from baseline 8 h after the meal.²⁰ Juxtaposing these results with the calcium-PTH data in this study leads to the conclusion that regulation of calcium by PTH is tighter than regulation of phosphorus by FGF-23. Our findings must be considered in the context of other studies that clearly demonstrated increased FGF-23 levels in response to several days of dietary phosphorus loading.^{10–12} Further studies are needed to define the minimum duration and increment in phosphorus intake that are required to trigger increased FGF-23 secretion and what stimulates the "switch" from acute to chronic regulation when it eventually occurs.

We were unable to quantify gastrointestinal absorption of phosphorus and calcium, but we minimized several other sources of confounding. We ensured adequate 25D levels as an entry criterion, standardized and validated the meal content with a detailed nutrient analysis, standardized the timing of the meals to minimize the effects of diurnal variation in phosphorus homeostasis,³³ and used only intact assays for FGF-23 and PTH to avoid confounding by cross-reactive catabolic fragments that accumulate preferentially in CKD. However, the main strength of this study is that we exclusively studied normophosphatemic, normocalcemic patients with early-stage CKD, which enabled us to focus on early mechanisms of sec-

ondary hyperparathyroidism. Indeed, there was not even a significant difference in fasting PTH levels between the patients with CKD and healthy subjects; important physiologic differences were exposed only in the postprandial state. Thus, it is noteworthy that fasting FGF-23 levels were already significantly increased in the patients with early CKD. Although there is a lack of consensus over when in the course of CKD FGF-23 begins to rise,^{16,19,34,35} these results suggest that increased FGF-23 predates increased PTH. Given the inhibitory effect of FGF-23 on renal 1,25D production^{8,13} and the adverse effects of 1,25D deficiency,^{36,37} early FGF-23 excess could have important implications for the pathogenesis and clinical management of secondary hyperparathyroidism. Whereas current practice guidelines recommend screening for secondary hyperparathyroidism in early CKD,³⁸ they do not specify when PTH levels should be measured or under what conditions. Our data suggest that fasting or random measurements of PTH levels may be insensitive to detect secondary hyperparathyroidism at its earliest stage and that perhaps random FGF-23 or postprandial PTH levels may prove to be superior screening tests for secondary hyperparathyroidism that could be integrated into clinical practice.

CONCISE METHODS

Study Population

We studied 21 healthy volunteers and 13 patients with CKD at the Mallinckrodt General Clinical Research Center at Massachusetts General Hospital. To be eligible, all individuals had to be ≥ 18 yr of age, normophosphatemic (2.5 to 4.6 mg/dl), and normocalcemic (8.5 to 10.0 mg/dl) without current or previous use of dietary phosphorus binders or active forms of vitamin D. The healthy volunteers had no history of kidney disease or other systemic illness, had a normal urinalysis at the screening evaluation, and had an estimated GFR >60 ml/min per 1.73 m² according to the abbreviated Modification of Diet in Renal Disease (MDRD) formula.³⁹ The CKD group included patients with a stable GFR between 15 and 60 ml/min per 1.73 m². Patients were excluded when they were anemic or had a history of primary parathyroid disease, gut absorption defects, malnutrition, or liver disease or were being treated with phosphorus binders, active vitamin D, or phenytoin, which induces vitamin D catabolism.⁴⁰ Pregnant or breastfeeding mothers, patients who were hospitalized within the previous 4 wk, and those who were unable to provide written informed consent or unwilling to consume the study meals in their entirety were also excluded.

Vitamin D deficiency is common in the general population and in patients with CKD.^{18,41,42} To minimize confounding by vitamin D deficiency, all participants were required to demonstrate 25-hydroxyvitamin D stores ≥ 20 ng/ml before they could proceed to the study. Those who were found to have lower levels at a screening visit were treated with ergocalciferol, 50,000 IU orally every other day for a total of four doses for levels 10 to 20 ng/ml or eight doses for levels <10 ng/ml. Thereafter, we repeated the 25D measure-

ments to reassess eligibility. All four participants (one healthy, three with CKD) who were initially found to be 25D deficient became eligible after a single course of ergocalciferol. The study adhered to the principles of the Declaration of Helsinki and was approved by the human research committee at Massachusetts General Hospital. All participants provided written informed consent at the screening visit.

Procedure

Participants consumed two isocaloric standardized breakfast meals at the General Clinical Research Center after an overnight fast. The menu and macronutrient content of the meals are presented in Table 2. Meal 1 contained 500 mg of phosphorus and 389 mg of calcium; meal 2 contained 250 mg of phosphorus and 272 mg of calcium. The phosphorus content of meal 1 was chosen to reflect one third of the daily intake on a typical 1500-mg/d phosphorus diet⁴³; meal 2 reflected one third of a phosphorus-restricted diet. We validated the meals' contents by performing a detailed nutrient analysis (Covance Laboratories, Madison, WI), which demonstrated $<10\%$ variation between the prescribed and administered meals' phosphorus and other macronutrient content. Participants consumed the meals in their entirety within 15 min on separate mornings at least 2 wk apart. They were encouraged to drink 1 to 2 L of water with the meal and throughout the postprandial period to maintain an adequate urine flow to support bihourly urine collections. We obtained blood and urine samples immediately before the meals (time 0) and every 30 min thereafter for 4 h.

Measurements/Assays

Standard assays for serum and urine phosphorus, calcium, and creatinine were performed using automated analyzers. Vitamin D levels were measured using radioimmunoassays (25D: Diasorin, Stillwater, MN; 1,25D: American Medical Laboratories, Chantilly, VA) with coefficients of variation (CV) $<7\%$. Our goal was to measure with precision the acute PTH and FGF-23 secretory responses to dietary intake. Because catabolic fragments of PTH and FGF-23 are known to accumulate in CKD, these fragments might have obscured our ability

Table 2. Menu and calculated nutritional components of the standardized meals

Parameter	Meal 1	Meal 2
Items	1 slice whole-wheat toast 25 g wheat bran cereal 0.5 cup applesauce 4 oz 1% milk 4 oz orange juice 8 oz coffee 2 pats butter 4 oz yogurt	1 English muffin 25 g Rice Krispies cereal 0.5 cup applesauce 4 oz 1% milk 4 oz orange juice 8 oz coffee 3 pats butter
Phosphorus (mg)	500	250
Calcium (mg)	389	272
Sodium (mEq)	23	30
Carbohydrate (%)	65	62
Fat (%)	23	29
Protein (%)	12	9
Calories (kcal)	503	502

to detect subtle changes in *de novo* hormone secretion, especially in the CKD group; therefore, we used an intact (1-84) PTH assay (Immutopics, San Clemente, CA; CV <6%), which does not cross-react with 7-84, 1-34, and other PTH fragments. Similarly, we used an intact FGF-23 assay (Kainos Laboratories Tokyo, Japan; CV <4%) that exclusively detects the intact hormone because the two epitopes recognized by the ELISA flank the metabolic cleavage site.¹¹ All samples were run in duplicate, and mean values are reported. Fractional excretion of mineral (phosphorus, calcium) was calculated as (urine mineral \times serum creatinine \times 100)/(serum mineral \times urine creatinine).

Statistical Analyses

Characteristics of the patients with CKD and healthy subjects were compared using two-sample *t* test or Wilcoxon rank sum test for continuous variables and Fisher exact test for categorical variables. We used mixed-model ANOVA for repeated measures to test for statistical differences in the postprandial response over time between the patients with CKD and healthy subjects. Separate analyses were performed for serum and urine phosphorus and calcium, PTH, and FGF-23. In the ANOVA models, time represented the repeated measures factor, individuals were represented as a random-effects term, and CKD status (present or absent) and meal (1 or 2) were treated as fixed-effects factors. To test whether there were postprandial differences according to CKD status, we tested for interaction between time and CKD. When no significant interaction was identified, we tested for main effects of CKD status and time. We localized individually significant postprandial time points within the groups by comparing them with the baseline fasting level using multiple linear regression. We used an identical analytic strategy to test for differences in response to the 500- and 250-mg phosphorus meals, first testing for a time-meal interaction followed by testing for main effects and then localizing individually significant, within-group time points. Fractional excretion of calcium was not normally distributed; therefore, we analyzed log-transformed values in the ANOVA and regression models. Although the patients with CKD were significantly older than the healthy volunteers and more likely to be men, age and gender were not associated with fasting or postprandial markers of mineral metabolism, and including age or gender in the analyses did not alter the results; therefore, age and gender-adjusted results are not presented. Means \pm SE of analytes are presented graphically according to CKD status and time. All analyses were performed using Intercooled Stata 7.0 (Stata Corp., College Station, TX). $P \leq 0.05$ was considered statistically significant.

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DISCLOSURES

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