

Pilot study of dietary phosphorus restriction and phosphorus binders to target fibroblast growth factor 23 in patients with chronic kidney disease

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Abstract

Background. High levels of fibroblast growth factor 23 (FGF23) are associated with mortality and progression of chronic kidney disease (CKD). Reducing dietary phosphorus intake lowers FGF23 secretion in healthy individuals, but there is little data on its effects in patients with pre-dialysis CKD.

Methods. Using a 2 × 2 factorial design, we randomly assigned 16 normophosphataemic CKD stage 3–4 patients to receive a 2-week treatment with either lanthanum carbonate 1000 mg three times daily or placebo, and to ingest a tightly controlled diet containing 750 or 1500 mg of dietary phosphorus daily. We analysed serial measurements of FGF23, parathyroid hormone, serum phosphate and calcium, and 24-h urinary phosphate and calcium excretion using repeated-measures analyses.

Results. Compared with the 1500-mg phosphorus diet, patients assigned to the 750-mg diet had greater reduction in 24-h urinary phosphate excretion (66% vs. 29%; $P < 0.0001$). Lanthanum-treated patients experienced a significant reduction in 24-h urinary phosphate excretion compared with baseline (64%; $P < 0.0001$), but the difference compared with placebo did not reach significance (64% vs. 31%). Despite the significant reductions in 24-h urinary phosphate excretion, no group demonstrated a significant reduction in FGF23 levels; FGF23 levels actually increased significantly in the 1500-mg diet plus placebo group, suggesting dietary phosphorus loading.

Conclusions. Although dietary phosphorus restriction and lanthanum lowered urinary phosphate excretion consistent with a rapid decrease in phosphorus absorption, inducing a reduction in FGF23 levels in CKD patients may require interventions with a longer duration than in healthy volunteers.

Keywords: chronic kidney disease; FGF23; phosphate; phosphate binders

Introduction

Fibroblast growth factor 23 (FGF23) is a phosphorus-regulating hormone that augments urinary phosphate excretion and decreases 1,25-dihydroxyvitamin D (1,25D) and parathyroid hormone (PTH) synthesis [1–3]. FGF23 levels increase progressively beginning as early as CKD stage 2 [4–6], presumably as a compensatory response to maintain normal phosphorus balance when dietary phosphorus intake stresses the renal capacity for phosphate excretion. As a result of the physiological actions of FGF23, disordered mineral metabolism in early CKD is characterized by FGF23 excess and normal serum phosphate levels [4–6]. Since a primary stimulus for FGF23 secretion is high dietary phosphorus intake [7–9], a high FGF23 level might help differentiate which early CKD patients with normal serum phosphate levels actually have a normal phosphorus metabolism from those who might benefit from interventions aimed at reducing dietary phosphorus loading.

Recent studies reported that increased FGF23 was far more powerfully associated with CKD progression, cardiovascular disease and death than increased serum phosphate, and FGF23 was particularly predictive when serum phosphate levels were normal [10–12]. These findings highlight the potential of FGF23 as a novel biomarker to guide the optimal timing of initiation and subsequent titration of therapies to target disordered phosphorus metabolism in early CKD. To date, a handful of studies have demonstrated a reduction in FGF23 levels in response to phosphorus binders in dialysis [13] and pre-dialysis CKD patients [14], but the impact on FGF23 of manipulating dietary phosphorus intake has not been studied in detail in CKD. The purpose of this pilot study was to examine the effects on FGF23 of tightly controlled dietary phosphorus restriction and phosphorus binders, alone and in combination, in normopho-

Table 1. Menu of the study diet

	Cycle 1	Cycle 2	Cycle 3
Breakfast	Scrambled egg English muffin Butter, unsalted Cranberry juice Total cereal with non-dairy creamer	Bagel Scrambled eggs Pancakes with syrup Total cereal with non-dairy creamer Cranberry juice Vegetarian burrito Water	Toast with butter and jelly Scrambled eggs Fruit cocktail Cranberry juice
Lunch	Turkey sandwich (with lettuce, tomato and mayo on white bread) Applesauce Ginger ale, reg		Tortilla chips with ground beef and kidney beans Fudge Cranberry juice Ginger ale
Dinner	Spaghetti carbonara Bread, white Butter, unsalted Salad with ranch dressing Cake, angel food Cranberry juice	Lemon chicken rice Graham crackers Ginger ale	Mushroom cheese quiche Fudge Cranberry juice Ginger ale

The menu for the 2-week study included three different days of meals that were rotated throughout the study period.

sphataemic patients with CKD stages 3–4. We hypothesized that decreasing phosphorus intake using dietary restriction, phosphorus binders or a combination of both would decrease FGF23 levels.

Materials and methods

Study design and participants

In this pilot study of pre-dialysis CKD patients, we randomly assigned participants to 750 or 1500 mg/day of dietary phosphorus intake paired with fixed-dose lanthanum carbonate 1000 mg three times daily with meals versus placebo using a 2 × 2 factorial design. The participants but not the investigators were blinded to treatment assignments. The primary end point was the effect of the randomized interventions on FGF23 levels during the 2-week follow-up.

Sixteen normophosphataemic (serum phosphate <4.6 mg/dL) CKD stages 3a, 3b and 4 patients (estimated glomerular filtration rate of 15–44 mL/min/1.73 m²), aged 18 years or older, were randomized to (i) 750-mg phosphorus diet plus lanthanum, (ii) 1500-mg phosphorus diet plus lanthanum, (iii) 750-mg phosphorus diet plus placebo or (iv) 1500-mg phosphorus diet plus placebo. Block randomization was used to ensure a balanced distribution of CKD stages in each intervention arm. Exclusion criteria included hyperphosphataemia, previous or current treatment with phosphorus binders or active vitamin D, rapidly advancing CKD, hospitalization within the previous 4 weeks, malnutrition (serum albumin <3.0 mg/dL), inflammatory bowel or liver disease, use of phenytoin, anaemia (haematocrit <27%), pregnancy or breastfeeding mothers, primary parathyroid disease, or previous parathyroidectomy. Because 25-hydroxyvitamin D deficiency may decrease dietary phosphorus absorption [15], all participants had to demonstrate 25-hydroxyvitamin D stores ≥20 ng/mL. Subjects found to be deficient (*n* = 3) were supplemented with ergocalciferol 50 000 IU every other day for a total of four doses when 25-hydroxyvitamin D levels were 10–20 ng/mL and eight doses when levels were <10 ng/mL. Prior to enrolment, participants were required to review the study menu and confirm that they were willing to consume only the prepared standardized meals in their entirety. The study was approved by the Institutional Review Board of the Massachusetts General Hospital, and all participants provided written informed consent.

Dietary intervention

A diet containing 1500 mg/day of phosphorus was chosen to represent 'usual' phosphorus intake based on published estimates of the typical Western diet [16]. The restricted diet contained 750 mg/day, which was chosen

based on the KDOQI recommendation for restriction in hyperphosphataemic CKD patients and previous studies of healthy volunteers in which this level of dietary restriction reduced FGF23 levels by 30% [9,17]. The diet was created with the use of ProNutra version 3.1.0.13 (Viocare Technologies, Inc.) and consisted of a 3-day rotating menu (Table 1). The diet was isocaloric with each individual participant's estimated pre-intervention intake and contained 750 mg of phosphorus per day. Additional components of the daily intake on a 2000-kcal diet included 75 g of protein, 277 g of carbohydrate, 66 g of fat, 1295 mg of calcium, 1664 mg of sodium and 1524 mg of potassium. To achieve the 'usual' phosphorus intake of 1500 mg while maintaining virtually the same intake of other macronutrients across the groups, one packet of Neutra-Phos was added to each meal (a total of three times daily providing 750 mg of additional phosphorus). All meals (breakfast, lunch, dinner and snacks) were prepared by the metabolic kitchen of the Mallinckrodt Clinical Research Center (CRC) at the Massachusetts General Hospital. No additional food was allowed during the 2-week intervention period. Participants returned to the CRC every 3 days to retrieve their packaged meals which they consumed as outpatients. Compliance with the interventions was monitored by meal weighbacks, self-reported intake in written study diaries, pill counts and measurements of 24-h urinary phosphate excretion.

Measurements and assays

Study visits coincided with the days that participants came to the CRC to retrieve their study meals. At each visit, blood and spot urine samples were collected to measure serum phosphate, calcium, FGF23, PTH, and urinary phosphate and calcium; 24-h urine samples from the prior day were also collected at each follow-up visit. Fractional excretion of phosphate (FePi) and calcium (FeCa) was calculated from both the spot and 24-h urine samples using the following formula: (urine mineral × serum creatinine) / (serum mineral × urine creatinine). Given the prior observations of high correlation between intact and C-terminal assays in CKD [10,12,18] and in inherited and acquired hypophosphataemic disorders [19,20] as well as a recent report that showed that all circulating FGF23 was intact and biologically active in peritoneal dialysis patients [21], we chose to measure FGF23 using the second generation C-terminal assay that detects two epitopes in the C terminus of FGF23 (cFGF23; Immotopics, CV <5%). PTH was measured using a bio-intact PTH assay that detects the intact 1–84 PTH peptide, (iPTH; Immotopics, San Clemente, CA, USA, CV <6%). Phosphate, calcium and creatinine concentrations were measured in blood and urine using standard assays.

Analysis

We used standard descriptive statistics to assess baseline laboratory data. For the main analyses, we used linear mixed-effects models to examine

Table 2. Baseline characteristics of the 16 participants overall and within each of the randomized groups (lanthanum versus placebo and 750-mg versus 1500-mg phosphorus diet)

	All participants, <i>n</i> = 16	Lanthanum + 750 mg, <i>n</i> = 4	Lanthanum + 1500 mg, <i>n</i> = 4	Placebo + 750 mg, <i>n</i> = 4	Placebo + 1500 mg, <i>n</i> = 4
Age	62 ± 16	63 ± 11	61 ± 13	56 ± 21	67 ± 16
CKD Stage 3a/3b/4 (<i>n</i>)	7/5/4	2/1/1	2/1/1	2/1/1	1/2/1
eGFR (mL/min/1.73 m ²)	40 ± 12	43 ± 13	41 ± 12	38 ± 18	36.5 ± 10
Creatinine clearance (mL/min)	54 ± 23	53 ± 23	51 ± 12	55 ± 26	59 ± 39
Serum phosphate (mg/dL)	3.2 ± 0.5	3.2 ± 0.5	3.4 ± 0.7	3.0 ± 0.3	3.0 ± 0.5
Serum calcium (mg/dL)	9.6 ± 0.4	9.4 ± 0.3	9.6 ± 0.4	9.3 ± 0.3	9.5 ± 0.2
24-h urinary phosphate (mg) ^a	704 (564–889)	557 (491–827)	756 (704–820)	727 (503–988)	701 (607–1262)
24-h urinary calcium (mg) ^a	48 (29–66)	51 (21–94)	51 (28–131)	46 (27–60)	47 (23–123)
FePi (%)	29 ± 9	28 ± 11	28 ± 8	23 ± 5	36 ± 12
FeCa (%)	0.6 (0.2–0.8)	0.7 (0.4–1.4)	0.8 (0.5–1.0)	0.2 (0.1–1.0)	0.4 (0.3–0.6)
25-hydroxyvitamin D (ng/mL)	32 ± 11	29 ± 8	35 ± 14	34 ± 10	30 ± 13
cFGF23 (RU/mL)	158 (95–223)	147 (77–252)	221 (158–368)	159 (103–254)	100 (85–158)
PTH (pg/mL)	48 (34–69)	40 (32–59)	32 (18–98)	64 (58–93)	53 (36–86)

Results are reported as mean ± standard deviation for normally distributed variables or median (interquartile range) for variables with skewed distributions.

eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone.

^aMean value of two 24-h urine collections prior to intervention.

post-intervention changes over time in cFGF23, iPTH, serum phosphate and calcium, FePi, FeCa, and 24-h urinary phosphate and calcium. The fixed-effects terms were intervention group, and time (Day 0, baseline; and Day 3, 6, 9, 12 and 15 post-intervention) and individual participants were coded as random-effects terms. We first tested for interaction between diet and lanthanum in relation to cFGF23 and the other outcome variables of interest. When significant interaction was excluded, we tested the main effects of each specific intervention in linear mixed models: 1500-mg versus 750-mg phosphorus diet and lanthanum versus placebo. For all analyses of changes over time between specific intervention groups, statistically significant differences were inferred when the interaction term between randomization group and time reached significance. Due to limited power related to the small sample size, we also tested for significant changes from baseline within the intervention groups. Natural log transformations were used for analyses of non-normally distributed dependent variables. For the analyses of baseline 24-h urinary mineral excretion, we analysed the mean of results from two separate collections during the pre-intervention period and compared the associations between fractional and total 24-h urinary excretions using Spearman correlation. Two-tailed *P*-values <0.05 were considered statistically significant. All analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the study population are presented in Table 2. The distribution of CKD stages within each of the four randomization groups was balanced. At baseline, the median cFGF23 of 158 RU/mL was elevated above the normal range (<50 RU/mL), but iPTH levels were normal. The median baseline 24-h urinary phosphate content was 704 (IQR 564–889) mg/day.

All participants completed the 2-week study with no losses to follow-up or withdrawals. Review of meal weigh-backs, study diaries and pill counts indicated a high level of compliance. Adherence to the study interventions was confirmed by the significant reductions in 24-h urinary phosphate excretion in all groups compared with those assigned to the 1500-mg phosphorus diet plus placebo (over-

all *P* for interaction between group and time = 0.015, Figure 1A). Participants assigned to 750 mg phosphorus plus lanthanum experienced the greatest mean reduction in 24-h urinary phosphate excretion of 78 ± 9% compared with baseline (*P* < 0.0001; Figure 1B). Intermediate reductions in 24-h urinary phosphate excretion were observed in the groups assigned to the less intensive interventions, either 1500 mg phosphorus plus lanthanum (49 ± 4% reduction from baseline) or 750 mg phosphorus plus placebo (53 ± 24% reduction from baseline). Given the lack of interaction between the dietary and pharmacological interventions, subsequent analyses focused on the main effects of each specific intervention compared with its respective control group: 750-mg versus 1500-mg phosphorus diet and lanthanum versus placebo.

Urinary phosphate excretion

Compared with the 1500-mg phosphorus diet, participants who consumed the 750-mg phosphorus diet had significantly greater reduction in 24-h urinary phosphate excretion [from 702 ± 262 mg/day at baseline to 249 ± 213 mg/day (66% decrease) at the end of study versus from 848 ± 372 to 607 ± 375 mg/day (29% decrease), *P* < 0.0001; Figure 2A]. Treatment with lanthanum was associated with a significant reduction in 24-h urinary phosphate excretion compared with baseline [from 710 ± 192 at baseline to 267 ± 140 mg/day (64% decrease) at the end of study; *P* < 0.0001], but the comparison with placebo [from 840 ± 416 mg/day at baseline to 588 ± 424 mg/day (31% decrease); Figure 3A] did not reach significance. The results were qualitatively similar when urinary phosphate excretion was analysed using FePi calculated from random or 24-h urine samples given the high cor-

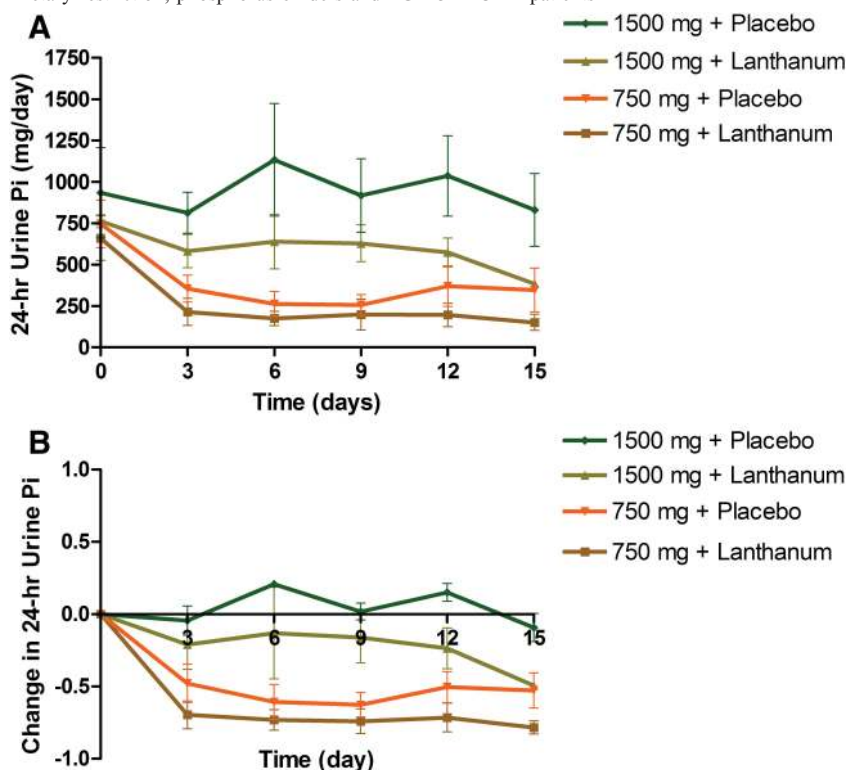


Fig. 1. Mean absolute (A) and percent change from baseline (B) in 24-h urinary phosphate excretion during the 2-week intervention period. Error bars represent standard errors.

relation between these measures and total 24-h urinary phosphate excretion ($r = 0.59$ and 0.77 , respectively, $P < 0.001$ for both).

Serum phosphate

There were no significant changes over time in serum phosphate levels between or within either of the diet or binder groups (Figures 2B and 3B). While there were no episodes of hypophosphataemia, one patient who was assigned to 1500 mg phosphorus plus placebo developed new-onset hyperphosphataemia with a serum phosphate of 5.1 mg/dL on Day 12.

FGF23

There were no significant differences in cFGF23 levels over time between the diet or binder groups (Figures 2C and 3C), but cFGF23 levels increased significantly from 150 ± 81 RU/mL at baseline to 206 ± 130 RU/mL on Day 12 within the placebo arm ($P = 0.004$). There was also a non-significant increase in cFGF23 levels on the 1500-mg phosphorus diet from 192 ± 139 RU/mL at baseline to 234 ± 133 RU/mL at Day 12. Further analysis of four-group models indicated that these increases in cFGF23 levels were driven by a significant early increase in cFGF23 ($53 \pm 25\%$ increase over baseline by Day 3) that peaked at Day 12 ($70 \pm 60\%$ increase over baseline) in the 1500-mg phosphorus diet plus placebo arm that was not observed in any of the other groups (Figure 4; overall P for interaction between group and time = 0.03).

Calcium and PTH

There were no significant differences between the diet or binder treatment groups in serum calcium, FeCa, 24-h urinary calcium excretion (data not shown) or PTH (Figures 2D and 3D).

Discussion

In this short-term pilot study of normophosphataemic pre-dialysis CKD patients, dietary phosphorus restriction and therapy with lanthanum carbonate reduced 24-h urinary phosphate excretion without accompanying changes in serum phosphate or FGF23 levels. In contrast, the group that received the higher phosphorus diet plus placebo demonstrated a significant increase in FGF23 levels, in support of an important effect of dietary phosphorus intake on FGF23 levels in CKD, albeit in the opposite direction of what we aimed to demonstrate. Thus, although we were unable to reduce FGF23 levels, this pilot study yields several important points that will help guide the design of future trials targeting FGF23 in CKD patients.

In steady states of normal bone turnover and in the absence of phosphate shift or deposition into compartments other than blood, urinary phosphate excretion matches dietary phosphate absorption, which is reported to be $\sim 66\text{--}75\%$ of intake [22]. Although we did not perform bone biopsies and therefore could not accurately assess bone turnover, nor did we have the capability to assess phosphate shift and thus total body phosphorus balance, the expected reduction in urinary phosphate excretion that we observed is likely indi-

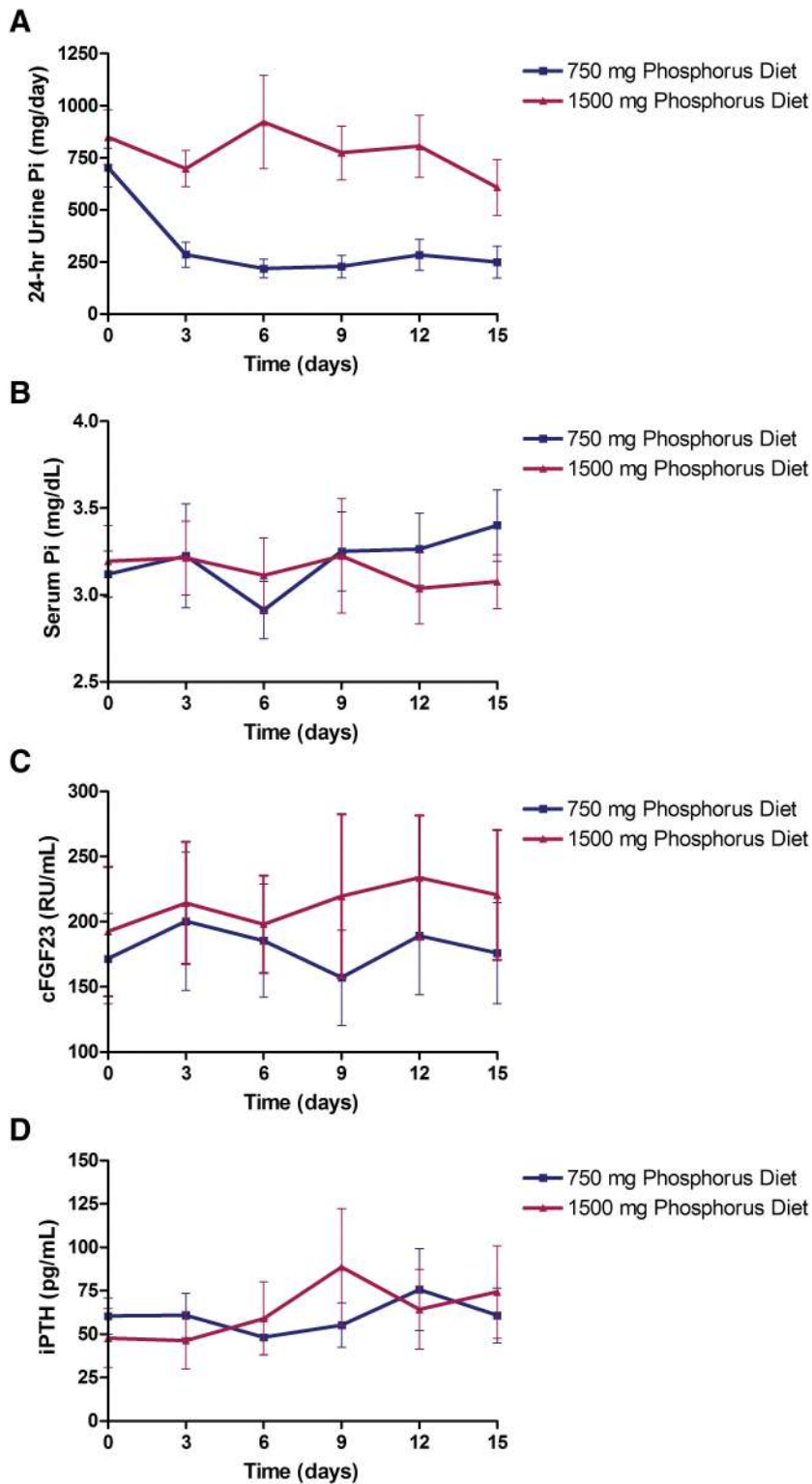


Fig. 2. Mean 24-h urinary phosphate excretion (A), serum phosphate (B), cFGF23 (C) and iPTH (D) in participants assigned to 750-mg ($n = 8$) versus 1500-mg phosphorus diet ($n = 8$). Error bars represent standard errors.

cative of decreased dietary phosphorus absorption. Yet, despite the clear evidence in healthy humans that reducing phosphorus absorption lowers FGF23 [7–9], we observed no reduction in FGF23 levels in response to binders or diet-

ary phosphorus restriction. There are several possible reasons for this discrepancy.

The simplest explanation is that phosphorus binders and dietary phosphorus restriction do not effectively reduce

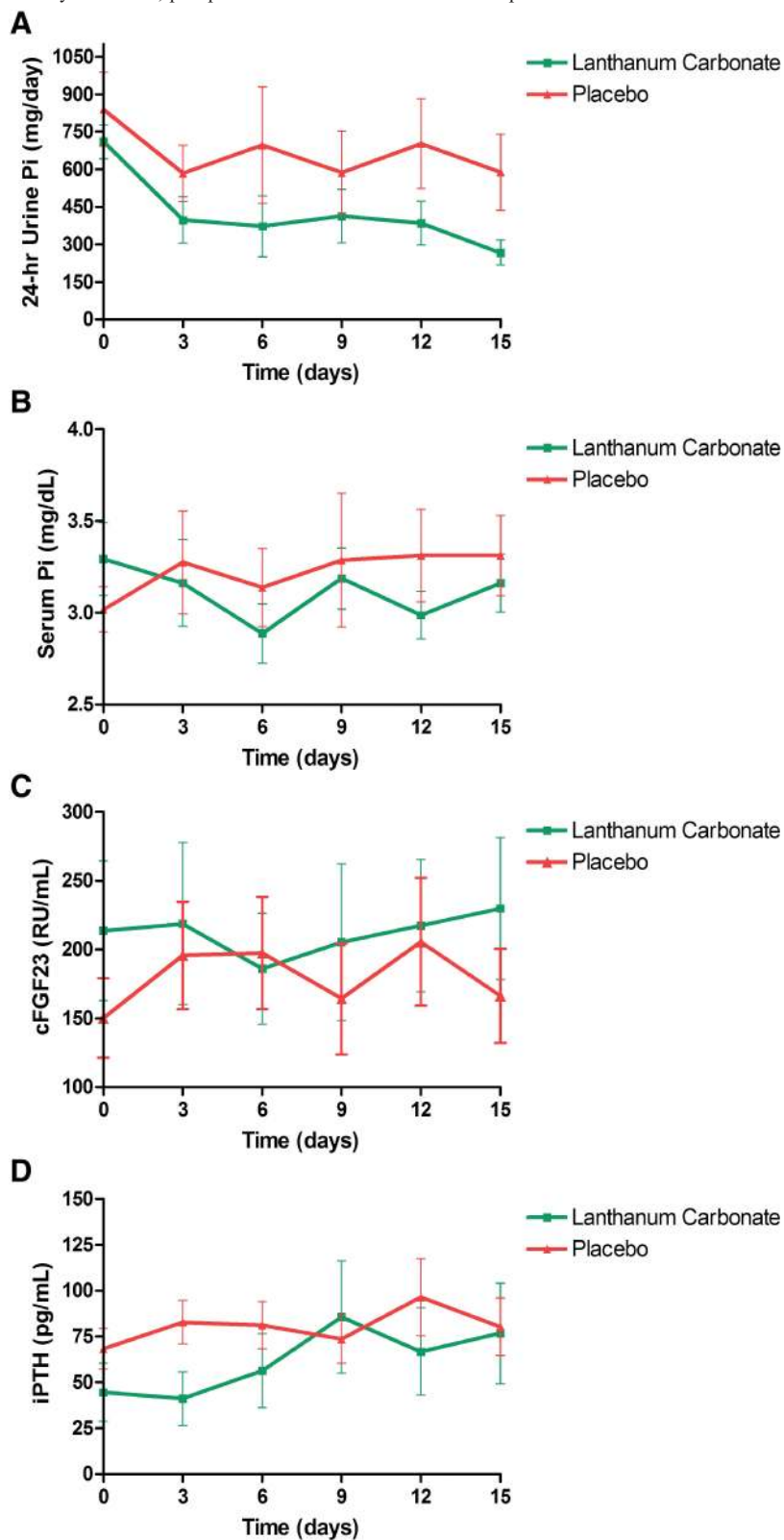


Fig. 3. Mean 24-h urinary phosphate excretion (A), serum phosphate (B), cFGF23 (C) and iPTH (D) in participants assigned to lanthanum carbonate ($n = 8$) versus placebo ($n = 8$). Error bars represent standard errors.

FGF23 levels in CKD patients, but this is unlikely given recent contrary reports in animal and human CKD studies [13,14,23]. Another possibility is that the interventions

were insufficiently intense to lower FGF23 levels, but this is also unlikely because of the rapid and sustained reduction in urinary phosphate excretion they induced. It is far

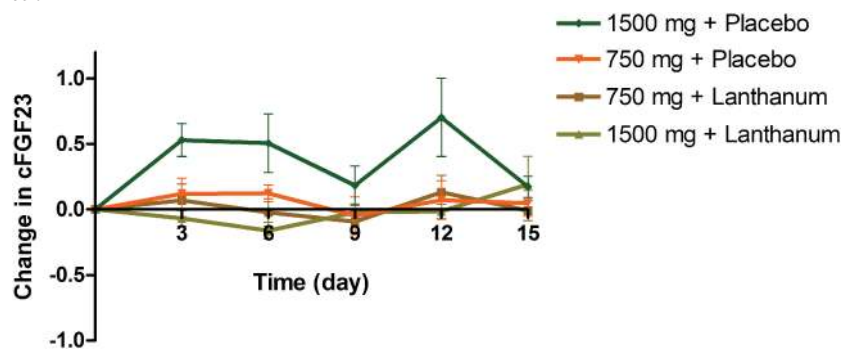


Fig. 4. Mean percent change from baseline in cFGF23 levels during the 2-week intervention period. Error bars represent standard errors.

more likely that the main limitation was the duration of the intervention period, which was probably too brief for a CKD population in whom FGF23 levels are chronically elevated. We chose a study duration of 2 weeks because we expected that it would be adequate to demonstrate an effect on FGF23 based on prior studies of healthy volunteers [7–9], while maximizing our ability to recruit participants and ensure their compliance with the arduous protocol. However, recent data indicate that 4 weeks of binder therapy were required to lower FGF23 levels in haemodialysis patients [13], and 6 weeks were required in pre-dialysis CKD patients [14] with similar baseline serum phosphate and FGF23 levels as the participants in the current study.

While 6 weeks of sevelamer therapy was needed to lower FGF23 levels in pre-dialysis CKD patients, FGF23 levels rebounded back to baseline within just 2 weeks after sevelamer was discontinued [14]. In addition, elevated FGF23 levels can persist up to 12 months following kidney transplantation despite a healthy allograft and frequent hypophosphataemia [24]. Collectively, these data suggest that reducing elevated FGF23 levels in CKD is a prolonged process that lags behind changes in serum phosphate and overall phosphorus balance, whereas rebound increases in FGF23 in CKD occur rapidly. This finding should inform future CKD studies to utilize long-term, sustained interventions. In addition, while dietary restriction appears to have reduced urinary phosphate excretion more potently than the pharmacological intervention, the greatest decrement in urinary phosphate excretion was observed in the group that received both a phosphorus-restricted diet and lanthanum. This highlights the importance of dual interventions for future studies.

An important strength of this study is the inclusion of a tightly controlled dietary intervention, but this pilot study also has several expected limitations. In addition to limited power, the small sample size led to imbalances in baseline laboratory tests, which added further variability to the analyses. While these baseline differences could have been minimized by a run-in period during which participants consumed identical standardized diets, this would have hindered recruitment and retention of participants by lengthening the time that they were required to eat a stringent study diet. Furthermore, choosing the duration of a run-in period would have been as difficult a guess as our choice for the duration of the intervention period.

An even more important and unexpected limitation is that several of the participants may have been unintentionally loaded with dietary phosphorus. Overall, participants' 24-h urinary phosphate excretion was only ~700 mg/day at baseline, or ~30% lower than the level we expected based on studies of individuals with normal kidney function consuming an American diet [25]. Furthermore, one patient who received the 1500-mg diet plus placebo developed new-onset hyperphosphataemia. Therefore, while we aimed to compare the effect of dietary phosphorus restriction versus 'usual' intake in a standardized fashion, we may have actually compared usual intake for a CKD patient with dietary phosphorus loading, which could have obscured important effects on FGF23. Indeed, a recent study of pre-dialysis CKD patients reported 24-h urinary phosphate excretion that was similar to what we observed [26]. The lower 'usual' intake in CKD than in healthy individuals could be due to subtle anorexia, decreased gut absorption due to calcitriol deficiency or perhaps violation of the steady state by positive phosphorus balance in CKD such that urinary excretion underestimates dietary absorption.

The recognition that increased FGF23 is independently associated with CKD progression, cardiovascular disease and mortality has the potential to revolutionize the management of phosphorus metabolism in early CKD. The ultimate goal is to prove that FGF23 screening leading to early initiation of phosphorus-related therapies will improve survival in pre-dialysis CKD patients with normal serum phosphate levels who otherwise would not be treated according to current standards. Before such a costly randomized trial can be initiated, however, we must demonstrate that FGF23 excess is an independent risk factor for mortality in normophosphataemic pre-dialysis CKD patients and that FGF23 levels can be successfully lowered in those patients. The results of the current pilot study and a previous single intervention study using phosphorus binders in which FePi decreased significantly but remained higher than in healthy volunteers [14] suggest that future studies should incorporate a double intervention of phosphorus binders and dietary restriction for a prolonged duration of longitudinal follow-up.

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Conflict of interest statement. M.W. has served as a consultant or received honoraria from Abbott Laboratories, Amgen, Davita, Genzyme, Lutipold, Novartis, Mitsubishi and Shire. T.I. has received honoraria from Shire. H. J. reports holding an active patent on the C-terminal FGF23 assay manufactured by Immunotopics.

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