RENAL FAILURE
2021, VOL. 43, NO. 1, 141–148
https://doi.org/10.1080/0886022X.2020.1870138

Taylor & Francis
Taylor & Francis Group

CLINICAL STUDY

OPEN ACCESS Check for updates

Acute effects of dietary phosphorus intake on markers of mineral metabolism in hemodialysis patients: post hoc analysis of a randomized crossover trial

Wan-Chuan Tsai[a,b], Hon-Yen Wu[a,c,d,e], Yen-Ling Chiu[a,d,f], Ju-Yeh Yang[a,b,d], Mei-Fen Pai[a,d], Yong-Ru Wu[g], Wan-Yu Lin[c], Kuan-Yu Hung[d], Kuo-Liong Chien[c,d], Shih-Ping Hsu[a,d]* and Yu-Sen Peng[a,d,h,i]*

[a]Division of Nephrology, Department of Internal Medicine, Far Eastern Memorial Hospital, New Taipei City, Taiwan; [b]Center for General Education, Lee-Ming Institute of Technology, New Taipei City, Taiwan; [c]Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei City, Taiwan; [d]Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei City, Taiwan; [e]Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei City, Taiwan; [f]Graduate Program in Biomedical Informatics, Yuan Ze University, Taoyuan City, Taiwan; [g]Dietary Department, Far Eastern Memorial Hospital, New Taipei City, Taiwan; [h]Department of Applied Cosmetology, Lee-Ming Institute of Technology, New Taipei City, Taiwan; [i]Department of Healthcare Administration, Oriental Institute of Technology, New Taipei City, Taiwan

ABSTRACT

Background: Long-term dietary phosphorus excess influences disturbances in mineral metabolism, but it is unclear how rapidly the mineral metabolism responds to short-term dietary change in dialysis populations.

Methods: This was a post hoc analysis of a randomized crossover trial that evaluated the short-term effects of low-phosphorus diets on mineral parameters in hemodialysis patients. Within a 9-day period, we obtained a total of 4 repeated measurements for each participant regarding dietary intake parameters, including calorie, phosphorus, and calcium intake, and markers of mineral metabolism, including phosphate, calcium, intact parathyroid hormone (iPTH), intact fibroblast growth factor 23 (iFGF23), and C-terminal fibroblast growth factor 23 (cFGF23). The correlations between dietary phosphorus intake and serum mineral parameters were assessed by using mixed-effects models.

Results: Thirty-four patients were analyzed. In the fully adjusted model, we found that an increase in dietary phosphorus intake of 100 mg was associated with an increase in serum phosphate of 0.3 mg/dL (95% confidence intervals [CI], 0.2–0.4, $p < .001$), a decrease in serum calcium of 0.06 mg/dL (95% CI, −0.11 to −0.01, $p = .01$), an increase in iPTH of 5.4% (95% CI, 1.4–9.3, $p = .01$), and an increase in iFGF23 of 5.0% (95% CI, 2.0–8.0, $p = .001$). Dietary phosphorus intake was not related to cFGF23.

Conclusions: Increased dietary phosphorus intake acutely increases serum phosphate, iPTH, and iFGF23 levels and decreases serum calcium levels, highlighting the important role of daily fluctuations of dietary habits in disturbed mineral homeostasis in hemodialysis patients.

ARTICLE HISTORY
Received 30 September 2020
Revised 20 November 2020
Accepted 22 December 2020

KEYWORDS
Acute; dietary phosphorus; FGF23; hemodialysis; phosphate; PTH

Introduction

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is very common in patients with end-stage kidney disease (ESKD), and emerging evidence suggests that mineral metabolism disorders, including hyperphosphatemia, fibroblast growth factor 23 (FGF23) excess, and secondary hyperparathyroidism, contribute to the high rates of adverse cardiovascular outcomes and premature death [1–4]. Phosphate retention plays an important role in the development of CKD-MBD [5,6]. In addition to adequate dialysis and pharmaceutical treatments, dietary phosphorus restriction has long been a cornerstone of therapy and is a recommended way to ameliorate phosphate retention [7–9]. Animal studies have demonstrated that dietary phosphorus regulates mineral metabolism markers [10–12]. In addition, in human studies, dietary phosphorus has been found to acutely change mineral parameters [13,14]. Previous clinical studies assessing the relationship

CONTACT Yu-Sen Peng taan70@gmail.com; Shih-Ping Hsu shihping999@yahoo.com.tw Division of Nephrology, Department of Internal Medicine, Far Eastern Memorial Hospital, No. 21, Section 2, Nanya South Rd, Banciao District, New Taipei City 220, New Taipei City, Taiwan
*Dr. Yu-Sen Peng and Dr. Shih-Ping Hsu contributed equally to this work.

between dietary phosphorus restriction and CKD-MBD marker levels have focused on non-dialysis populations [15,16]. There is scant evidence to suggest that dietary phosphorus restriction positively affects CKD-MBD markers in patients with CKD [17]. For hemodialysis patients, who have limited compensatory mechanisms to maintain normal phosphate levels and thus have high hyperphosphatemia rates and elevated FGF23 levels, little is known about the association between acute changes in dietary phosphorus and changes in these mineral markers, especially FGF23 [15].

In a previous randomized active-controlled crossover trial conducted on ESKD patients receiving hemodialysis, we found no benefit of a 2-day dietary intervention with a very-low-phosphorus diet (8 mg/g phosphorus-to-protein ratio, PPR) compared with a low-phosphorus diet (10 mg/g PPR) on the outcome of an FGF23 change from baseline [18,19]. However, serum intact FGF23 levels were lower than the baseline in both diet groups. In addition, a greater reduction in serum phosphate level and a greater increase in serum calcium level were found in the 8 mg/g diet group than in the 10 mg/g diet group. Based on these observations, we hypothesized that elevated dietary phosphorus intake acutely and negatively affects markers of mineral metabolism. For clinical application, there is concerned that day-to-day variations in nutritional intake may not be captured by current dialysis practice, which adopted monthly assessment of laboratory measures, and these snapshots of laboratory values are potentially misinterpreted and occasionally of questionable relevance [20]. In order to examine the relationship between dietary phosphorus intake and serum mineral parameters, a *post hoc* analysis was conducted on existing data from our previous study to evaluate the short-term effects of dietary phosphorus intake on markers of mineral metabolism in patients with ESKD undergoing hemodialysis.

Methods

Study design

Figure 1 shows the study design and outcome assessments. We performed a *post hoc* analysis of a randomized active-controlled crossover single-center clinical trial in which we randomly assigned participants (at an allocation ratio of 1:1) to receive a very-low-phosphorus diet with a PPR of 8 mg/g or a low-phosphorus diet with a PPR of 10 mg/g [18]. Within each 9-day period, each patient consumed each study diet regimen for 2 days; the order of the diets was random, and the two diets were separated by a 5-day washout period. The study protocol, sample size calculation method, and primary outcomes have previously been published in detail [18]. The original study was approved by the institutional review board at Far Eastern Memorial Hospital (FEMH-106108-F) and was registered online before study initiation (ClinicalTrials.gov ID = NCT03367338).

Study population

Subjects with (1) ages greater than 20 years, (2) ESKD and a history of thrice-weekly hemodialysis for more than three months, (3) adequate dialysis (urea reduction ratios [URRs] equal to or greater than 65%), (4) most recent serum phosphate levels greater than 5.5 mg/dL or between 3.5 and 5.5 mg/dL with regular phosphate binder use, (5) serum intact parathyroid

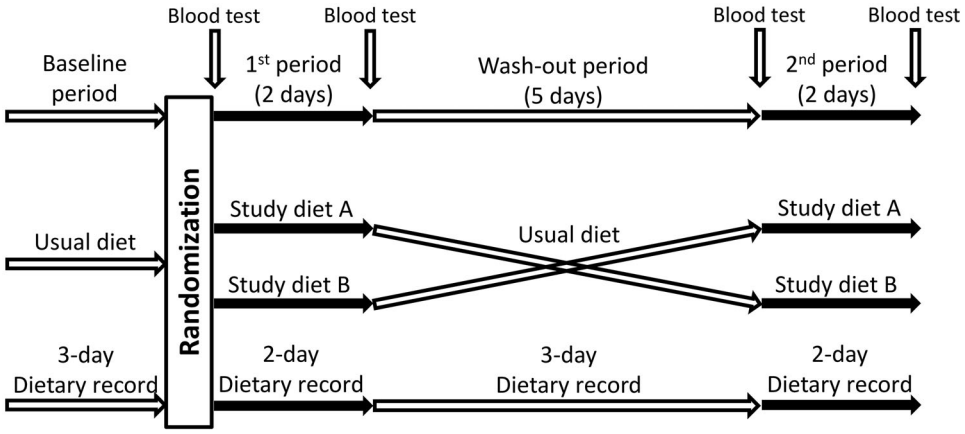

Figure 1. Study design and outcome assessments. Participants were randomly assigned to receive study diet A (with a phosphorus-to-protein ratio [PPR] of 8 mg/g) or study diet B (with a PPR of 10 mg/g) during 2-day study periods separated by a 5-day washout period. A total of 4 repeated measurements for the study outcomes were attained before and after each study period. Before each study phase, each participant kept a 3-day dietary record to enable estimation of the nutrient content of his or her usual diet. During the study periods, dietary compliance was assessed by evaluation of 2-day dietary records.

hormone (iPTH) levels less than 800 pg/mL, and (6) dry weights between 42.5 kg and 67.5 kg were included in the study. The exclusion criteria included serum albumin levels less than 2.5 g/dL, hospitalization within the past 4 weeks, psychiatric disorders, mental retardation, dislike of the study meals and poor dietary adherence. All participants provided written informed consent. We enrolled 35 patients, of whom 34 completed the first study period and 29 completed the second study period, contributing the relevant data for the present analysis.

Dietary assessment

The daily dietary intake of participants was estimated at baseline and during the study periods. The dietitians educated the participants and instructed them to complete standard daily food-recording forms with entries on the meal time, meal type, brand of food, amount of food in standard measuring units, preparation style (homemade or not), and recipe. Before each study period, the participants prospectively maintained a dietary record of their daily intake for three days, including a dialysis weekday, a non-dialysis weekday and a non-dialysis weekend day, allowing us to estimate the nutrient content of their usual diet as well as their dietary compliance.

As described in our previous study [18], the nutrient compositions of the study diets were chemically measured and used by dietitians to calculate the daily nutrient intake of the participants during the study periods. In addition, the participants maintained a 2-day dietary record of their consumption of portions of the assigned study diets and of foods outside of the study diets during the study periods. The completeness, consistency, and clarity of the food diaries were reviewed by the dietitians.

Clinical data collection

The following demographic and clinical data were recorded: age; sex; dry weight; body mass index; duration of dialysis therapy; history of parathyroidectomy; interdialytic weight gain; dialysis unit blood pressure; type of arteriovenous shunt; dialysate calcium concentration; URR; hemoglobin; ferritin; alkaline phosphatase; albumin; 25-hydroxyvitamin D; and amounts, frequencies, and types of medications, including phosphate-binding agents and vitamin D analogs. The phosphate binder doses among study participants were compared by calculating the phosphate-binding equivalent dose as described by Daugirdas [21].

Biochemical assessment

Venous blood samples were taken under nonfasting conditions prior to the dialysis session at the beginning and end of each study period. Standard assays for serum phosphate and calcium were performed using automated analyzers. iPTH (reference range 8–76 pg/mL) was analyzed in serum using an immunoradiometric assay (ELSA-PTH, Cisbio Bioassays, France); the intra- and interassay coefficients of variation (CVs) ranged from 2.1 to 7.5% and from 2.7 to 6.8%, respectively. 25-Hydroxyvitamin D (reference range 5.3–47 ng/mL) was analyzed in serum using an electrochemiluminescence immunoassay analyzer (ECLIA) (Roche Diagnostics GmbH, Germany); the intra- and interassay CVs ranged from 2.2 to 6.8% and from 3.4 to 13.1%, respectively. In light of the absence of any standardized commercial FGF23 assays, we simultaneously performed two available FGF23 assays: an assay for intact FGF23 (iFGF23) and an assay for C-terminal fragments of FGF23 (cFGF23). iFGF23 was assessed in serum using an enzyme-linked immunosorbent assay (ELISA) (Kainos Laboratories, Tokyo, Japan); the intra- and interassay CVs ranged from 2.0 to 3.0% and from 2.1 to 3.8%, respectively. cFGF23 was assessed in EDTA-plasma using a sandwich ELISA (Immutopics, San Clemente, CA) according to the manufacturer's instructions; the intra- and interassay CVs ranged from 1.4 to 2.4% and from 2.4 to 4.7%, respectively. Each sample was run in duplicate, and the mean values are presented.

Statistical analysis

Analyses were conducted on data from 34 individuals in the first study period and 29 in the second study period for whom samples were available for dietary assessment and measurement of mineral parameters. To evaluate the relationship between dietary phosphorus intake and the levels of mineral markers, including serum phosphate, calcium, iPTH, iFGF23 and cFGF23, we employed separate mixed-effects models, which allowed us to use a total of 4 repeated measurements for each of the outcome variables of interest. In each mixed-effects model, the dependent variable was one of the markers of mineral metabolism, the participant was included as a random effect, and the main independent variable was dietary phosphorus intake of 100 mg. In contrast to serum phosphate and calcium, which were normally distributed, iPTH, iFGF23 and cFGF23 were non-normally distributed and were thus log-transformed before being entered into the model, and the estimated means and 95% confidence intervals

(CIs) are presented as percentage changes, as suggested by Benoit [22].

For each of the outcome analyses, we used 4 types of models based on the level of multivariate adjustment: model 1 included dietary phosphorus as the only independent variable; model 2 included the additional covariates of age, sex, body mass index, and randomized group; model 3 included the additional covariates of baseline vitamin D, baseline iPTH, baseline URR, and phosphate-binding equivalent dose; and model 4 included all of the abovementioned covariates plus 2 dietary record data items: total calorie intake and dietary calcium intake. A two-sided p value of less than .05 was considered to indicate statistical significance. All analyses were performed with SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics

Table 1 presents the baseline characteristics of the 34 participants included in the present *post hoc* analysis. The mean age of the participants was 64 ± 7 years, 38% of the participants were men, and the mean dialysis vintage was 10 ± 7 years. The mean dry weight was 55 ± 7 kg, and the interdialytic weight gain was 2.1 (1.7, 2.6) kg. Approximately 90% of participants had arteriovenous fistulae, and approximately 60% used low dialysate calcium.

Table 1. Baseline demographic and clinical characteristics.

Characteristic	($n = 34$)
Age (years)	64 ± 7
Male sex, *n* (%)	13 (38)
Vintage (years)	10 ± 7
Dry weight (kg)	55 ± 7
Body mass index (kg/m^2)	22 ± 2
Interdialytic weight gain (kg)	2.2 (1.7, 2.6)
Systolic BP (mm Hg)	134 ± 30
Diastolic BP (mm Hg)	71 ± 15
Use of AVF, *n* (%)	30 (88)
Low dialysate calcium[a], *n* (%)	20 (59)
Use of iron agent, *n* (%)	10 (29)
Phosphate-binding equivalent dose (g/day)	3.1 (2.0, 4.9)
Use of vitamin D analogs, *n* (%)	15 (44)
Parathyroidectomy, *n* (%)	14 (41)
Urea reduction ratio (%)	74 ± 4
Ferritin (ng/mL)	360 (216, 537)
Hemoglobin (g/dL)	11.4 ± 1.1
Albumin (g/dL)	4.0 ± 0.4
Alkaline phosphatase (IU/L)	80 (63, 99)
25OHVitD (ng/mL)	30 (23, 40)
Phosphate (mg/dL)	5.2 ± 1.1
Calcium (mg/dL)	9.3 ± 0.6
iPTH (pg/mL)	150 (78, 341)
iFGF23 (pg/mL)	2996 (506, 9821)
cFGF23 (RU/mL)	5750 (1698, 12539)

25OHVitD: 25-hydroxy vitamin D; AVF: arteriovenous fistula; BP: blood pressure; cFGF23: C-terminal fibroblast growth factor 23; iFGF23: intact fibroblast growth factor 23; iPTH: intact parathyroid hormone. Continuous data are shown as means (±SDs) or median (1st and 3rd quartiles), and categorical variables as counts and percentages.
[a]Low dialysate calcium means a dialysate calcium concentration of ≤ 2.5 mEq/L.

Changes in dietary record data and changes in markers of mineral metabolism

Table 2 describes the dietary record data and the levels of mineral markers at baseline and at the end of each study period. At baseline, the mean total calorie intake of the participants was 1558 ± 295 kcal per day, the mean dietary phosphorus intake was 763 ± 289 mg per day, and the mean dietary calcium intake was 284 ± 128 mg per day. Prior to dietary intervention, the mean serum phosphate was 5.0 ± 1.1 mg/dL, the mean serum calcium was 9.2 ± 0.7 mg/dL, the median iPTH was 124 (65, 325) pg/mL, the median iFGF23 was 2996 (506, 9821) pg/mL, and the median cFGF23 was 5750 (1698, 12539) RU/mL. Compared with the baseline data, dietary phosphorus intake decreased and dietary calcium intake increased during the study periods. The participants exhibited reductions in serum phosphate, iPTH and iFGF23 levels and increases in calcium levels after each of the dietary interventions, whereas they exhibited progressive decreases in cFGF23 levels throughout the 9-day study period.

Relationships between dietary phosphorus intake and markers of mineral metabolism

Table 3 demonstrates the relationships between dietary phosphorus intake and markers of mineral metabolism. Higher dietary phosphorus intake was strongly correlated with a higher serum phosphate level in models 1 (the univariate model) through 4 (the fully adjusted model); multivariate adjustment did not mitigate the positive association between dietary phosphorus intake and serum phosphate level. Every increase in dietary phosphorus intake of 100 mg was significantly related to an increase in serum phosphate level of 0.28 mg/dL (95% CI, 0.21–0.35, $p < .001$). In contrast, there was an inverse correlation between dietary phosphorus intake and serum calcium level; an increase in dietary phosphorus intake of 100 mg was significantly related to a decrease in serum calcium level of 0.06 mg/dL (95% CI, −0.11 to −0.01, $p = .01$) in the fully adjusted model.

For log-transformed variables including iPTH, iFGF23 and cFGF23, the estimates are presented as percentage changes for every 100 mg increase in dietary phosphorus intake. The iPTH level increased in response to elevated dietary phosphorus intake; in the fully adjusted model, an increase in dietary phosphorus

Table 2. Changes in dietary and serum mineral parameters in the study population.

	Usual diet[a] (baseline) $n = 34$	After first study period[b] (2 days after baseline measurement) $n = 34$	Usual diet[a] (7 days after baseline measurement) $n = 29$	After second study period[b] (9 days after baseline measurement) $n = 29$
Dietary parameters				
Calories (kcal)	1558 ± 295	1590 ± 327	1525 ± 359	1574 ± 350
Phosphorus (mg)	763 ± 289	602 ± 120	671 ± 185	618 ± 156
Calcium (mg)	284 ± 128	382 ± 86	227 ± 93	386 ± 106
Markers of mineral metabolism				
Phosphate (mg/dL)	5.0 ± 1.1	4.3 ± 1.1	4.7 ± 0.9	4.0 ± 0.9
Calcium (mg/dL)	9.2 ± 0.7	9.3 ± 0.7	9.3 ± 0.6	9.6 ± 0.6
iPTH (pg/mL)	124 (65, 325)	105 (63, 262)	105 (75, 296)	83 (65, 280)
iFGF23 (pg/mL)	2996 (506, 9821)	1915 (484, 8588)	2345 (496, 8540)	1776 (614, 6875)
cFGF23 (RU/mL)	5750 (1698, 12539)	5485 (1984, 13488)	4737 (1550, 6197)	2680 (1140, 5741)

cFGF23: C-terminal fibroblast growth factor 23; iFGF23: intact fibroblast growth factor 23; iPTH: intact parathyroid hormone.
[a]We calculated a 3-day average value for the estimated daily dietary intake before the study.
[b]We calculated a 2-day average value for the estimated dietary intake during the study period.

Table 3. Associations between an increase in dietary phosphorus intake of 100 mg and changes in serum mineral parameters.

Outcome variables	Phosphate (mg/dL)			Calcium (mg/dL)			iPTH (%)			iFGF23 (%)			cFGF23 (%)		
	Estimate	95% CI	*p*	Estimate	95% CI	*p*	Estimate	95% CI	*p*	Estimate	95% CI	*p*	Estimate	95% CI	*p*
Model 1[a]	0.19	0.12 to 0.25	<.001	−0.03	−0.07 to 0.01	.10	3.1	−0.3 to 6.5	.06	3.1	0.5 to 5.7	.02	4.3	0.2 to 8.4	.03
Model 2[b]	0.18	0.12 to 0.25	<.001	−0.03	−0.07 to 0.01	.11	3.0	−0.4 to 6.4	.08	3.1	0.5 to 5.7	.02	4.1	0.02 to 8.1	.04
Model 3[c]	0.19	0.12 to 0.25	<.001	−0.04	−0.08 to 0.002	.06	3.0	−0.4 to 6.4	.07	3.0	0.4 to 5.6	.02	3.8	−0.2 to 7.9	.06
Model 4[d]	0.28	0.21 to 0.35	<.001	−0.06	−0.11 to −0.01	.01	5.4	1.4 to 9.3	.01	5.0	2.0 to 8.0	.001	3.7	−1.2 to 8.6	.13

cFGF23: C-terminal fibroblast growth factor 23; CI: confidence intervals; iFGF23: intact fibroblast growth factor 23; iPTH: intact parathyroid hormone; URR: urea reduction ratio.
[a]Univariate model.
[b]The adjusted variables included age, sex, body mass index, and randomized group.
[c]The adjusted variables included age, sex, body mass index, randomized group, baseline vitamin D, baseline iPTH, baseline URR, and phosphate-binding equivalent dose.
[d]The adjusted variables included age, sex, body mass index, randomized group, baseline vitamin D, baseline iPTH, baseline URR, phosphate-binding equivalent dose, total calorie intake, and dietary calcium intake.

intake of 100 mg was significantly correlated with an increase in the iPTH level of 5.4% (95% CI, 1.4–9.3, $p = .01$). Similarly, the iFGF23 level increased as dietary phosphorus intake increased; there was a significant association in both the unadjusted and adjusted models. An increase in dietary phosphorus intake of 100 mg was significantly related to an increase in iFGF23 level of 5.0% (95% CI, 2.0–8.0, $p = .001$). In contrast to the levels of the abovementioned mineral markers, the cFGF23 level did not change in response to variations in dietary phosphorus intake.

Discussion

Although there is considerable evidence for non-dialysis patients with CKD, this is, to our knowledge, the first study to describe acute variations in biomarkers of mineral metabolism (including FGF23, as measured with iFGF23 and cFGF23 assays) in response to variations in dietary phosphorus intake in hemodialysis patients. We conducted a *post hoc* analysis on existing data from our previous randomized crossover trial to investigate the acute responses of mineral metabolism markers to dietary phosphorus changes. A total of 4 repeated measurements of dietary and biochemical data obtained during a 9-day period were used, and mixed-effects models indicated that acute variations in 4 mineral parameters occurred in response to variations in dietary phosphorus intake. Specifically, dietary phosphorus intake was positively associated with serum phosphate, iPTH and iFGF23 levels and inversely associated with serum calcium levels. These findings support the evidence that dietary phosphorus intake rapidly and positively affects CKD-MBD markers, highlighting the important role of daily fluctuations in dietary habits in the clinical management of CKD-MBD in dialysis populations.

Our study showed that acute changes, i.e., within days, in dietary phosphorus intake led to corresponding changes in markers of mineral metabolism. Within a 9-day period, we obtained a total of 4 repeated measurements for each participant regarding dietary and biochemical data. We demonstrated that even a small change in dietary phosphorus intake, i.e., 100 mg, caused a significant change in markers of mineral metabolism within days. Including mainly natural food sources and higher percentage of plant-derived protein, low-phosphorus diets used in our study facilitated

acute change in markers of mineral metabolism. These hyper-acute changes in mineral metabolism reflect the day-to-day fluctuations of dietary phosphorus intake. Such diet-induced changes to markers of mineral metabolism may not be completely detected by the monthly surveillance of laboratory-based measures, which is the current clinical practice of dialysis. As stated in the recent review by Tibor Fülöp and his colleagues [20], nephrologists have been largely relied on objective laboratory measures that are performed on a monthly basis, and these laboratory measures, such as pre-dialysis serum phosphorus and calcium, provide a snapshot view that may not adequately capture the potentially large daily fluctuations in dietary habits. Using serum concentrations of clinical mineral parameters assumes a chronic steady state, but it is hardly true in the dialysis population because these laboratory parameters are both largely and rapidly dependent on variations in daily diet and hemodynamic instabilities during dialysis procedure. Tibor Fülöp and his colleagues [20] also pointed out that assessment of the individual patient's clinical status based on snapshots of laboratory values are often misinterpreted and occasionally of questionable relevance. Thus, monthly snapshots of mineral parameters may not adequately characterize fluctuations in dietary intake, and cautions should be taken when interpreting these results.

There are limited data concerning the association of dietary phosphorus intake with FGF23 in dialysis populations. Our systematic review and meta-analysis [15] of the currently available randomized clinical trials has demonstrated that (1) low-phosphorus diets tend to lower FGF23 levels, (2) the FGF23-lowering effects are more prominent when measured with the iFGF23 assays, and (3) no studies have included dialysis patients. In an attempt to fill this knowledge gap, we included only hemodialysis patients in a randomized crossover clinical trial comparing the FGF23-lowering effects of two low-phosphorus diets: a diet with a PPR of 8 mg/g and a diet with a PPR of 10 mg/g [18]. Notably, both low-phosphorus diets lowered the mean serum iFGF23 level by approximately 1000 pg/mL (a decrease of 18%–19%) after only 2 days, and cFGF23 was unaffected. In the current study, which pooled data from both low-phosphorus diets regarding FGF23-lowering effects, we found that every increase in dietary phosphorus intake of 100 mg was significantly associated with an increase in iFGF23 of 5% and had no effect on cFGF23 level. In accordance with the results from our systematic review and meta-analysis [15], this finding supports the involvement of a regulatory pathway in diet-mediated phosphate control in which dietary phosphorus regulates iFGF23 levels without concomitantly changing cFGF23 levels in patients with CKD.

Even though low-phosphorus diets had no effect on cFGF23 within 2 days, it is noteworthy that the study participants exhibited progressive declines in median cFGF23 levels after 7 days (baseline cFGF23, 5750 [1698, 12539] vs. those after 7 days, 4737 [1550, 6197], $p = .0002$; baseline cFGF23 vs. those after 9 days, 2680 [1140, 5741], $p < .0001$) independent of the study diet interventions during the 9-day study period. As stated in a recent review by Daniel Edmonston and Myles Wolf [23], the results of iFGF23 and cFGF23 assays can be totally different depending on the balance between production and cleavage of FGF23. We did observe lags in cFGF23 level changes in response to variations in dietary phosphorus intake. Based on this observation, we hypothesize that catabolism or removal of cFGF23 from the circulation may take a few days or perhaps a week in hemodialysis patients. Future studies should be undertaken to investigate the effect of dietary phosphorus intake on FGF23 catabolism in dialysis populations.

Similar to previous studies [24,25], our study showed that dietary phosphorus intake was well correlated with serum phosphate, calcium and iPTH levels. The associations were independent of baseline iPTH levels, dialysis adequacy, phosphate binder dosages, and dietary parameters including total calorie intake and dietary calcium intake, as we adjusted for these factors in the mixed-effects models. Contrary to previous studies that have assessed the long-term effects of phosphorus restriction on mineral parameters in non-dialysis populations [24,25], our study assessed short-term changes in hemodialysis patients. We obtained 4 repeated measurements of parameters within 9 days in a dialysis population with a vintage of 10 years and found significant associations between dietary phosphorus intake and mineral parameters. Of note, in a previous study enrolling non-dialysis patients [26], short-term (5-day) dietary intervention did not alter serum phosphate and calcium levels. Owing to the limited compensatory mechanisms for maintenance of serum phosphate levels in hemodialysis patients, the effects of dietary phosphorus intake on mineral metabolism markers are more rapid and stronger in these patients than in non-dialysis patients with CKD. As previously reported [27–29], we found that elevated dietary phosphorus intake acutely, directly and strongly increased serum phosphate levels and reciprocally decreased serum calcium levels. In addition to increased dietary phosphorus intake, which directly increased iPTH levels, both the resulting increases in serum phosphate levels and the simultaneous

decreases in serum calcium levels increased iPTH levels [30,31] .

Our study has a few limitations that deserve mentioning. First, the results of our study should be considered hypothesis-generating, because we performed a *post hoc* analysis of our previous randomized crossover trial. Second, our study included only hemodialysis patients with a mean vintage of 10 years and high FGF23 levels; caution should be taken when extrapolating our findings to different populations. Third, the small number of participants limited our ability to analyze all of the factors that may have been associated with dietary phosphorus intake or influenced the changes in the mineral parameters. However, this limitation was partially compensated for by the use of mixed-effects models, in which participants act as their own controls in a crossover design, and by the use of 4 repeated measurements of the changes in each individual over time. Finally, the short duration of this study did not allow us to study potential effects on clinical outcomes. Our study was able to evaluate only diet-mediated changes in biomarkers of mineral metabolism, which are considered intermediate outcomes.

In conclusion, dietary phosphorus acutely regulates CKD-MBD markers in hemodialysis patients. Serum phosphate, iPTH, and iFGF23 levels increase and serum calcium levels decrease in response to elevated dietary phosphorus intake.

Acknowledgments

We would like to thank Shu-Min Tsai, the former director of the Dietary Department of Far Eastern Memorial Hospital for gathering the dietary data and helping to conduct the original study.

The funders had no role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; the preparation, writing, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

Authors' contributions

Research idea and study design: WCT, HYW, YLC, SPH and YSP; data acquisition: WCT, YRW, MFP, and KLC; data analysis/interpretation: WCT, HYW, YLC, MFP, KYH, KLC, SPH and YSP; statistical analysis: WCT, HYW, JYY, YRW, and WYL; supervision or mentorship: WYL, KYH, KLC, SPH, and YSP. Each author contributed important intellectual content during manuscript drafting or revision and agreed to be held accountable for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by research grants to Dr. Wan-Chuan Tsai from Far Eastern Memorial Hospital, New Taipei City, Taiwan [FEMH-2020-C-018, FEMH-2019-C-031 and FEMH-2018-C-014].

References

[1] Block GA, Klassen PS, Lazarus JM, et al. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. J Am Soc Nephrol. 2004;15(8):2208–2218.

[2] Slinin Y, Foley RN, Collins AJ. Calcium, phosphorus, parathyroid hormone, and cardiovascular disease in hemodialysis patients: the USRDS waves 1, 3, and 4 study. J Am Soc Nephrol. 2005;16(6):1788–1793.

[3] Isakova T, Xie H, Yang W, et al. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. JAMA. 2011;305(23):2432–2439.

[4] Scialla JJ, Xie H, Rahman M, et al. Fibroblast growth factor-23 and cardiovascular events in CKD. J Am Soc Nephrol. 2014;25(2):349–360.

[5] Bricker NS. On the pathogenesis of the uremic state. An exposition of the "trade-off hypothesis". N Engl J Med. 1972;286(20):1093–1099.

[6] Moe S, Drueke T, Cunningham J, et al. Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 2006;69(11):1945–1953.

[7] Tsai WC, Yang JY, Luan CC, et al. Additional benefit of dietitian involvement in dialysis staffs-led diet education on uncontrolled hyperphosphatemia in hemodialysis patients. Clin Exp Nephrol. 2016;20(5):815–821.

[8] Tsai WC, Peng YS, Wu HY, et al. Accuracy of a nutrient database in estimating the dietary phosphorus-to-protein ratio and using a boiling method in low-phosphate hospital diets. Sci Rep. 2018;8(1):15246.

[9] Slatopolsky E, Bricker NS. The role of phosphorus restriction in the prevention of secondary hyperparathyroidism in chronic renal disease. Kidney Int. 1973;4(2):141–145.

[10] Grabner A, Amaral AP, Schramm K, et al. Activation of cardiac fibroblast growth factor receptor 4 causes left ventricular hypertrophy. Cell Metab. 2015;22(6):1020–1032.

[11] Finch JL, Lee DH, Liapis H, et al. Phosphate restriction significantly reduces mortality in uremic rats with established vascular calcification. Kidney Int. 2013;84(6):1145–1153.

[12] Thomas L, Bettoni C, Knopfel T, et al. Acute adaption to oral or intravenous phosphate requires parathyroid hormone. J Am Soc Nephrol. 2017;28(3):903–914.

[13] Scanni R, vonRotz M, Jehle S, et al. The human response to acute enteral and parenteral phosphate loads. J Am Soc Nephrol. 2014;25(12):2730–2739.
[14] Burnett SM, Gunawardene SC, Bringhurst FR, et al. Regulation of C-terminal and intact FGF-23 by dietary phosphate in men and women. J Bone Miner Res. 2006;21(8):1187–1196.
[15] Tsai WC, Wu HY, Peng YS, et al. Effects of lower versus higher phosphate diets on fibroblast growth factor-23 levels in patients with chronic kidney disease: a systematic review and meta-analysis. Nephrol Dial Transplant. 2018;33(11):1977–1983.
[16] Isakova T, Gutierrez OM, Smith K, et al. Pilot study of dietary phosphorus restriction and phosphorus binders to target fibroblast growth factor 23 in patients with chronic kidney disease. Nephrol Dial Transplant. 2011;26(2):584–591.
[17] Liu Z, Su G, Guo X, et al. Dietary interventions for mineral and bone disorder in people with chronic kidney disease. Cochrane Database Syst Rev. 2015;(9):Cd010350.
[18] Tsai WC, Wu HY, Peng YS, et al. Short-term effects of very-low-phosphate and low-phosphate diets on fibroblast growth factor 23 in hemodialysis patients: a randomized crossover trial. Clin J Am Soc Nephrol. 2019;14(10):1475–1483.
[19] Hill Gallant KM. Dietary phosphorus and FGF23: is more restriction better? Clin J Am Soc Nephrol. 2019;14(10):1424–1426.
[20] Zsom L, Zsom M, Abdul Salim S, et al. Subjective global assessment of nutrition, dialysis quality, and the theory of the scientific method in Nephrology practice. Artif Organs. 2020;44(10):1021–1030.
[21] Daugirdas JT, Finn WF, Emmett M, et al. The phosphate binder equivalent dose. Semin Dial. 2011;24(1):41–49.
[22] Benoit K. Linear regression models with logarithmic transformations. Methodology Institute, London School of Economics; March 17, 2011; [cited 2020 Mar 10]. Available from:https://kenbenoit.net/assets/courses/ME104/logmodels2.pdf.
[23] Edmonston D, Wolf M. FGF23 at the crossroads of phosphate, iron economy and erythropoiesis. Nat Rev Nephrol. 2020;16(1):7–19.
[24] Combe C, Morel D, de Precigout V, et al. Long-term control of hyperparathyroidism in advanced renal failure by low-phosphorus low-protein diet supplemented with calcium (without changes in plasma calcitriol). Nephron. 1995;70(3):287–295.
[25] Combe C, Aparicio M. Phosphorus and protein restriction and parathyroid function in chronic renal failure. Kidney Int. 1994;46(5):1381–1386.
[26] Portale AA, Booth BE, Halloran BP, et al. Effect of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. J Clin Invest. 1984;73(6):1580–1589.
[27] Reiss E, Canterbury JM, Bercovitz MA, et al. The role of phosphate in the secretion of parathyroid hormone in man. J Clin Invest. 1970;49(11):2146–2149.
[28] Slatopolsky E, Caglar S, Gradowska L, et al. On the prevention of secondary hyperparathyroidism in experimental chronic renal disease using "proportional reduction" of dietary phosphorus intake. Kidney Int. 1972;2(3):147–151.
[29] Karkkainen M, Lamberg-Allardt C. An acute intake of phosphate increases parathyroid hormone secretion and inhibits bone formation in young women. J Bone Miner Res. 1996;11(12):1905–1912.
[30] de Francisco AL, Cobo MA, Setien MA, et al. Effect of serum phosphate on parathyroid hormone secretion during hemodialysis. Kidney Int. 1998;54(6):2140–2145.
[31] Slatopolsky E, Finch J, Denda M, et al. Phosphorus restriction prevents parathyroid gland growth. High phosphorus directly stimulates PTH secretion *in vitro*. J Clin Invest. 1996;97(11):2534–2540.