Supplemental Material

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Supplemental Table 3. Assessment of Carryover Effects and Period Effects by Mixed Effects Models

This supplemental material has been provided by the authors to give readers additional information about their work.

Supplemental Appendix 1.

Study Protocol

Short-Term Effects of Very-Low-Phosphate and Low-Phosphate Diets on Fibroblast Growth Factor 23 in Hemodialysis Patients: A Randomized Crossover Trial

Contents for Study Protocol

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Background and Rationale

In patients with hemodialysis, the prevalence of cardiovascular disease (CVD) is high, and is the leading cause of death.[1, 2] Among several cardiovascular risk factors of hemodialysis patients, elevated fibroblast growth factor 23 (FGF23) level is common, and plays major role in the development of CVD with independent pathophysiologic mechanisms.[3, 4]

Evidence from animal studies demonstrated that low-phosphate diet reduced FGF23 level.[5] Clinical trials assessing the effect of dietary phosphate restriction on FGF23 focused on non-dialysis population.[6, 7] However, little is known about the effect of low-phosphate diet on FGF23 in hemodialysis patients who have higher prevalence of hyperphosphatemia and severely elevated FGF23 level.

Based on evidence from observational studies of nondialysis population, the Kidney Disease Outcomes Quality Initiative (K/DOQI) clinical practice guidelines have recommended that dietary phosphate intake should be restricted to 800-1000 mg/day (adjusted for dietary protein needs) when serum phosphate levels are greater than 5.5 mg/dL in those with kidney failure.[8] To limit dietary phosphate intake while ensuring adequate protein intake, the K/DOQI guidelines recommend a low-phosphate diet with phosphate-to-protein ratio (PPR) of 10-12 mg/g. For hemodialysis population, the optimal amount of dietary phosphate restriction has not been determined.

Purpose

The aims of this study are to evaluate the effect of low-phosphate diet on FGF23 level and to determine the optimal amount of dietary phosphate restriction in hemodialysis patients. In particular, we will compare the FGF23- and phosphate-lowering effects of a very-low-phosphate diet (PPR value of 8 mg/g) with those of a low-phosphate diet

(PPR value of 10 mg/g) in hemodialysis patients.

Study Population

Patients with maintenance hemodialysis, who meet the following inclusion criteria, but not violate the following exclusion criteria.

Study settings

The eligible participants will be recruited from a hemodialysis unit of a tertiary teaching hospital, in northern Taiwan.

Subject screening

Patients with maintenance hemodialysis who develop hyperphosphatemia will be screened by routine monthly laboratory surveillance system.

Subject inclusion criteria

Each patient must meet the following criteria to be enrolled in this study:

1) Aged older than 20 years
2) Having end-stage renal disease and having undergone thrice-weekly hemodialysis for more than three months
3) Having adequate dialysis (urea reduction ratio equal to or greater than 65%)
4) Having the most recent serum phosphate level greater than 5.5 mg/dL or between 3.5 and 5.5 mg/dL with regular phosphate binder use
5) Serum intact parathyroid hormone (PTH) level less than 800 pg/mL
6) In accordance with available study diets restricted to body sizes between 42.5 kg and 67.5 kg, we did not enroll participants with a dry weight outside of this range.

Subject exclusion criteria

Patients who meet any of the following criteria will be excluded from the study:

1) Serum albumin level less than 2.5 g/dL
2) Hospitalization within the past 4 weeks
3) History of psychiatric disorders
4) Having mental retardation
5) Those who dislike of the study meals
6) Soft diet requirement
7) Vegetarian
8) Poor dietary adherence

Characteristics and Preparation of Study Diets

Characteristics of study diets

1) A total of 20 highly consumed dishes will be selected from the hospital menu, comprising 2 fruit dishes, 4 grain-based dishes, 5 meat dishes, 4 side dishes, 4 vegetable dishes and 3 kind of nutritional supplements.
2) Since food additives include readily absorbable inorganic phosphorus, only natural food ingredients without any processed food or additive sources of phosphate will be chosen for both study diets.
3) All study food items have the following unique characteristics:
    A. composed of locally produced raw materials
    B. compliance with health and safety requirements
    C. compliance with national quality standards
4) The study diets are prepared according to the food hygiene practices of the

Hazard Analysis and Critical Control Points (HACCP) system at the hospital cafeteria.

5) Our hospital kitchen has received and renewed HACCP certification periodically from the Food and Drug Administration, Ministry of Health and Welfare, Taiwan.

6) To enhance nutrition and reduce phosphate amount and bioavailability, the study diets are designed to fulfill the following criteria[9]:

   A. adequate calories (≥ 30 kcal/kg/day)

   B. high protein (≥ 1.2 g/kg/day)

   C. low phosphate-to-protein ratio (≤ 10 mg/g)

   D. low phosphate content (≤ 800 mg/day)

   E. increased protein source of phosphate from plant in origin[10]

   F. meats boiled for 30 minutes before cooking.[11]

Methods for chemical analysis of dietary composition of the study meals

Prior to patient enrollment, the compositions of the study diets will be subjected to chemical analysis.

1) Following Association of Official Analytical Communities (AOAC) Official Method 984.27, phosphorus and calcium will be determined by inductively coupled plasma-optical emission spectrometer (ICP-OES) analysis with a detection limit of 0.1 mg/L.[12] In brief, the sample weights will be obtained, and the edible portions of samples will be ashed at high temperature and digested in nitric acid. Then, inductively coupled plasma will be used to determine the actual contents of phosphorus and calcium.

2) Following Taiwanese official methods, the study diets are analyzed for protein,[13]

fat,[14] saturated fat,[15] sugar,[16] moisture,[17] and ash.[7] Carbohydrates are calculated by the formula: 100 - (Protein + Fat + Moisture + Ash) (g/100 g). Calories are calculated by the formula: Protein (g) x 4 kcal + Fat (g) x 9 kcal + Carbohydrate (g) x 4 kcal.

Preparation of study diets

1) Based on the measured values of the food items, the dietitian will craft two kinds of low-phosphate diets: a very-low-phosphate diet, with a PPR value of 8 mg/g, equivalent to an intake of 10 mg phosphate per kg of body weight, and a low-phosphate diet, with a PPR value of 10 mg/g, equivalent to an intake of 12.6 mg phosphate per kg of body weight.
2) The two diets will be designed to have minimal variation between them in total calories, protein and calcium contents while differing in phosphate and PPR contents.
3) The study diet will be provided in 3 meals per day.
4) Breakfast includes steamed bread and nutritional supplements; lunch and dinner each include 1 meat item, 1 side dish, 1 vegetable item, 1 grain-based item and 1 fruit item.

Individualized diets for participants

1) To facilitate food preparation and supply, 5 different kinds of study diet, i.e., diets for body weights of 45 ± 2.5 kg, 50 ± 2.5 kg, 55 ± 2.5 kg, 60 ± 2.5 kg and 65 ± 2.5 kg, will be crafted to fulfill the requirements of body sizes from 42.5 kg to 67.5 kg.
2) For participants with dry weights between 95% and 115% of the ideal body

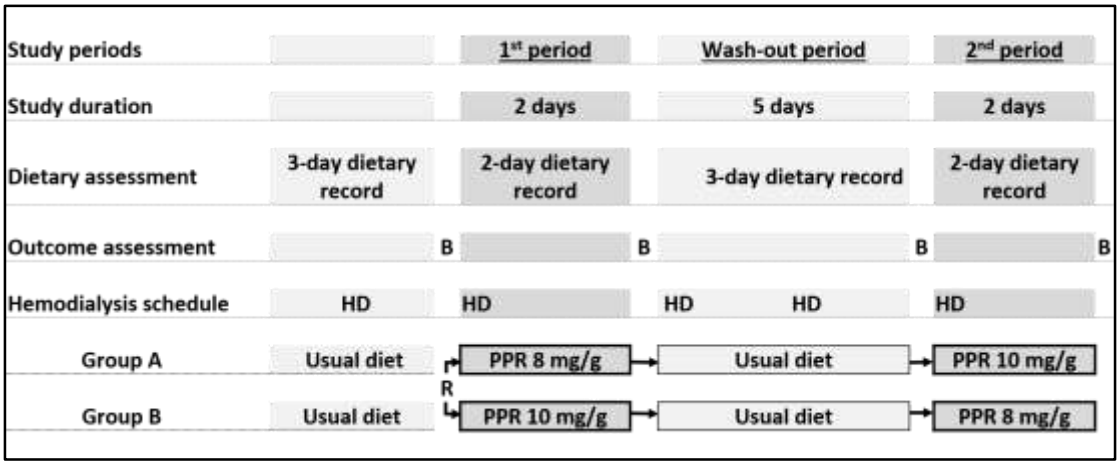
weight, nutritional prescription will be based on actual dry weight, and for those with dry weights outside this range, adjusted dry weight will be used to prescribe the energy and protein requirements.

3) We will use the formula [height (meter) x height (meter) x 22] to calculate ideal body weight. We modified the equation suggested by K/DOQI clinical guidelines to calculate adjusted dry weight (aDW) as follows: aDW = DW + [(IBW - DW) x 0.25], where DW is actual dry weight, and IBW is ideal body weight.

Study Design

1) As shown in the following Figure 1, it is to conduct a randomized, active-controlled trial with a crossover design.
2) With an allocation ratio of 1:1, the participants will be randomly assigned to two interventions.
3) Half of the participants will be assigned to receive a 2-day very-low-phosphate diet with a PPR of 8 mg/g, followed by a 2-day low-phosphate diet with a PPR of 10 mg/g, while the other half will receive the opposite order of study diets.
4) The timetable of the study protocol will be accommodated within the routine hemodialysis schedule.

Figure 1. Study design and outcome assessments.



Abbreviations: B, blood test; HD, hemodialysis; PPR, phosphate-to-protein ratio; R, randomization.

Approval by institutional review board (IRB)

The study was approved by the institutional review board at Far Eastern Memorial Hospital (FEMH-106108-F).

Online registration of the study

The study was registered online before study initiation (ClinicalTrials.gov ID = NCT03367338).

Study interventions

1) As shown in Figure 1, each participant will consume two low-phosphate diets over 3 meals a day.
2) Each diet will be consumed for 2 days, and diet order will be randomized.
3) Participants in group A will consume a very-low-phosphate diet, with a PPR of 8 mg/g, for 2 days, followed by a 5-day washout period in which they will adhere to their usual diets. Then, they will follow a 2-day low-phosphate diet, with a PPR of 10 mg/g.
4) Those in group B will consume the study diets in the opposite order.

5) No additional food will be allowed during each study period.

Type of dietary interventions

1) A very-low-phosphate diet, with a PPR value of 8 mg/g, equivalent to an intake of 10 mg phosphate per kg of body weight
2) A low-phosphate diet, with a PPR value of 10 mg/g, equivalent to an intake of 12.6 mg phosphate per kg of body weight.

Rationale for selecting dietary phosphate amount in two intervention arms

1) Based on the recommendations of K/DOQI clinical practice guidelines, the amount of dietary phosphate restriction should be less than 800 mg per day.
2) Among 5 trials including in our systematic review and meta-analysis, FGF23-lowering effects of low phosphate diets are significant in two studies.[10, 18]
3) The differences in dietary phosphate amount between low-phosphate diet and very-low-phosphate diet in these studies are approximately 200 mg per day.
4) Hence, we defined low-phosphate diet as phosphate content less than 800 mg per day, and very-low-phosphate diet as phosphate content less than 600 mg per day.
5) To restrict dietary phosphate amount while providing adequate protein amount, we chose foods by the metric PPR as suggested by the K/DOQI guidelines.
6) In this study, we ethically defined the low-phosphate diet with a PPR of 10 mg/g as the active comparator, which is the lower limit of 10-12 mg/g as suggested by K/DOQI guidelines, and that value is recommended for hemodialysis patients with hyperphosphatemia.[8]
7) To test our hypothesis that lowering dietary phosphate intake has a better FGF23-lowering effect, we crafted the very-low-phosphate diet with a PPR of 8 mg/g as

the experimental treatment.[19]

8) Taking together, we defined low-phosphate diet with phosphate content less than 600 mg/d, selecting foods with a PPR value of 8 mg/g and very-low-phosphate diet with phosphate content less than 800 mg/d, containing foods with a PPR value of 10 mg/g.

Duration of dietary interventions

There is evidence that changes to FGF23 levels through dietary modulation can occur within 2 days of changing diet.

1) Burnett *et al* showed that manipulation of dietary phosphate alters circulating FGF-23 levels within 48 h of the manipulation.[6]
2) Vervloet *et al* revealed that variation in dietary phosphate and calcium intake induces changes in FGF23.[20] In this study, these changes are detectable within a day after the change in the phosphate content of meals.
3) Patients with ESRD have a higher prevalence of hyperphosphatemia and elevated FGF23 levels. Therefore, changing diet may induce a significant change to FGF23 level within 2 days.
4) Based on the fact that FGF23 levels change after dietary phosphate intervention within 2 days, we defined 2-day duration as the intervention period.

Delivery of study meals

The delivery of study meals will be assimilated into the routine hemodialysis schedule.

1) During the intradialytic period, study meal boxes will be provided at the hemodialysis unit.

A. Participants with the morning shift (7 a.m. to 12 p.m.) will receive takeout boxes of study meals for lunch after dialysis

B. Participants with the afternoon shift (12 p.m. to 5 p.m.) consume study lunch after beginning dialysis and receive takeout boxes of study meals for dinner at the end of dialysis.

2) During the interdialytic period, the packaged study meal boxes will be retrieved at the hospital cafeteria or delivered to the residences via a pre-existing home delivery service and consumed by the participants as outpatients.

A. The delivery vehicles are equipped with a real-time tracking system to ascertain delivery status and provide insulated food transport.

Randomization

After recruitment, the participants will be equally stratified by intact PTH level and dialysis shift prior to randomization.

1) The intact PTH level is used as one of the stratification factors because it is a routine laboratory item and correlates with the severity of renal hyperparathyroidism.

2) According to the study protocol, participants with the afternoon shift (12 p.m. to 5 p.m.) will consume study lunch during hemodialysis. There is concern about postprandial hypotension while participants with the afternoon shift consume study lunch during hemodialysis, and participants are therefore unable to adhere to the study protocol, leading to the unwanted drop-out rate and the subsequent variability to the analyses. To minimize drop-out rate due to the above reason, participants will also be stratified by dialysis shift.

3) Within each stratum, a random allocation sequence will be generated by

computer-based randomization and used to randomly allocate the participants to the interventions.

4) The investigator will send the random allocation sequences to the dietitian, who prepares individualized study meal boxes for the participants according to assigned group.

Masking

1) The participant, primary care provider, and laboratory technicians who performed the outcome measurements will be blinded to the allocation sequence.
2) The study meals will be prepared by the same cook and with similar natural food materials so that the participants cannot infer their allocation group from the appearance or taste of the study meals.

Non-allowed treatment during the study

To avoid interference with the effects of low-phosphate diets, the following prescriptions are not allowed to change during the entire study period:

1) Hemodialysis therapy associated regimens including dialysis duration, dialysis frequency, dialysis shift, and dialysate calcium concentration
2) Medications including phosphate binder, anti-acid agents, vitamin D analogs, calcimimetic agent, and iron agents.

Promoting dietary adherence

To enhance adherence with the low-phosphate diets and minimize the dropout rate, we have planned the following actions:

1) The cook will tailor the food preparation and cooking methods to improve the

palatability of the boiled meats and the feasibility of the study.

2) Free individualized meals will be provided as incentives for adherence.
3) Prior to enrollment, intensive counseling and ample time will be provided to ensure the participants understood the dietary interventions.
4) Participants will review the study menu and confirm their willingness to consume the prepared study meals in their entirety. Foods outside of the study diets will not be allowed.
5) During the study period, dietary adherence will be monitored by self-reported intake in written diaries. Participants will be asked to record consumed portions in their study diaries and any deviations from the recommendations.
6) For the convenience of participants, packaged study meals will be insulated for transport and delivered to the participants' residences.
7) Collection of blood specimen via dialysis assess will be done during regular hemodialysis session without the necessity for any additional venipuncture.
8) Delivery of the meals and consumption of the study diets will be verified by telephone.
9) We will enroll a sufficient number of participants, assuming a power of 90% and a dropout rate of 25%, to have adequate power to detect differences between the two dietary interventions.

Dietary assessment

The daily dietary intake of participants will be estimated at baseline and during the study periods.

1) The dietitians will educate the participants and instruct them to complete standard daily food-recording forms with entries on meal time, type, the brand of

food and amount in standard measuring units, preparation style (homemade or not), and the recipe.

2) Before each study period, each participant will maintain a dietary record of his or her daily intake of typical food items for three days, including a dialysis weekday, a nondialysis weekday and a nondialysis weekend day, allowing us to estimate the nutrient content of his or her typical diet as well as dietary compliance.
3) During the study periods, the participants will maintain a 2-day dietary record of their consumption of portions of the assigned study diets and foods outside of the study diets.
4) The completeness, consistency, and clarity of the food diaries will be reviewed by the dietitians.

Data Collection

The following data will be recorded:

1) Age, gender, and body mass index
2) Hemodialysis unit data, including dry weight, duration of dialysis therapy, history of parathyroidectomy, interdialytic weight gain, dialysis-unit blood pressure, type of arteriovenous shunt, and dialysate calcium concentration.
3) Data on medications used, including phosphate-binding agents and vitamin-D analogs will also be collected.
    A. The amount, frequency, and type of medications will be registered.
    B. The phosphate-binder doses among study participants will be compared by calculating phosphate-binding equivalent dose (PBED) values as described by Daugirdas.[21]

C. Briefly, the PBED per tablet in grams is 0.50 for 500 mg of calcium carbonate, 0.67 for 667 mg of calcium acetate, 0.98 for 650 mg of aluminum hydroxide, 0.60 for 800 mg of sevelamer carbonate or hydrochloride, and 0.76 for 500 mg of ferric citrate.

4) Laboratory data regarding urea reduction ratio, hemoglobin, ferritin, alkaline phosphatase, albumin, glucose, and 25-hydroxy-vitamin D will also be collected.

Laboratory Measurements

Timing of blood sampling

Nonfasting venous blood assessments will be performed prior to dialysis sessions at baseline and at the beginning and end of each study period.

1) The timing of venous sampling during the study periods will be assimilated into the routine hemodialysis schedule.
2) Blood samples will be drawn at around 7:30 a.m. for participants with the morning shift (7 a.m. to 12 p.m.) and at around 12:30 p.m. for those with the afternoon shift (12 p.m. to 5 p.m.).
3) At the baseline and the beginning of each study period, blood samples will be collected prior to 2nd dialysis session of the week. At the end of each study period, blood samples will be drawn before 3rd dialysis session of the week.
4) Participants with the morning shift will receive predialysis blood tests after consuming their usual breakfast, and those with the afternoon shift undergo predialysis blood tests and consume the study-diet lunch after beginning dialysis.

Type of laboratory assays

1) Standard assays for serum phosphate and calcium will be performed using

automated analyzers.

2) Intact PTH (reference range 8 to 76 pg/mL) will be analyzed in serum using an immunoradiometric assay (ELSA-PTH, Cisbio Bioassays, France): intra and interassay coefficients of variation (CVs) are 2.1 to 7.5% and 2.7 to 6.8%, respectively.

3) The 25-hydroxyvitamin D (reference range 5.3 to 47 ng/mL) will be analyzed in serum using an electrochemiluminescence immunoassay analyzer (ECLIA) (Roche Diagnostics GmbH, Germany): intra and interassay CVs are 2.2 to 6.8% and 3.4 to 13.1%, respectively.

4) In light of the absence of any standardized commercial FGF23 assays, two available FGF23 assays will be performed: intact FGF23 and C-terminal fragments of FGF23.

   A. Serum and plasma samples will be stored at -72℃ and batch analyzed for intact FGF23, and C-terminal FGF23.

   B. Intact FGF23 will be assessed in serum using an enzyme-linked immunosorbent assay (ELISA) (Kainos Laboratories, Tokyo, Japan); the intra and interassay CVs are 2.0 to 3.0% and 2.1 to 3.8%, respectively.

   C. The C-terminal fragments of FGF23 will be assessed in EDTA-plasma using a sandwich ELISA (Immutopics, San Clemente, CA) according to the manufacturer's instructions: the intra and interassay CVs are 1.4 to 2.4% and 2.4 to 4.7%, respectively.

   D. Each sample will be run in duplicate.

Outcome Measures

1) The primary and secondary outcome measures will be recorded at the beginning

and end of each study period.

2) A total of 4 repeated measurements for each participant will be obtained.
3) The laboratory technicians who performed the outcome measurements will be blinded to the allocation sequence.

Primary outcome measure

Difference in change-from-baseline intact FGF23 level between two low-phosphate diets, a very-low-phosphate diet with a PPR value of 8 mg/g, and a low-phosphate diet with a PPR value of 10 mg/g.

Secondary outcome measures

Difference in change-from-baseline serum phosphate, intact parathyroid hormone and C-terminal FGF23 levels between two intervention groups.

Safety and Adverse Events

Any adverse events, whether be serious or not, observed or reported by participants during the study, regardless of the causality with study diets, will be recorded.

Sample Size Determination

1) Our recent systematic review and meta-analysis showed that lower-phosphate diets tended to reduce FGF23 levels (standardized mean difference, -0.74; 95% CI, -1.54 to 0.07, $P = 0.07$) relative to higher-phosphate diets.[22]
2) To conduct a crossover trial, a total sample size of 29 was required to achieve 90% power and a type I error of 0.05, based on the standardized mean difference in FGF23 levels between two low-phosphate diets of 0.74 and a drop-out rate of 25%.

Statistical Analysis Plan (SAP)

1) Continuous measures are evaluated as means (±SDs) or median (1^{st} and 3^{rd} quartiles), and categorical variables are evaluated as counts and percentages.
2) For each study period, we will calculate a 3-day average value of estimated daily dietary intake at baseline and a 2-day average value of estimated daily dietary intake during the study period.
3) For between-group comparisons at baseline, Student's t test will be used for normally distributed variables, the Wilcoxon-Mann-Whitney test for nonnormally distributed variables, and Chi-square and Fisher's exact tests for categorical variables.
4) For primary and secondary outcomes, Wilcoxon signed-rank tests will be performed for non-normally distributed data, and paired t tests will be performed for normally distributed data.
5) We will perform intention-to-treat (ITT) analysis. Each participant should have at least two laboratory measurements, i.e. before and after the study period, to calculate difference in change-from-baseline values.
6) No imputation will be done for missing data using calculation or estimation.
7) In a crossover trial, each subject serves as his or her own control, and repeated measurements from the same subject are correlated.
    A. To account for this correlation, we will employ mixed effects models to examine the difference in treatment effect of the low-phosphate diets on outcomes and the presence of potential bias resulting from carryover and period effects.
    B. In each mixed effects model, the dependent variable is a primary or secondary outcome, the participant is included as a random effect, and the

independent variables are diet, group, and study period.

C. To take account of the stratified randomization, the stratification factors will be included as covariates in the model.

D. A two-sided *P* value of less than 0.05 indicates statistical significance.

E. All analyses will be performed with SAS version 9.4 software (SAS Institute).

Protocol Amendment

Date: 19 September 2018		
Change	Rationale	Affected Protocol Sections
Updated primary and secondary outcomes: (1) Specified the primary outcome (FGF23) measured with intact assay, and added C-terminal FGF23 as the secondary outcome, (2) Cancelled serum 1,25-dihydroxyvitamin D_3 from the list of the secondary outcomes.	(1) Intact FGF23 is biologically active and can more precisely reflect dietary response than C-terminal FGF23, (2) 1,25-dihydroxyvitamin D_3 measurement is no longer available.	Outcome measures ● Primary outcome measure ● Secondary outcome measures
Note. 1) We changed the pre-specified primary and secondary outcomes based on the biological reason.		

2) At the time of study initiation, the difference in change-from-baseline FGF23 levels between the two diets was designated as the primary outcome measure, without specifying the assay type.
3) After the study participants were enrolled, we specified the primary outcome (FGF23) measured with intact assay, removed 1,25-dihydroxyvitamin D_3 from the list of the secondary outcome, and added C-terminal FGF23 as the secondary outcome.
4) The rationale for the changes are (1) intact FGF23 is biologically active and can more precisely reflect dietary response than C-terminal FGF23, (2) 1,25-dihydroxyvitamin D_3 measurement was unavailable at our central laboratory.
5) We registered the change on clinicaltrials.gov on 29 September 2018.

Expected Effects

1) This study has clinical applicability, and has developed a special healthy diet for dialysis population, which can provide important reference information for clinical education.
2) The dialysis-specific special healthy diets are characterized by adequate calorie and protein intake, natural food ingredients without any processed food, higher portions of plant-based food, and boiling meats to remove the phosphorus content.
3) To understand the relationship between the recommended dietary phosphorus intake and intermediate cardiovascular outcomes in the dialysis population
4) To provide information that lead to reduce cardiovascular risk in dialysis population in terms of limiting daily phosphorus intake

5) To explore diet-mediated pathophysiological mechanism of cardiovascular disease in dialysis patients by analyzing the interactions between low-phosphate diet and FGF23 level
6) To establish the daily recommended phosphorus intake in Taiwanese dialysis patients
7) To inspire dialysis patients and healthcare professionals about the routine clinical practice of phosphorus-restricted diet and adequate protein intake
8) Highlighting the significance of limiting dietary phosphate intake in dialysis patients, starting from the Far Eastern Memorial Hospital and moving towards the international nephrology community

Funding

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Supplemental Appendix 2. Characteristics of the Study Diets

The dietitian selected a total of 20 highly consumed dishes from the hospital menu, comprising 2 fruit dishes, 4 grain-based dishes, 5 meat dishes, 4 side dishes, 4 vegetable dishes and 3 nutritional supplements. Since food additives included readily absorbable inorganic phosphorus, only natural food ingredients without any processed food or additive sources of phosphate were chosen for both study diets.

All study food items had the following unique characteristics: (1) composed of locally produced raw materials, (2) compliance with health and safety requirements, and (3) compliance with national quality standards. The study diets were prepared according to the food hygiene practices of the Hazard Analysis and Critical Control Points (HACCP) system at the hospital cafeteria.

To enhance nutrition and reduce phosphate amount and bioavailability, the study diets were designed to fulfill the following criteria: (1) adequate calories (≥ 30 kcal/kg/day), (2) high protein (≥ 1.2 g/kg/day), (3) low phosphate-to-protein ratio (≤ 10 mg/g), (4) low phosphate content (≤ 800 mg/day), (5) increased protein source of phosphate from plant in origin, and (6) meats boiled for 30 minutes before cooking.[1-3]

Supplemental Appendix 3. Methods for Chemical Analysis of Dietary Composition of the Study Meals

Prior to patient enrollment, the compositions of the study diets were subjected to chemical analysis; the measurement methods are described here.

Following Association of Official Analytical Communities (AOAC) Official Method 984.27, phosphorus and calcium were determined by inductively coupled plasma-optical emission spectrometer (ICP-OES) analysis with a detection limit of 0.1 mg/L.[4] In brief, the sample weights were obtained, and the edible portions of samples were ashed at high temperature and digested in nitric acid. Then, inductively coupled plasma was used to determine the actual contents of phosphorus and calcium.

Following Taiwanese official methods, the study diets were analyzed for protein,[5] fat,[6] saturated fat,[7] sugar,[8] moisture,[9] and ash.[7] Carbohydrates were calculated by the formula: 100 - (Protein + Fat + Moisture + Ash) (g/100 g). Calories were calculated by the formula: Protein (g) x 4 kcal + Fat (g) x 9 kcal + Carbohydrate (g) x 4 kcal.

Supplemental Appendix 4. Actions to Promote Dietary Adherence

To enhance adherence with the low-phosphate diets and minimize the dropout rate, we summarized the following actions:

1) The cook tailored the food preparation and cooking methods to improve the palatability of the boiled meats and the feasibility of the study.
2) Free individualized meals were provided as incentives for adherence.
3) Prior to enrollment, intensive counseling and ample time were provided to ensure the participants understood the dietary interventions.
4) Participants reviewed the study menu and confirmed their willingness to consume the prepared study meals in their entirety. Foods outside of the study diets were not allowed.
5) During the study period, dietary adherence was monitored by self-reported intake in written diaries. Participants were asked to record consumed portions in their study diaries and any deviations from the recommendations.
6) For the convenience of participants, packaged study meals were insulated for transport and delivered to the participants' residences.
7) Collection of blood specimen via dialysis assess was done during regular hemodialysis session without the necessity for any additional venipuncture.
8) Delivery of the meals and consumption of the study diets were verified by telephone.
9) We enrolled a sufficient number of participants, assuming a power of 90% and a dropout rate of 25%, to have adequate power to detect differences between the two dietary interventions.

Supplemental Table 1. Ingredients of the Study Diets

Menu item	Category	Ingredients
Breakfast[a]	Grain	Steamed bread (mantou)
	Nutritional supplement	LPF, low protein formula (Sentosa Co., Ltd) Whey protein (Sentosa Co., Ltd) MCT, medium-chain triglyceride formula (Sentosa Co., Ltd).
Lunch or dinner[b]	Meat[c]	Grilled lean meat with white sesame seeds Sliced boiled pork with garlic sauce Black pepper foreshank Poached chicken with scallion oil Braised chicken cutlets
	Side dish[d]	Braised tofu with black fungus Stir-fried cucumber and bean curd skin Stir-fried sweet pepper and bean curd noodles Stir-fried Chinese chive flower and soybean curd
	Vegetable	Fried sweet potato leaves Fried green bean Fried broccoli Fried pak-choi
	Grain	Steamed rice Fried bean thread noodles with celery cabbage Fried rice stick noodle with celery cabbage
	Fruit	Orange Apple

Note. [a]Breakfast included steamed bread and a mixture of low protein formula, whey protein and

medium-chain triglyceride formula. [b]Lunch or dinner included 1 meat, 1 side dish, 1 vegetable, 1 grain and 1 fruit. [c]The meats were boiled in water for 30 minutes before subsequent cooking to meet the low-phosphate requirements. [d]To increase proportion of plant-based foods, all side dishes were made up of soybean based foods.

Supplemental Table 2. Nutrient Compositions of the Study Diets According to Participant Body Weight

Category	Body Size (kg)	Calorie (kcal/day)	Protein (g/day)	Phosphorus (mg/day)	Calcium (mg/day)	PPR (mg/g)
PPR = 8 mg/g						
	45	1368	54	452	343	8.4
	50	1502	57	481	387	8.4
	55	1662	69	546	349	7.9
	60	1808	73	585	418	8.0
	65	1950	78	651	431	8.4
PPR = 10 mg/g						
	45	1341	57	591	359	10.3
	50	1492	61	627	381	10.3
	55	1609	64	662	387	10.3
	60	1768	70	752	480	10.7
	65	1937	76	824	508	10.9

Note. To facilitate food preparation and supply, 5 different kinds of study diet, i.e., diets for body weights of 45 ± 2.5 kg, 50 ± 2.5 kg, 55 ± 2.5 kg, 60 ± 2.5 kg and 65 ± 2.5 kg, were crafted to fulfill the requirements of body sizes from 42.5 kg to 67.5 kg.

Abbreviations: PPR, phosphate-to-protein ratio

Supplemental Table 3. Assessment of Carryover Effects and Period Effects by Mixed Effects Models

Outcome	Carryover Effect (Group: A *vs.* B)[b]	Period Effect (Period: 2nd *vs.* 1st)
	***P* value**	***P* value**
Intact FGF23 (pg/mL)[a]	0.40	0.73
Phosphate (mg/dL)	0.13	0.75
Intact PTH (pg/mL)[a]	0.20	0.80
C-terminal FGF23 (RU/mL)[a]	0.14	< 0.001[c]

[a]Log transformed data.

[b]Participants in group A received a 2-day diet with a phosphate-to-protein ratio of 8 mg/g, followed by a 5-day washout period, and then received a 2-day diet with a phosphate-to-protein ratio of 10 mg/g. Those in group B received the diets in the opposite order.

[c]The C-terminal FGF23-lowering effect did not differ between group A and group B ($P = 0.47$), indicating that there was no differential period effect regarding C-terminal FGF23 level.

Abbreviations. FGF23, fibroblast growth factor 23; PPR, phosphate-to-protein ratio; PTH, parathyroid hormone

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