Phosphatemic Index Is a Novel Evaluation Tool for Dietary Phosphorus Load: A Whole-Foods Approach

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Objective: Dietary phosphorus (P) restriction is crucial to treat hyperphosphatemia and reduce cardiovascular disease risk and mortality in patients with chronic kidney disease (CKD) and the wider population. Various methods for dietary P restriction exist, but the bioavailability of P in food should also be considered when making appropriate food choices to maintain patients' quality of life. Here, we propose the "Phosphatemic Index" (PI) as a novel tool for evaluating dietary P load based on P bioavailability; we also evaluated the effect of continuous intake of different PI foods in mixed meals on serum intact fibroblast growth factor 23 concentration.

Design and Methods: A 2-stage crossover study was conducted: Study 1: 20 healthy participants consumed 10 different foods containing 200 mg of P, and the PI was calculated from the area under the curve of a time versus serum P concentration curve; Study 2: 10 healthy participants consumed 4 different test meals (low, medium, or high PI meals or a control) over a 5-day period.

Results: Study 1 showed milk and dairy products had high PI values, pork and ham had medium PI values, and soy and tofu had low PI values. In Study 2, ingestion of high PI test meals showed higher fasting serum intact fibroblast growth factor 23 levels and lower serum 1,25-dihydroxyvitamin D levels compared with ingestion of low PI test meals.

Conclusion: These findings suggest that the PI can usefully evaluate the dietary P load of various foods and may help to make appropriate food choices for dietary P restriction in CKD patients.

Introduction

FOR CORRECT BIOLOGICAL function, the serum phosphorus (P) level is maintained within an appropriate range by a complex interplay among the processes of intestinal absorption, bone formation, and renal excretion.[1] Parathyroid hormone (PTH), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), and fibroblast growth factor 23 (FGF23) are the principal hormones responsible for maintaining P homeostasis.[2] Serum PTH levels have a bearing on the rapid regulation of P loading. On the other hand, serum FGF23 levels do not rapidly increase to adapt to dietary P loading, whereas increase in response to continual high P loading. Thus, FGF23 could be an indicator of P loading in humans.[3,4]

High dietary P intake and elevated serum P levels, albeit within the normal range, have been associated with an increased risk of cardiovascular disease (CVD)[5-9] and mortality[10,11] in both the general population and patients with chronic kidney disease (CKD). Our previous study demonstrated that high dietary P intake, even in a single meal challenge, resulted in postprandial elevation of serum P levels to more than the normal range.[12] Postprandial elevation of serum P levels has also been shown to impair endothelial function in healthy young men.[13] Thus, controlling hyperphosphatemia and excessive P loading is important to prevent CVD and improve survival rates among the whole population.

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Epidemiological studies have shown that postprandial hyperglycemia is also a risk factor for CVD.[14,15] The Glycemic Index (GI) is a quantitative assessment of foods based on their resulting postprandial blood-glucose levels.[16] It is expressed as a percentage of the response to an equivalent carbohydrate portion of a reference food. The GI of a particular food is determined primarily by the nature of the carbohydrate and by other dietary cofactors that affect nutrient digestibility or insulin secretion. Therefore, the GI can accurately reflect the bioavailability of carbohydrate in foods.

Dietary P restriction is the primary therapeutic treatment for hyperphosphatemia. In addition to the quantity of dietary P, the P-to-protein rations, and type (organic vs. inorganic) and source (animal vs. plant-derived) of P are of considerable importance.[17] However, dietary P restriction is often associated with a reduction in protein intake, which is correlated with protein energy wasting and poor prognosis in CKD patients.[18] Furthermore, these conventional strategies for P management focus simply on the amount of intestinal absorption, and the bioavailability of P in most foods remains unclear.[19]

In this study, we developed a "Phosphatemic Index" (PI) based on serum P levels following the ingestion of various foods. A further validation study demonstrated that the continuous intake of mixed meals consisting of high PI foods over a 5-day period resulted in higher serum intact FGF23 levels.

Methods

Participants

Volunteers with no apparent health problems were recruited for Study 1 (n = 20; aged 21-26 years) and Study 2 (n = 10; aged 21-27 years). The participants showed no evidence of kidney disease, diabetes mellitus, glucose intolerance, obesity, hypertension, CVD, dyslipidemia, or any other bone or mineral disorders. All participants were nonsmokers, consumed <30 g/day alcohol, and took no medication or antioxidant supplements. The eligibility of participants for this study was determined as we previously reported.[12,13] Demographic data for the participants are shown in Table 1.

Study Design

Study 1

Study 1 used an open-label crossover design on 11 different days separated by at least 7 days (Fig. S1A). On the day before each study day, participants were asked to abstain from food and beverages (other than water that did not contain P) after 1400 hours. They were served a standard dinner (644 kcal, 100 g carbohydrate, 23 g protein, 16 g fat, 517 mg P, and 238 mg Ca) at 2000 hours, then instructed to go to bed until 2400 hours. On each study day, participants consumed a standard breakfast (617 kcal, 100 g carbohydrate, 21 g protein, 16 g fat, 332 mg P, and 201 mg Ca). At 1200 hours, the participants were served one of 10 test foods or sodium P, which contained 200 mg of P. All foods were consumed over a 7- to 14-minute period. Blood samples were collected immediately before (0 hours) and at 0.25, 0.5, 1, 2, 4, and 6 hours after ingestion of the test food. Urine samples after ingestion of test food were also collected every hour from 1200 to 1800.

Study 2

Study 2 used an open-label crossover design of 4 different test meals separated by at least 7 days (Fig. S1B). The study was carried out for 7 consecutive days for 1 test meal: an adjustment phase of 2 days followed by a test meal phase of 5 days. During the study period, participants consumed only the meals and beverages provided. All foods were consumed over an approximately 15-minute period. On the day before each study day, participants were asked to abstain from food and beverages (other than water not containing P) after 1400 hours. They were instructed to go to bed until 2400 hours. On each day during the study period, participants had breakfast at 0800, lunch at 1200, and dinner at 1800 hours. Blood and 24-hour urine samples were obtained on days 2 (preintervention) and 7 (postintervention). Blood samples were collected immediately before (0 hours) and at 2, 4, 6, 10, 12, 14, and 24 hours after the ingestion of test food. Each sample was taken between meals (2, 6, 12, and 14 hours) or before meals at 0800 (0 hours), 1200 (4 hours), and 1800 (10 hours), with the last one taken before the meal at 0800 (24 hours) on the following morning.

Test Foods and Meals

Study 1

The test foods were as follows: soybeans (soy), tofu, buckwheat noodles (soba), broccoli, pork, ham, milk, processed cheese (cheese), red sea bream (fish), and eggs. We used 200 mg neutral sodium phosphate (a mixture of Na_2HPO_4 and NaH_2PO_4 dissolved in 22 mL water) as the reference food (referred to as "suppl"). The composition of the test foods is shown in Table S1. Standard tables of food composition from Japan 2010 were used to estimate and design the nutritional composition of the study diets.

Study 2

All meals were designed to be similar in total caloric content (2,000 kcal/day for males, 1,800 kcal/day for females); protein:fat:carbohydrate ratios in terms of percentage of energy (15:25:60); protein (77 mg/day for males, 71 mg/day for females); P (1,200 mg/day); and sodium (3,500 mg/day). One-half of the P sources (600 mg/day) were varied by different test foods. The test foods were as follows: plant sources such as soybeans and tofu (low PI test meals; "Low"); animal sources such as pork and ham (medium PI test meals; "Medium"); and dairy products such as milk and processed cheese (high PI test meals; "High"). We used neutral sodium P as a reference food

Table 1. Baseline Characteristics of Participants in Study 1 and 2

Characteristic	Study 1	Study 2
Total (male/female)	20 (10/10)	10 (5/5)
Age (y)	22.8 ± 0.4	22.9 ± 0.5
Body mass index (kg/m^2)	21.4 ± 0.5	21.2 ± 0.7
Systolic blood pressure (mm Hg)	113 ± 2.0	110 ± 3.7
Diastolic blood pressure (mm Hg)	67 ± 1.8	67 ± 3.1
Serum creatinine (mg/dL)	0.8 ± 0.0	0.7 ± 0.0
Serum uric acid (mg/dL)	5.1 ± 0.3	4.7 ± 0.4
Blood glucose (mg/dL)	90 ± 1.3	92 ± 1.7
Triglyceride (mg/dL)	64 ± 4.6	74 ± 10.0
HDL cholesterol (mg/dL)	65 ± 3.0	63 ± 5.1
LDL cholesterol (mg/dL)	94 ± 6.8	95 ± 5.3
Serum sodium (mEq/L)	139 ± 0.3	140 ± 0.3
Serum potassium (mEq/L)	4.2 ± 0.0	4.2 ± 0.1
Serum chloride (mEq/L)	105 ± 0.3	106 ± 0.7
Serum calcium (mg/dL)	9.7 ± 0.1	9.6 ± 0.1
Serum phosphorus (mg/dL)	4.0 ± 0.1	3.8 ± 0.1

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data are presented as mean ± standard error of the mean.

(control test meal; "Control"). Differences in protein content between the test meals were normalized using egg whites. Each test food was served with standard food including steamed rice or bread and vegetables such as cabbage, carrots, spinach, corn, bean sprouts, and edamame. The composition of the test foods is shown in Table S2.

Biochemical Analysis

Venous blood was taken from participants' median cubital veins to measure levels of serum inorganic P, Ca, Na, K, Cl, intact PTH (iPTH), insulin, uric acid, creatinine, glucose, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol in both studies, and intact FGF23 (iFGF23) and 1,25$(OH)_2$D in Study 2. Aliquots of serum were stored at −80°C until assays could be performed. Urine samples were also collected to measure urinary concentrations of P, Ca, and creatinine, and the volume of each urine sample was recorded. The volumes of 24-hour urine collected were recorded on days 2 and 7 from 0800 to 0800 the following morning. Urine samples were stored at −30°C until assays could be performed. All biochemical measurements and analyses were performed by LSI Medience Corporation (Tokyo, Japan). The in vitro measurement of digestive P and total P content of each test food was performed using inductively coupled plasma-optical emission spectrometry at the Saga Nutraceuticals Research Institute of the Otsuka Pharmaceutical Co., Ltd. (Saga, Japan), according to a previously reported method.[20] Serum iFGF23 levels were measured using an iFGF23 kit (Kainos Laboratories Inc., Tokyo, Japan).

Calculation of Phosphatemic Index, Digestible P, and Kinetic Parameters

The PI value was calculated as the area under the curve (AUC) for serum P levels following the consumption of each test food divided by the AUC for the suppl containing the same amount of P. The equation is given as follows:

$$\mathrm{PI} = (\text{AUC for test food}) \cdot 100 / (\text{AUC for 200 mg sodium P})$$

The average of the PI ratings from all 20 participants is published as the PI for each food. AUCs were calculated to reflect the levels of serum P more than 6 hours after the ingestion of each test food, which equals the sum of all areas for each segment divided by the time of blood collection and includes all areas below each curve, including the area below the fasting concentration. Digestible P was measured according to the previous study.[20] Each test food was digested enzymatically in principle in the same way as in the alimentary canal before P analyses. The fasting level was used as a baseline; the area beneath the fasting level was treated as a negative value. Kinetic parameters were derived for each individual participant from their serum P levels. The C_{max} of serum P was directly obtained from the experimental data, while T_{max} was defined as the time of the first occurrence of C_{max}.

Calculation of TmP/GFR

In Study 2, the ration of the renal tubular maximum reabsorption rate of P to the glomerular filtration rate (TmP/GFR) was calculated for each test meal.[21] First, the fractional tubular reabsorption of P (TRP) was calculated using the following equation: $\mathrm{TRP} = 1 - \{(U_p/P_p) \times (P_{cr}/U_{cr})\}$.

Then, TmP/GFR was calculated using the following equation, obeying the rules of the algorithm and depending on the value of TRP (TRP ≧ 0.86):

$$\mathrm{TmP/GFR} = 0.3 \cdot \mathrm{TRP} / \{1 - (0.8 \cdot \mathrm{TRP})\} \cdot P_p.$$

where P_p represents plasma P; U_p, urine P; P_{cr}, plasma creatinine; and U_{cr}, urine creatinine.

Statistical Analysis

Continuous data were expressed as means ± standard errors of the means for normally distributed variables, or medians (interquartile ranges) for non-normally distributed variables. In Study 2, comparisons between preinterventions and postinterventions were performed after the same time period, 24 hours, of the levels of each serum or urine biomarker. Preintervention biochemical data were represented by the average of each preintervention value. All data were tested to see if variables of interest were normally distributed, using the Shapiro-Wilk normality test, before employing further parametric or nonparametric statistical analyses. Statistical significance among multiple groups was determined using one-way repeated-measures analysis of variance with a post hoc Bonferroni's multiple comparison test, or a Friedman test

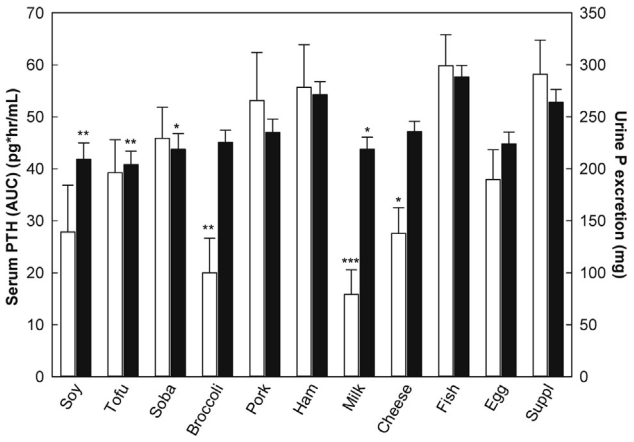

Figure 1. The effect of dietary P intake from different test foods on serum iPTH AUCs and urine P excretion levels for 6 h following the ingestion of each test food. Open square, serum iPTH AUC 0-6 h; closed square, urine P excretion 0-6 h. Data are shown as mean ± SEM. Statistical significance among multiple groups was determined by one-way repeated-measures ANOVA with a *post hoc* Bonferroni's multiple comparison test, or a Friedman test with Dunn's multiple comparison test, depending on the distribution of the data. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ versus sodium phosphate. ANOVA, analysis of variance; AUC, area under the curve; Cheese, processed cheese; Fish, red sea bream; iPTH, intact parathyroid hormone; SEM, standard error of the mean; Soba, buckwheat noodles; Soy, soybeans; Suppl, sodium phosphate.

with Dunn's multiple comparison test depending on the distribution of the data. Pearson's correlation coefficients were estimated to identify associations between PI and digestive P (%), C_{max}, and T_{max}. Two-way repeated-measures analysis of variance was used to evaluate the presence of diurnal variation. Repeated factors were test meal (Low, Medium, High, and Control PI test meals) and hour of day (0800, 1000, 1200, 1400, 1800, 2000, 2200, and 0800). Test meal versus time interactions were tested to determine whether diurnal patterns of serum parameters differed among test meals. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA). A P value of <0.05 was taken to indicate significance.

Results

Study 1

Effect on P Homeostasis of Dietary P Originating From Different Foods

First, we examined serum P levels after the ingestion of test foods in 20 healthy volunteers (Table 1). Serum P levels rapidly increased following the ingestion of pork, ham, milk, cheese, broccoli, fish, and eggs (Fig. S2). Furthermore, the ingestion of milk, cheese, and eggs led to a prolonged and significant increase in serum P levels through the postprandial period. On the other hand, only a slight increase in serum P levels was seen following the ingestion of tofu.

Serum P levels are mainly regulated by renal P excretion. The immediate response of renal P excretion to dietary P intake is dependent on PTH.[12] Thus, we investigated postprandial changes in urinary P excretion and serum iPTH levels. The AUC for urine P excretion was significantly lower following the ingestion of milk, soy, or tofu compared with ingestion of the suppl (Fig. 1). Furthermore, serum iPTH levels following the ingestion of test foods increased compared with the baseline level. The AUC for iPTH was significantly lower in milk and broccoli compared with the suppl. We did not measure serum iFGF23 levels because an earlier study demonstrated that they remained unchanged immediately after the ingestion of a meal, even in the case of high P meals.[12]

Establishment of the PI Based on Postprandial Changes in Serum P Levels

The PI for each test food was calculated according to postprandial changes in serum P levels based on the bioavailability of P. As shown in Table 2, PI values tend to be lower for plant-based foods (soy, tofu, soba, and broccoli) compared with animal-based foods (pork, ham, cheese, milk, fish, and eggs). Interestingly, soba and broccoli had the higher PI values observed among the plant foods. When comparing natural and processed foods, the PI tended to be lower for pork than ham, whereas there was no difference between soybean-based (soy and tofu) or dairy (milk and cheese) products.

Subsequently, we compared the PI with digestible P, which indicates the proportion of digestible P to total P or P-to-protein ratio (PPR). As shown in Figure 2, a significant positive association was observed between the PI and the percentage of digestible P ($r = 0.659$, $P = .0273$). However, this was not consistent across all foods, e.g., the PIs of milk and cheese differed markedly from their digestive P values. In addition, no significant association was shown between the PI and PPR ($r = 0.632$, $P = .0503$).

We also assessed the relationship between PI and C_{max} or T_{max} to ascertain whether higher maximum concentration or more rapid increase in postprandial serum P levels contributed most to the PI value (Table 2). A significant positive association was observed between PI and C_{max} ($r = 0.733$, $P = .0103$), while no significant negative association was observed between PI and T_{max} ($r = -0.596$, $P = .0532$).

Study 2

Effects on P Homeostasis of Continuous Ingestion of Foods With Different PIs

To confirm the effects of continuous ingestion of foods with different PIs on P homeostasis, a crossover dietary intervention using a control and 3 meals of different PIs was performed in 10 healthy volunteers (Table 1). After 5 days of the intervention, urinary P excretion decreased significantly following Low PI meals compared with the control and High PI meals, whereas fasting serum P levels

Table 2. Serum Phosphorus AUC, PI, PPR, Digestible P%, C_{max}, and T_{max} in Study 1

Test Food	Serum P AUC[a]	PI[a]	PPR	Digestive P%	C_{max}[a]	T_{max}[a]
Soy	1.02 ± 0.31	25 ± 9	13.48	19.7	4.1 ± 0.1	3.3 ± 0.4
Tofu	0.6 ± 0.36	19 ± 14	16.89	16.4	4.0 ± 0.1	2.5 ± 0.4
Soba	1.33 ± 0.35	49 ± 14	16.89	21.9	4.2 ± 0.1	3.5 ± 0.4
Broccoli	2.01 ± 0.28	71 ± 12	20.76	49.9	4.2 ± 0.1	1.7 ± 0.2
Pork	1.66 ± 0.27	51 ± 11	8.94	49.9	4.3 ± 0.1	1.3 ± 0.2
Ham	2.32 ± 0.37	82 ± 12	18.18	78.2	4.6 ± 0.1	1.0 ± 0.1
Milk	2.97 (2.62-3.34)	127 ± 12	28.18	75.1	4.5 ± 0.1	1.6 ± 0.2
Cheese	3.06 ± 0.27	118 ± 17	32.16	57.4	4.4 ± 0.1	1.8 ± 0.3
Fish	2.63 ± 0.25	99 ± 12	11.26	76.3	4.5 ± 0.1	1.3 ± 0.2
Egg	2.13 ± 0.42	78 ± 16	14.81	11.3	4.2 ± 0.1	2.7 ± 0.4
Suppl	3.09 ± 0.34	100	–	90.2	4.8 ± 0.1	1.2 ± 0.2

AUC, area under the curve; Cheese, process cheese; C_{max}, maximum concentration of serum phosphorus; Digestive P%, digestive phosphate/total phosphate in each test food (%); Fish, red sea bream; PI, Phosphatemic Index; PPR, phosphorus-protein ratio (mg/g); SEM, standard error of the mean; Soba, buckwheat noodle; Soy, soybean; Suppl, sodium phosphate; T_{max}, time to maximum concentration of serum phosphorus.
[a]Data are presented as mean ± SEM or median (interquartile range).

were unchanged (Fig. 3A and 3B). In contrast, TmP/GFR significantly increased following Low PI meals compared with the preintervention, control, High, and Medium PI meals (Fig. 3C). Fasting serum Ca levels were significantly higher following High PI meals compared with the preintervention values (Table S4). In addition, urinary Ca excretion was significantly higher following High PI meals compared with the preintervention, Medium, and Low PI meals (Table S4). There was no difference in fasting serum or urine creatinine levels, or creatinine clearance levels (Table S4).

Fasting serum iPTH levels significantly decreased following Low and Medium PI meals compared with the preintervention values (Fig. 3D). Fasting serum iFGF23 levels significantly decreased following Low PI meals compared with preintervention, Control, High, and Medium PI meals (Fig. 3E). Furthermore, fasting serum $1,25(OH)_2D$ levels significantly decreased following High PI meals compared with preintervention and High PI meals (Fig. 3F).

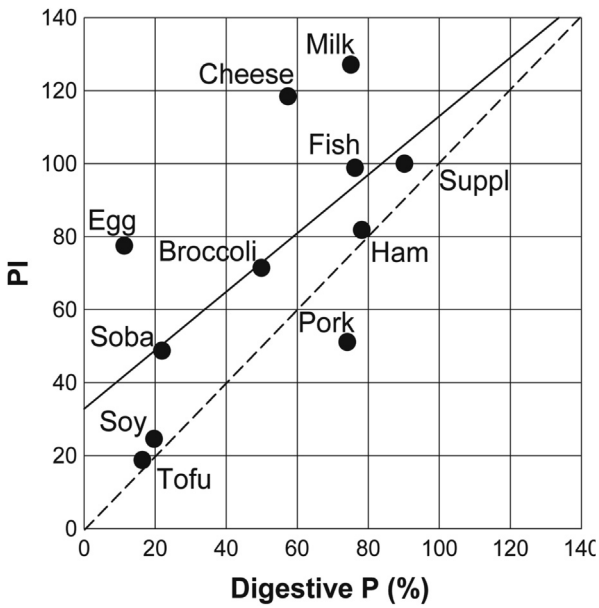

Figure 2. Correlation between the PI and percentage digestive P in each test food. Pearson's correlation coefficient r and its P-value for $r = 0$ are presented in the association. Solid line, the approximate line of correlation; dashed line, the line when the phase becomes completely consistent ($r = 1$). Cheese, processed cheese; Fish, red sea bream; PI, Phosphatemic Index; Soba, buckwheat noodles; Soy, soybeans; Suppl, sodium phosphate.

Effects of Continual Ingestion of Different PI Foods on Diurnal Variation of P Homeostasis

Serum P levels undergo diurnal variation so we examined the impact of continuous ingestion of different PI meals on this diurnal variation of P homeostasis. We observed a test meal versus time interaction ($P < .01$), which indicated that the diurnal pattern of serum P levels differed by meal. Serum P levels were at their lowest at 1000 and 2000 hours for all 4 test meals and also showed a peak value at 1800 hours (Fig. 4A). In addition, serum P levels were lower throughout the day following Low PI meals compared with other test meals. A test meal versus time interaction was also found for serum iPTH ($P < .05$; Fig. 4B) and iFGF23 levels ($P < .001$; Fig. 4C). Serum iFGF23 levels were significantly lower throughout the day following Low PI meals compared with other meals, whereas serum iPTH levels were only slightly affected by different PI meals. Meanwhile, there were no significant interactions observed for serum Ca (Fig. 4D), glucose (Fig. 4E), or insulin levels (Fig. 4F).

Discussion

In the present study, we developed a PI for P loading based on changes in serum P levels following the ingestion of various test foods. We demonstrated that the PI was mainly affected by the amount of digestible P in foods,

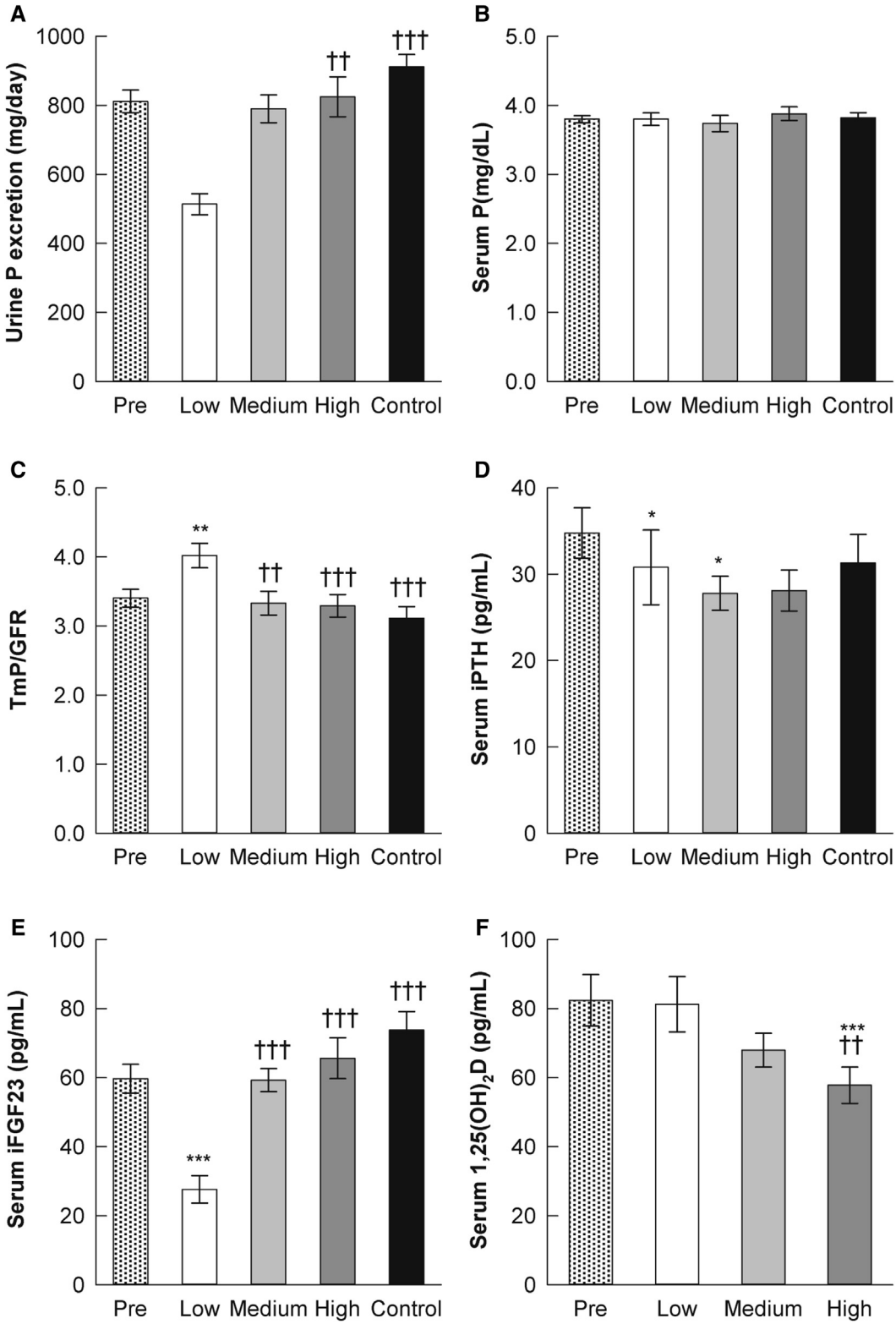

Figure 3. The effect of continual ingestion of various PI test meals over a 5-day period. The effect of continual ingestion of low or high PI food over a 5-day period on (A) urine P excretion, (B) serum P levels, (C) TmP/GFR, (D) serum iPTH levels, (E) serum iFGF23 levels, and (F) serum 1,25$(OH)_2$D levels. Data are shown as mean ± SEM for 10 participants in (B), (C), (D), and (E) and for 8 participants in (A). Statistical significance among multiple groups was determined by one-way repeated-measures ANOVA with a *post hoc* Bonferroni's multiple comparison test or a Friedman test with Dunn's multiple comparison test depending on the distribution of the data. *$P < .05$, **$P < .01$, ***$P < .001$ versus Pre. †$P < .05$, ††$P < .01$, †††$P < .001$ versus Low. ANOVA, analysis of variance; Control, postintervention with a Control test meal; GFR, glomerular filtration rate; High, postintervention with a High PI test meal; iFGF23, intact fibroblast growth factor 23; iPTH, intact parathyroid hormone; Low, postintervention with a Low PI test meal; Medium, postintervention with a Medium PI test meal; PI, Phosphatemic Index; Pre, average of preintervention collections done before each intervention with 4 test meals; SEM, standard error of the mean; TmP, tubular maximum reabsorption rate of P.

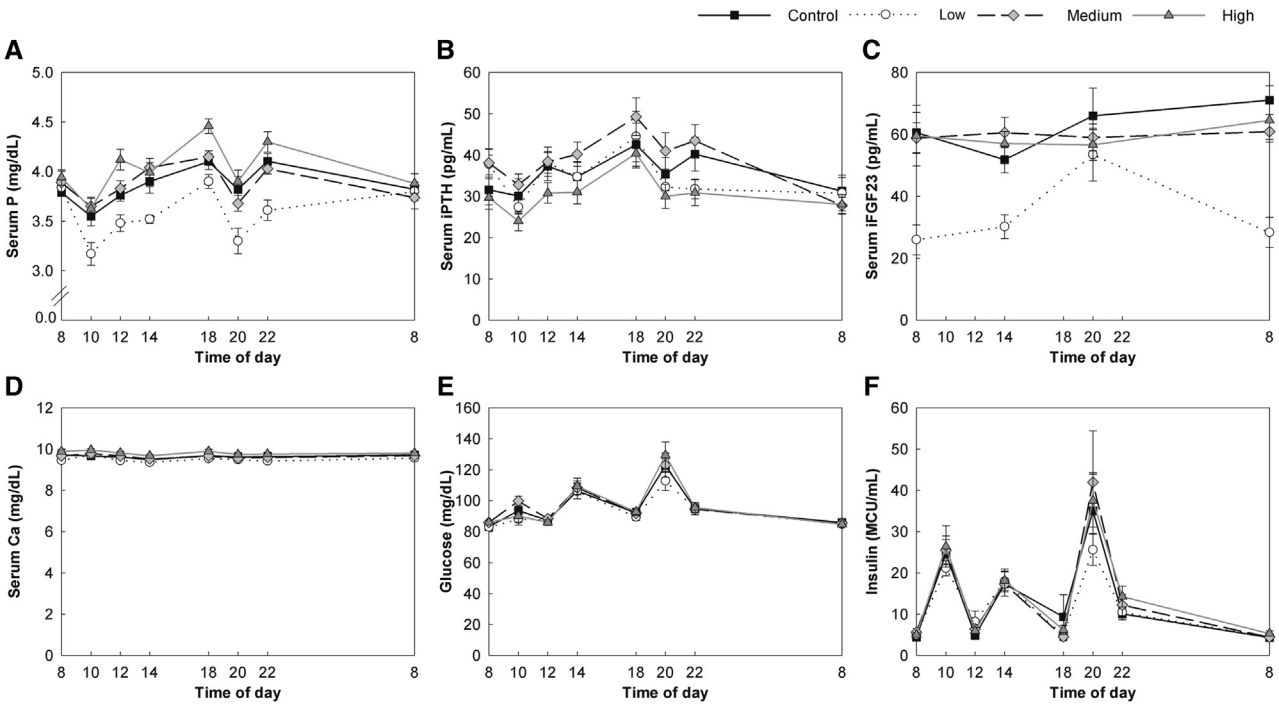

Figure 4. The effect of continual ingestion of different PI test meals over a 5-day period on the diurnal variation of P homeostasis. The effect of continual ingestion of low or high PI food over a 5-day period on diurnal variation in (A) serum P levels, (B) serum iPTH levels, (C) serum iFGF23 levels, (D) serum Ca levels, (E) glucose levels, and (F) insulin levels. Data are shown as mean ± SEM for 10 participants in (A), (B), (D), (E), and (F), and for 8 participants in (C). Two-way repeated-measures ANOVA was used to evaluate the presence of diurnal variation. ANOVA, analysis of variance; Control; postintervention with a Control test meal; High, postintervention with a High PI test meal; iFGF23, intact fibroblast growth factor 23; iPTH, intact parathyroid hormone; Low, postintervention with a Low PI test meal; Medium, postintervention with a Medium PI test meal; PI, Phosphatemic Index.

but also reflected physiological responses including P distribution associating postprandial insulin surge, urine P excretion, and iPTH secretion. Furthermore, an intervention study to investigate meals with different PIs consumed over a 5-day period showed that the PI can reflect the magnitude of P loading as assessed by urine P excretion and serum iFGF23 levels. Our data therefore provide a novel index with which to classify foods according to their magnitude of P loading.

In addition to dietary P restriction,[22] current strategies for the nutritional management of P focus on intestinal absorption properties relating to the type[17,20,23-26] and source of P. In natural foods, organic P is the main source of P, whereas inorganic P is the main component of many food additives found in processed foods. The majority of inorganic P is rapidly absorbed in the intestine, as opposed to only 40%-60% absorption of organic P.[17,27,28] Furthermore, the absorbability of P from plant-derived foods is lower than that of animal-derived foods, because humans do not express the degrading enzyme of phytate which is found in plant-derived P.[29] Our data also showed that the PI values of processed foods and animal-derived foods were significantly higher than those of natural foods and plant foods. Moreover, a significant positive association between PI and digestive P estimated from in vitro digestion studies was observed. These findings indicate that the PI is largely based on the absorbability of dietary P. However, the PI values of dairy products were higher than those of meat products, despite having comparable levels of digestible P. Although digestibility is a key determinant of P bioavailability, this correlation may be altered by other nutrients in phosphate-containing food.[19] Dairy products contain high levels of calcium. High calcium intake can suppress postprandial PTH secretion, which results in a reduction in urinary P excretion.[23] Our results showed that serum iPTH levels and urine P excretion were suppressed following the ingestion of milk and cheese. Therefore, the prolonged increase in serum P levels after the ingestion of milk and cheese could be affected by postprandial serum iPTH levels and urinary P excretion. Additionally, there was no significant association between PI and PPR, which is one of the strategies of nutritional management for hyperphosphatemia in CKD patients.[30] This may be due to the limitations of PPR that the P content in food is not always associated with the protein content. Our results emphasize the importance of evaluating bioavailability based on postprandial metabolic responses in vivo, including intestinal absorption and renal excretion. The PI can reflect not

only the absorbability of dietary P but also the bioavailability of dietary P, including the amount of urinary P excretion.

Because we initially assessed PI based on the ingestion of a single food, it was important to investigate the effects of PI on P metabolism when these foods were ingested as part of a mixed meal. Therefore, we conducted a short-term dietary intervention study where mixed meals consisting of different PI foods were ingested. Our study demonstrated that continuous ingestion of different PI meals showed little impact on fasting serum iPTH levels, whereas serum iFGF23 levels and urine P excretion significantly increased following the ingestion of high PI meals. We previously reported that acute oral P loading did not rapidly affect serum iFGF23 levels.[12] However, longer term ingestion of a high P diet induced changes in serum FGF23 levels, suggesting FGF23 may not be associated with rapid adaptation but with chronic adaptation to P loading.[3,31] Therefore, increased iFGF23 probably mediates the increased renal P excretion observed following continuous ingestion of high PI meals.

Several studies have shown that serum P levels in healthy individuals exhibit a diurnal variation,[29,32-34] with lowest P levels present during the morning and higher levels in the afternoon. Furthermore, the diurnal variation of serum P levels varies by individuals' intake of dietary P and their kidney function. Consistent with these studies, we confirmed a similar diurnal variation that indicated a nadir of serum P levels at 1000 hours and a peak value at 1800 hours with all 4 types of meals. Moreover, we demonstrated that serum P and iFGF23 levels were lower throughout the day when Low PI meals were consumed, as were urinary P excretion levels. These results indicate that the bioavailability of P in Low PI meals was lower, leading to suppression of P loading. A previous study demonstrated that these variations are similar between healthy individuals and patients with CKD, but the magnitude of change in P was blunted in the latter group. Thus, the interactions of different PI meals and these variations will require further study.

In several prior studies, serum P concentrations generally decreased following meals in healthy individuals.[23,32,34] This decrement is due to diurnal change in serum P level: serum P level decreases following the breakfast, then rises following the lunch meal. It is hard to evaluate the effect of test food on serum P level in the decrement phase. Therefore, we conducted the study 1 experiment in the increment phase following lunch as our previous study.[12] In addition, to exclude interexperimental variation in diurnal increment of serum P levels, the dinner on the day before and the breakfast on the experimental day were consistent across test foods.

There are some limitations to this study. First, a limited number of test foods was investigated. Further studies to investigate other foods, e.g., different types or parts of meat, are needed to improve the quality of PI evaluation and to apply the PI to dietary instructions in a clinical setting. In our study, we found that high PI food is likely to have not only a sustained increase but also a greater and more rapid increase in postprandial P levels based on different bioavailabilities of P. Further research will also enable us to obtain some common profiles or discover a formula for estimating PI values. Second, we assessed the effects of bioavailability of P in a limited number of healthy participants. Studies that include patients with CKD should be carried out to determine whether an assessment based on the PI is applicable for these patients. Third, we did not assess the results for all participants and at all sampling points due to a lack of samples for some evaluations, such as the effect of different PI meals on urine P excretion or the control on serum $1,25(OH)_2D$ levels, and the diurnal variation in serum iFGF23 levels. However, our results do provide useful evidence for the effects arising from the bioavailability of P in different foods.

In conclusion, the PI is a novel index that can reflect the bioavailability of P including its intestinal absorption, tissue distribution, and renal excretion. Habitual ingestion of low PI food decreases P loading despite it containing an equivalent amount of P to other foods. The PI can help to evaluate the bioavailability of P in various foods and achieve a more sensitive classification of foods depending on the magnitude of P loading associated with them. Additionally, the PI must be applied for a nutritional assessment of patients with hyperphosphatemia.

Practical Application

Similar to GI, PI can be available as an index of dietary P load from foods or meals based on postprandial increase in serum P which reflects not only the absorbability of P in each food but also the various physical responses such as distribution to tissues and renal excretion. Practically, PI is applicable for evaluation of dietary P load, and for food choices to prevent hyperphosphatemia or excessive P load in CKD patients. This application may require the evaluation of additional foods as well as clinical trials in CKD patients for future studies.

CRediT authorship contribution statement

Yoko Narasaki: Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization. **Michiyo Yamasaki:** Methodology, Investigation, Resources, Data curation. **Sayaka Matsuura:** Investigation, Resources, Data curation. **Mayumi Morinishi:** Investigation, Resources, Data curation. **Tomomi Nakagawa:** Investigation, Resources, Data curation. **Mami Matsuno:** Investigation, Resources, Data curation. **Misaki Katsumoto:** Investigation, Resources, Data curation. **Sachi Nii:** Investigation, Resources, Data curation. **Yuka Fushitani:** Investigation, Resources, Data curation. **Kohei Sugihara:** Validation, Formal analysis. **Tsuneyuki Noda:**

Investigation, Resources, Data curation. **Takeshi Yoneda:** Investigation, Resources, Data curation. **Masashi Masuda:** Validation, Formal analysis. **Hisami Yamanaka-Okumura:** Validation, Formal analysis. **Eiji Takeda:** Investigation, Resources, Writing - review & editing, Supervision. **Hiroshi Sakaue:** Investigation, Resources, Writing - review & editing, Supervision. **Hironori Yamamoto:** Investigation, Resources, Writing - review & editing, Supervision. **Yutaka Taketani:** Conceptualization, Methodology, Writing - review & editing, Project administration, Funding acquisition.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1053/j.jrn.2020.02.005.

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