

Effects of a High-Protein Diet on Regulation of Phosphorus Homeostasis

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Context: High-protein diets, which are popular for weight loss, contain large quantities of phosphorus. Phosphorus excess and consequent changes in phosphorus regulatory hormones are implicated in vascular calcification and cardiovascular disease.

Objective: We tested the hypothesis that a moderate increase in dietary phosphorus during a high-protein diet leads to changes in phosphorus-responsive hormones.

Design, Participants, and Setting: We conducted a post hoc analysis of a sequential dietary modification trial in 19 healthy volunteers in the general community.

Intervention: Participants received 2 weeks of a weight-maintaining, low-protein (15%) diet, followed by 2 weeks of an isocaloric, high-protein (30%) diet, followed by 12 weeks of an ad libitum high-protein (30%) diet.

Main Outcome Measures: Using previously collected samples, plasma concentrations of fibroblast growth factor-23 (FGF-23), PTH, 1,25-dihydroxyvitamin D, and 24,25-dihydroxyvitamin D were measured at 8 time points to assess 24-hour variability and in 24-hour pooled samples to delineate changes at the end of each diet period.

Results: Mean dietary phosphorus intake during each study period was 1556, 2071, and 1622 mg/d, respectively. Plasma concentrations of FGF-23 and vitamin D metabolites varied in a diurnal pattern; plasma PTH concentrations varied in a bimodal pattern. After changing from a low- to high-protein isocaloric diet, plasma FGF-23 concentrations decreased slightly (mean -4.48 pg/mL, 95% confidence interval 1.88–7.07). There were no other statistically significant changes in phosphorus regulatory hormones in response to diet modifications.

Conclusions: Among healthy people, an approximate 33% increase in dietary phosphorus after institution of a high-protein diet does not cause large changes in measured concentrations of phosphorus regulatory hormones. (*J Clin Endocrinol Metab* 98: 1207–1213, 2013)

A high-protein diet has several potential health benefits. Observational data suggest a reduced risk of cardiovascular disease associated with high dietary protein (1). High-protein diets have proven efficacy for weight

loss (2–4), possibly through increasing satiety (5) or resting energy expenditure (6). However, the safety of increasing dietary protein is not proven. Increasing dietary protein necessarily increases dietary phosphorus.

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Abbreviations: BMI, body mass index; CV, coefficient of variation; eGFR, estimated glomerular filtration rate; FGF, fibroblast growth factor; GCRC, General Clinical Research Center; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D.

Phosphorus excess is a novel factor implicated in the development of cardiovascular disease. In the Framingham Offspring Study, higher serum phosphorus concentrations within the normal laboratory range are associated with left ventricular mass, incident heart failure (7), and cardiovascular events (8). Fibroblast growth factor (FGF)-23, a master regulatory hormone of phosphorus metabolism, is associated with cardiovascular events and mortality in populations with chronic kidney disease (9) and stable coronary heart disease (10). Moreover, phosphorus excess impairs the enzymatic activation of 25-hydroxyvitamin D [25(OH)D] to 1,25-dihydroxyvitamin D [1,25(OH)₂D]. Low circulating 1,25(OH)₂D is associated with death among coronary angiography patients (11) and with death and dialysis initiation among patients with chronic kidney disease (12).

Associations of metabolic markers of phosphorus metabolism with cardiovascular risk suggest the possibility that dietary phosphorus might play a role in cardiovascular disease. Previous studies have demonstrated modest changes in serum FGF-23 and 1,25(OH)₂D concentrations among healthy volunteers in response to large changes in dietary phosphorus (13–15). However, previous studies manipulated dietary phosphorus over an extreme range using phosphorus binders and supplements and were of relatively short duration. Although useful for demonstrating a physiologic principle, this approach does not inform the potential impact of realistic changes in dietary phosphorus, such as that found in high-protein diets used for weight loss.

We tested the hypothesis that a modest increase in dietary phosphorus from natural food sources as part of a high-protein diet alters primary phosphorus-responsive hormones: FGF-23, PTH, and 1,25(OH)₂D as well as the primary vitamin D metabolite 24,25-dihydroxyvitamin D [24,25(OH)₂D]. To test this hypothesis, we conducted a post hoc evaluation of blood samples from 19 healthy participants from a sequential trial of 3 controlled diets: a weight-maintaining moderate protein diet (period 1), an isocaloric high-protein low-fat diet (period 2), and an ad libitum high-protein low-fat diet (period 3), each with known quantities of dietary phosphorus (5).

Subjects and Methods

Study population

We used plasma samples that were previously collected as part of a sequential dietary modification trial for weight loss (5). A total of 19 healthy adults were recruited by newspaper advertisement from Seattle, Washington, and Portland, Oregon. The research protocol was approved by the Human Subjects Review Committee at the University of Washington and at Oregon Health and Science University. Inclusion criteria included stable weight for at least 3 months and currently at lifetime maximum weight. Exclusion criteria included body mass index (BMI) >30,

regular aerobic exercise, tobacco use, consumption of more than 2 alcoholic beverages daily, diabetes, chronic medical illness, or pregnancy. Subjects were not enrolled if they expressed expectation of losing weight as a direct result of the study. All subjects provided written informed consent before enrollment.

Study protocol and nutritional analysis

The nutritional protocol of this study has been described in detail elsewhere (5). Briefly, the study consisted of 3 periods. Period 1 was a 2-week run-in period in which participants were provided a diet resembling the average American diet, containing 50% of total calories from carbohydrate, 35% from fat, and 15% from protein. During this period, study dietitians adjusted total calories provided to keep weight constant. Period 2 was a 2-week high-protein period, isocaloric with the first period, in which participants were provided with a diet containing 50% of total calories from carbohydrate, 30% from protein, and 20% from fat. The total calorie content of meals was designed to be identical with the first period, and subjects were instructed to eat all of the food items. Period 3 was a 12-week high-protein, ad libitum period, in which the diet macronutrient composition remained 50% of total calories from carbohydrate, 30% from protein, and 20% from fat, but subjects were instructed to eat only until satiety. The period 1 diet included food items such as low-fat dairy products and oil-based condiments, whereas the period 2 and 3 diets substituted items such as fat-free dairy products, egg whites, and larger servings of lean meat. Throughout the study, participants maintained a daily food log, recorded information regarding appetite, and returned to the General Clinical Research Center (GCRC) every 2 to 3 days to meet with the study dietitian and pick up their next group of meals. All uneaten food items were returned to the GCRC and weighed to determine actual nutrient intake. At the end of each study period, participants were admitted to the GCRC for 24 hours and serial blood draws were performed every 30 minutes from 8:00 AM until 9:00 PM, then every hour until the following 8:00 AM. In the parent study, there was a mean weight loss of 4.9 kg during period 3. We reassessed the provided study diets for mineral content. Study dietitians used the U.S. Department of Agriculture 21 standard food composition database and the National Data System for Research 2010 to calculate the amount of phosphorus and calcium consumed, in milligrams per day, using the provided menus and records of returned foods.

Measurement of chemical and mineral metabolism biomarkers

From the serial blood draws performed in the parent study, we used samples obtained at 8 AM, 12 PM, 4 PM, 6 PM, 8 PM, 10 PM, 12 AM, and 2 AM to measure each mineral metabolism biomarker. We used the individual timed samples collected after period 1 to assess diurnal variation in biomarkers. In addition to the timed samples, a pooled 24-hour integrated sample was created by combining 50- μ l aliquots from each 30-minute time point and 100- μ l aliquots from each 60-minute time point to determine average 24-hour values. We used these pooled samples to evaluate changes in biomarkers after each study period. All samples were stored and maintained at -70°C . Due to limited sample volume, plasma 1,25(OH)₂D measurements were performed among only 16 of the 19 study participants at the end of each study period, and among only 14 of 19 subjects for the diurnal

Table 1. Energy and Micronutrient Consumption in the 15% and 30% Protein Diets

	Period 1	Period 2	Period 3
Energy composition	Weight-maintaining	Iso-caloric	Ad libitum
Total daily calories	2328 ± 318	2294 ± 338	1837 ± 507
Calorie source, %			
Protein	15 ± 0.1	29.5 ± 0.4	29.6 ± 0.3
Fat	35 ± 0.2	20.2 ± 0.6	20.2 ± 0.6
Carbohydrate	50 ± 0.3	50.3 ± 0.4	50.2 ± 0.4
Micronutrient composition			
Phosphorus, mg	1556 ± 271	2071 ± 346	1622 ± 442
Calcium, mg	1166 ± 284	1537 ± 286	1332 ± 356

Values are shown as means ± SD. Values are derived from measured food intake from all 19 study participants. Period 1 lasted 13 days, period 2 lasted 14 days, and period 3 lasted 101 to 115 days.

pattern of 1,25(OH)₂D variation. We used fasting morning samples to measure plasma phosphorus, calcium, and creatinine.

The University of Washington Nutrition and Obesity Research Center performed all laboratory measurements from frozen plasma samples. Calcium was measured using atomic absorption on a PerkinElmer Analyst 200 spectrometer (PerkinElmer, Waltham, Massachusetts). Phosphorus and creatinine were measured using the Beckman Synchron DxC automated clinical chemistry analyzer (Beckman Coulter, Inc, Brea, California). Phosphorus was measured using molybdate, and creatinine was measured using an isotope dilution-mass spectrometry-traceable Jaffe picric acid method. Intact FGF-23 was measured using a commercially available sandwich ELISA (Kainos Co, Tokyo, Japan) that measures the full-length (intact) molecule by recognizing both mid-molecule and distal epitopes. The coefficient of variation (CV) was 12.4%. Intact PTH was quantified using a 2-site immunoassay on a Beckman Unicel DxI clinical analyzer, with a CV of 6.1%. Plasma total 1,25(OH)₂D was measured by immunoaffinity enrichment HPLC-tandem mass spectrometry (Xevo TQ by Waters Corp, Milford, Massachusetts) with a CV of 13%. Total 24,25(OH)₂D was measured using HPLC-tandem mass spectrometry on a Waters Quattro Micro mass spectrometer (CV 14.7%). Plasma 25(OH)D was measured using HPLC-tandem mass spectrometry on a Waters Quattro Micro mass spectrometer. Interassay coefficient of variation was <3.4%.

Statistical analysis

We computed mean and SD for baseline characteristics and daily intakes of phosphorus, calcium, and total kilocalories. We used repeated-measures ANOVA, clustered by individual study subject, to test for differences in geometric mean values of plasma FGF-23, PTH, 1,25(OH)₂D, and 24,25(OH)₂D concentrations across the 8 sample collection points during the 24-hour period. We tested geometric means because the values of biomarkers were not normally distributed and the sample size was not large. We used repeated-measures ANOVA, clustered by individual study subject, to test for differences in micronutrient intake. When the ANOVA test indicated significant differences across the 3 diets, we compared individual pairs of biomarkers using the paired *t* test. We used repeated-measures ANOVA, clustered by individual study subject, to test for differences in mean values of plasma calcium, phosphorus, creatinine, and estimated glomerular filtration rate (eGFR), as well as mean values of integrated plasma FGF-23, PTH, 1,25(OH)₂D, and 24,25(OH)₂D concentrations at the end of each diet period. All *P* values were 2-sided.

Analyses were performed using STATA version 11IC (STATA Corp, College Station, Texas).

Results

Baseline characteristics and nutritional analysis

Among the 19 study participants, the mean age was 41.4 ± 10.8 years and mean BMI was 26.2 ± 2.1. Sixteen of 19 study participants (84.2%) were female. At baseline, mean plasma 25(OH)D level was 23.6 ng/mL (interquartile range 17–28.6 ng/mL). Three of 19 participants (15%) had plasma 25(OH)D level <15 ng/mL. Seven of 19 participants (36%) had plasma 25(OH)D level <20 ng/mL. Glomerular filtration rate, estimated by the Chronic Kidney Disease Epidemiology Collaboration equation (16), was >60 ml/min/1.73 m² for all participants. Mean daily energy intake during period 1, period 2, and period 3 were 2328 ± 318, 2294 ± 338, and 1837 ± 507 kcal, respectively (Table 1). Dietary phosphorus intake increased by an estimated 33% during period 2 and then returned to approximately baseline values during period 3. Dietary calcium intake roughly paralleled that of dietary phosphorus. Changes in dietary intake of phosphorus, calcium, and energy were all statistically significant (*P* < .001) by ANOVA.

Diurnal variation in phosphorus biomarkers

Plasma FGF-23 concentrations varied in a diurnal pattern with a relative peak occurring at 8:00 AM and relative nadir at 8:00 PM (Figure 1). Plasma PTH concentrations varied in a bimodal pattern, with two relative peaks occurring at 4:00 PM and 2:00 AM. Vitamin D metabolites followed a diurnal pattern, with 4:00 PM and 6:00 PM relative peaks for plasma 1,25(OH)₂D, and 24,25(OH)₂D concentrations, respectively.

Response to dietary changes

Plasma calcium, phosphorus, and creatinine did not vary between study periods (Table 2). Switching from a

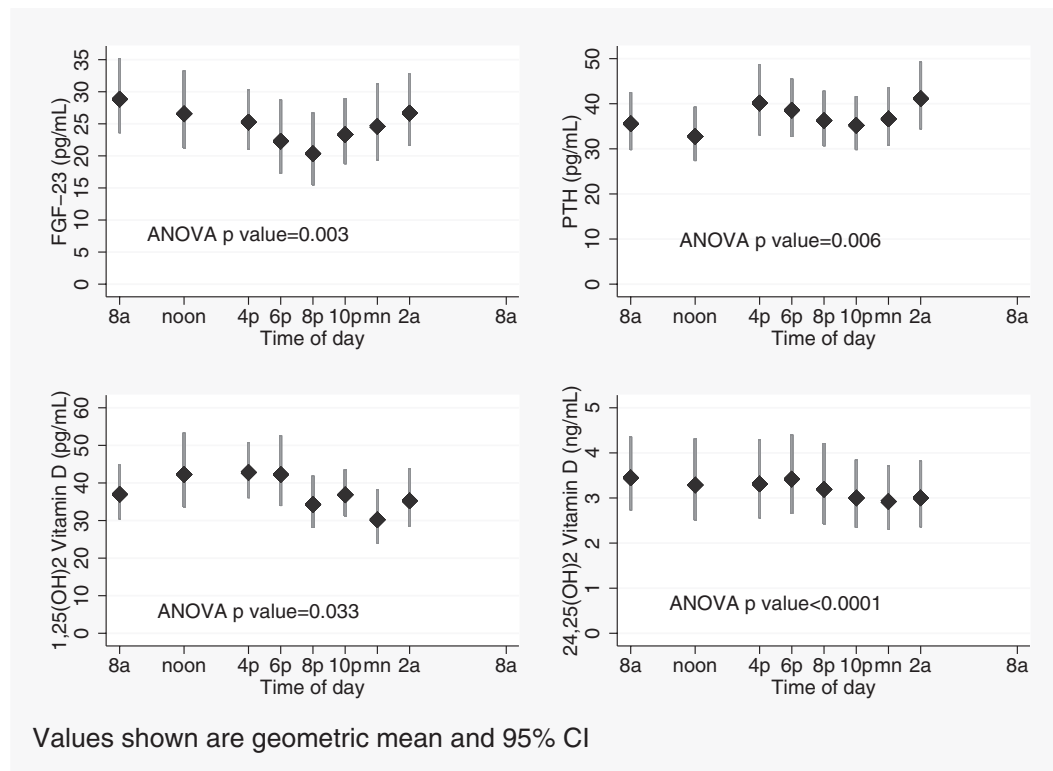


Figure 1. Diurnal variation in phosphorus-responsive hormones at end of period 1 (15% protein, 35% fat, 50% carbohydrate diet). The y-axes depict geometric mean and 95% confidence interval of each hormone; x-axes depict time of day. *P* values are from repeated-measures ANOVA performed on measured biomarker values.

15% weight-maintaining to a 30% protein isocaloric diet resulted in a decrease in mean plasma FGF-23 concentrations from 33.6 ± 7.0 to 29.1 ± 5.7 pg/mL (Table 3 and Figure 2; *P* value for period 2 vs period 1 = 0.002). The ad libitum diet, which returned dietary phosphorus intake close to baseline levels, resulted in an intermediate plasma FGF-23 concentration that was not significantly different from either of the other dietary periods (final mean FGF-23 value 32.6 ± 10.7 pg/mL). Plasma PTH concentration did not significantly change during period 2 or period 3. Similarly, there were no significant changes in vitamin D metabolites 1,25(OH)₂D or 24,25(OH)₂D (Table 3).

Discussion

We found that moderate changes in dietary phosphorus intake from natural food sources did not have a large im-

act on phosphorus-responsive hormone blood levels among healthy subjects. Specifically, 2 weeks of a high-protein diet, which increased dietary phosphorus by 33%, did not cause a large change in plasma concentrations of intact FGF-23, intact PTH, 1,25(OH)₂D, or 24,25(OH)₂D. Subsequently, an approximately 30% reduction in dietary phosphorus during a 12-week ad libitum diet did not affect plasma concentrations of any of these phosphorus metabolism hormones. During the ad libitum period, subjects lost an average of 4.9 kg of body weight and 3.7 kg of fat mass, with no significant weight loss during the first 2 periods (5). Although our findings are limited to a relatively small number of selected individuals, they suggest that realistic changes in dietary phosphorus consumption that are part of a high-protein diet for weight loss are unlikely to provoke a harmful metabolic response in terms of the hormones measured in this study. It is notable that

Table 2. Plasma Calcium, Phosphorus, Creatinine, and Estimated GFR at End of Study Periods

	n	15% Protein Weight-Maintaining (13 d) ^a	30% Protein Isocaloric (14 d) ^a	30% Protein Ad Libitum (112 d) ^a	ANOVA <i>P</i> Value
Plasma phosphorus, mg/dL	19	2.9 ± 0.7	2.8 ± 0.5	2.8 ± 0.6	.71
Plasma calcium, mg/dL	19	8 ± 1	8 ± 0.6	8.3 ± 0.8	.16
Plasma creatinine, mg/dL	19	0.61 ± 0.2	0.59 ± 0.17	0.62 ± 0.19	.11
Estimated GFR, mL/min/1.73 m ²	19	118 ± 24	119 ± 18	114 ± 18	.06

^a Values shown as mean \pm SD.

Table 3. Phosphorus-Related Biomarkers at End of Study Periods

Biomarker	n	15% Protein Weight-Maintaining (13 d) ^a	30% Protein Isocaloric (14 d) ^a	30% Protein Ad Libitum (112 d) ^a	ANOVA P Value
FGF-23, pg/mL	19	33.6 ± 7	29.1 ± 5.7	32.6 ± 10.7	.02
PTH, pg/mL	19	35.4 ± 11.1	35 ± 11	35.4 ± 11.7	.94
1,25(OH) ₂ D, pg/mL	16	49.1 ± 10.7	50 ± 10.2	46.9 ± 12.2	.42
24,25(OH) ₂ D, ng/mL	19	3.8 ± 2.3	3.8 ± 2	4.1 ± 2.3	.55

^a Values shown as mean ± SD.

the observed increase in dietary calcium did not suppress plasma PTH levels.

Serum phosphorus concentrations were first associated with mortality and cardiovascular risk among individuals who have chronic kidney disease. These observations were subsequently extended to the general population. For example, higher serum phosphorus concentrations within the normal laboratory range are associated with incident cardiovascular events (8) and heart failure (7) among 3368 disease-free individuals in the Framingham Offspring Cohort Study and with coronary events and mortality among 4127 participants in the Cholesterol and Recurrent Events

Trial (17). Moreover, higher serum phosphorus concentrations are associated with aortic valve calcification among older adults in the Cardiovascular Health Study (18) and with coronary artery calcification among young adults in the Coronary Artery Risk Development in Young Adults (CARDIA) study (19). Dystrophic calcification has emerged as a candidate mechanism to explain these findings, because phosphorus directly transforms cultured vascular smooth muscle cells into osteoblast-like cells that calcify surrounding matrix proteins (20).

Other biomarkers of phosphorus metabolism are also linked with cardiovascular disease outcomes. High phos-

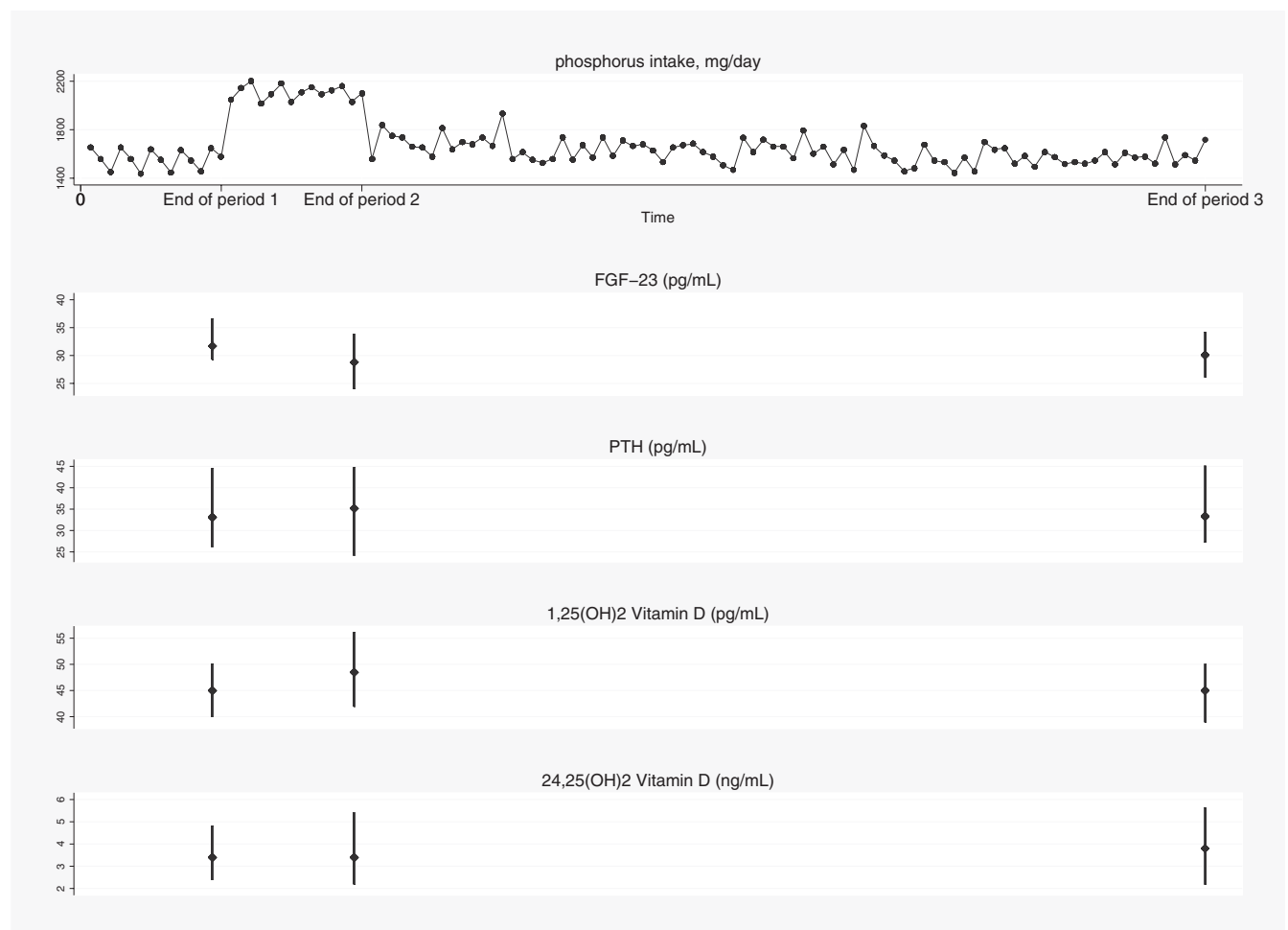


Figure 2. Change in phosphorus-responsive hormones in response to dietary changes. Values shown are median and interquartile range. Time is shown on the x-axis. Separate graphs show daily phosphorus intake, plasma FGF-23, plasma PTH, plasma 1,25(OH)₂D, and plasma 24,25(OH)₂D.

phorus concentrations impair conversion of 25(OH)D to 1,25(OH)₂D, the active form of the vitamin D. In mice, inadequate 1,25(OH)₂D stimulates the renin-angiotensin system (21). In prospective human studies, lower circulating concentrations of 25(OH)D are associated with inflammatory pathways and with cardiovascular outcomes (22). FGF-23, a bone-derived hormone that stimulates phosphorus excretion through the kidneys, is associated with all-cause mortality among chronic kidney disease patients in the Chronic Renal Insufficiency Cohort (9), among stable coronary heart disease patients in the Heart and Soul study (10), and among community-living older persons in the Cardiovascular Health Study (23). Collectively, these findings raise concern that dietary phosphorus excess could have potentially harmful effects on the cardiovascular system.

Some previous studies have observed an impact of dietary phosphorus on FGF-23. However, these studies involved extreme and arduous changes in diet. In chronic kidney disease, reduction in protein intake to 0.3 g/kg/d (and phosphorus intake <500 mg/d) leads to a decline in FGF-23 level (24). Among healthy volunteers, FGF-23 increases with the use of large doses of phosphorus supplements (13, 14, 25) or with the consumption of more than double the typical American phosphorus intake (15). In a study of healthy men (14), FGF-23 levels did not change when phosphorus intake increased from 1500 mg daily to 2300 mg daily, a dietary change similar to that observed in our study. Although unexpected, our study showed a biologically small, but statistically significant decrease in plasma FGF-23 concentration with dietary phosphorus increase. Our study is the first to examine phosphorus regulatory hormones in subjects after a particular diet for more than 9 days. Within limitations of a small sample size and restricted generalizability, our data suggest that among healthy subjects, plasma levels of the phosphorus regulatory hormones FGF-23, PTH, and 1,25(OH)₂D do not substantially change in response to realistic changes in dietary phosphorus, implying that the response to dietary phosphorus in healthy people is regulated through mechanisms that do not involve these hormones. Dietary-protein-induced increase in the GFR (26) could lower serum FGF-23 concentrations independently; however, estimated GFR did not change with conversion to a high-protein diet in our study.

Contrary to our findings, a previous study of 8 healthy volunteers reported that FGF-23 does not vary over the course of the day (27). Similar to the data presented here, that study also found mean FGF-23 concentrations varied from 20 to 30 pg/mL over 24 hours. Whereas that study did not find diurnal variation in FGF-23 concentration, we find that there is a statistically significant difference in values measured at different time points, with a clear nadir at 8:00 PM. Although the reason for this diurnal variation

is not clear, the practical implication is that clinical studies of FGF-23 levels should use consistent sampling times for valid interindividual comparisons.

Our study has several strengths. All foods consumed by study subjects over the entire 16-week study period were prepared by dietitians in a research kitchen, which included weighing all food items that were provided as well as those returned by subjects. Study personnel met with participants at least twice weekly to ensure compliance with the dietary regimen. The prevalence of 25(OH)D deficiency at study entry was consistent with National Health and Nutrition Examination Survey assessments between 2001 and 2006 (28). Plasma biomarker concentrations were measured on pooled samples prepared from aliquots of plasma collected during a 24-hour period, reducing intra-individual variability. The sequential study design permitted evaluation of dietary changes within the same individual, mitigating the potential for confounding. The 12-week ad libitum diet period was considerably longer than that of any previously described study. Finally, manipulation of dietary phosphorus was performed using only naturally occurring foods, without phosphorus supplements. Therefore, our results are more directly applicable to typical changes in dietary patterns, such as increasing dietary protein for the goal of weight loss.

Our study also has some important limitations. The relatively small sample size increases variability of estimated effects of diet on phosphorus-responsive hormones; however, our results exclude large changes. Our study population was Caucasian and predominantly female; it is unknown whether study findings would apply to more diverse populations. However, restricting our analysis to females only did not change the findings. It is possible that other dietary components that coexist with phosphorus could counter the impact on regulatory hormones. However, the use of natural foods to raise phosphorus in this study is practically relevant. Phosphorus restriction was not part of the initial trial design; therefore, we could compare only moderate versus high phosphorus intake. We did not have urine samples to measure excretion of calcium, phosphorus, and creatinine. Given published cross-sectional associations of BMI and FGF-23 (29), it is possible that the weight loss experienced during the final period of the trial may have attenuated the final measurement of FGF-23.

In summary, we found that a high-protein diet used for weight loss does not cause a large change in phosphorus-responsive hormones in a small group of healthy participants. If these results are confirmed in a larger study, one interpretation is that high-protein diets that use naturally occurring protein sources are generally safe in terms of not provoking an adverse phosphorus hormonal response

while providing the benefit of decreased body weight and body fat mass. A second interpretation is that yet undiscovered mechanisms are responsible for the regulation of phosphorus homeostasis in the context of relatively modest changes in dietary phosphorus consumption.

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