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# Dietary Phosphorus Regulates Serum Fibroblast Growth Factor-23 Concentrations in Healthy Men

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**Context:** Fibroblast growth factor 23 (FGF-23) is important in the regulation of phosphorus and vitamin D metabolism. States of excess circulating FGF-23 are associated with renal phosphate wasting and inappropriately low serum 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] concentrations. Conversely, states of absent or biologically inactive circulating FGF-23 are associated with increased serum phosphorus and 1,25$(OH)_2$D concentrations. Restriction of the dietary intake of phosphorus increases renal phosphate reabsorption and 1,25$(OH)_2$D production, whereas the opposite occurs when dietary phosphorus is supplemented.

**Objective:** We sought to determine whether serum FGF-23 concentration is regulated by dietary phosphorus and thereby mediates the physiological response of serum 1,25$(OH)_2$D to changes in dietary phosphorus.

**Design, Setting, and Participants:** We studied 13 healthy men as inpatients during a 4-wk dietary phosphorus intervention study.

**Intervention:** Subjects consumed a constant diet that provided 500 mg of phosphorus per day, which was supplemented to achieve three phosphorus intakes, each of 9 d: 1) control = 1500 mg/d; 2) supplemented = 2300 mg/d; 3) restricted = 625 mg/d. Intakes of calcium, sodium, potassium, magnesium, and energy were constant.

**Main Outcome Measure:** Serum FGF-23, 1,25$(OH)_2$D, phosphorus, and calcium concentrations were measured.

**Results:** Serum FGF-23 concentrations decreased significantly from 30.7 ± 8.7 pg/ml during phosphorus supplementation to 19.6 ± 7.0 pg/ml during phosphorus restriction. Serum 1,25$(OH)_2$D concentrations increased significantly from 29 ± 10 pg/ml (75 ± 26 pmol/liter) during phosphorus supplementation to 40 ± 16 pg/ml (104 ± 42 pmol/liter) during phosphorus restriction ($P < 0.001$). Serum 1,25$(OH)_2$D concentrations varied inversely with those of serum FGF-23 ($r = -0.67$, $P < 0.001$).

**Conclusions:** We conclude that in healthy men, changes in dietary phosphorus within the physiological range of intakes regulate serum FGF-23 concentrations and suggest that dietary phosphorus regulation of 1,25$(OH)_2$D production is mediated, at least in part, by changes in circulating FGF-23. **(*J Clin Endocrinol Metab* 91: 3144–3149, 2006)**

PHOSPHORUS HOMEOSTASIS IS determined by the dietary intake, intestinal absorption, and renal tubular reabsorption of phosphorus. Phosphorus is abundant in the typical Western diet, and its gastrointestinal absorption is efficient. An increase in dietary phosphorus induces a decrease in renal phosphate reabsorption, whereas dietary phosphorus restriction results in an increase in phosphate reabsorption. Diet-induced changes in renal phosphate reabsorption are mediated by changes in the abundance of the type 2a sodium-phosphate cotransporter ($NaP_i$-IIa) protein on the apical surface of renal proximal tubule cells (1, 2). The type 2c sodium-phosphate cotransporter, which also is expressed in proximal tubular cells and regulated by dietary phosphorus, may account for up to 30% of sodium-dependent phosphate transport in mice fed a low-phosphorus diet (1–6). Although PTH can modulate the abundance of $NaP_i$-IIa in the apical membrane, PTH does not play a role in regulating $NaP_i$-IIa expression in response to variation in dietary phosphorus intake because urinary phosphorus excretion in parathyroidectomized rats is regulated appropriately in response to variation in dietary phosphorus (7). Similarly, renal phosphate handling responds appropriately to variation in phosphorus intake independently of vitamin D status (8–10). It has therefore long been postulated that other regulators of renal phosphate reabsorption exist. In recent years, considerable molecular and genetic evidence has accrued to suggest that one such factor is fibroblast growth factor 23 (FGF-23).

FGF-23 is a circulating 26-kDa peptide produced by osteogenic cells (11, 12). States of excess circulating FGF-23, such as tumor-induced osteomalacia, X-linked hypophosphatemia, or autosomal dominant hypophosphatemic rickets are characterized by severe hypophosphatemia due to renal phosphate wasting, rickets or osteomalacia, and inappropriately normal or low serum concentrations of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] (13–17). Conversely, patients with tumoral calcinosis, in which secretion of intact FGF-23 is impaired, and FGF-23 null mice are characterized by hyperphosphatemia and increased serum 1,25$(OH)_2$D concentrations, demonstrating the importance of FGF-23 in the physiological regulation of 1,25$(OH)_2$D metabolism (18–23).

Published studies of the effects of dietary phosphorus on

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Abbreviations: FGF-23, Fibroblast growth factor 23; $NaP_i$-IIa, type 2a sodium-phosphate cotransporter; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; TmPi/GFR, renal tubular reabsorption of phosphate.

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serum FGF-23 concentrations in humans have yielded disparate results. One study found that a high dietary phosphorus intake was associated with an increase in serum FGF-23 concentration (24), whereas another observed no change in serum FGF-23 in men ingesting a high-phosphorus diet (25). Similarly, the effects of restricting dietary phosphorus on serum FGF-23 have been varied because phosphorus restriction has been associated with either a decrease (24) or no effect on serum FGF-23 concentration (25). Studies in experimental animals, on the other hand, have yielded more consistent results and indicate that circulating FGF-23 is regulated by dietary phosphorus intake. In normal rats and mice, serum FGF-23 concentrations vary directly with dietary phosphorus intake (26, 27) and serum phosphorus concentrations (26, 28).

Given the data in animals and the conflicting reports in humans on the effects of dietary phosphorus on serum FGF-23 concentrations, we sought to determine whether altering dietary phosphorus intake in healthy men, while maintaining all other nutrients constant, would affect the serum concentration of intact, biologically active FGF-23 and, if so, whether dietary phosphorus-induced changes in serum $1,25(OH)_2D$ concentrations could be linked to changes in serum FGF-23 concentrations.

## Subjects and Methods

### *Study population*

We studied 13 healthy men (aged 28–43 yr) to determine the effect of changes in dietary phosphorus intake on the serum concentration of FGF-23. All subjects were hospitalized for the duration of the study on the General Clinical Research Center under a protocol approved by the Committee on Human Research at the University of California, San Francisco. Written informed consent was obtained from each subject.

### *Treatment*

All subjects consumed a constant whole-food diet prepared by the General Clinical Research Center's metabolic kitchen. The diet provided 500 mg phosphorus, 200 mg calcium, 100 mg magnesium, and 70 meq sodium per 70 kg body weight per day (dietary intakes are subsequently reported per 70 kg of body weight). Throughout the study, the intakes of magnesium and calcium were constantly maintained at 350 and 850 mg/d by supplementing the diet with orally administered magnesium sulfate and calcium carbonate, respectively. The 4-wk study included three periods of equal duration, each with differing dietary intakes of phosphorus. Goal total oral phosphorus intake was achieved by supplementing the whole-food diet with a solution of neutral sodium and potassium phosphate (4:1 mixture of $Na_2HPO_4/K_2HPO_4$ and $NaH_2PO_4/KH_2PO_4$, 31 mg phosphorus, 0.9 meq sodium, and 0.9 meq potassium per 5 ml). During the first study period (control), 1000 mg/d supplemental phosphorus was administered in divided doses with each meal; the total phosphorus intake was 1500 mg/d. During the second period (supplemented), dietary phosphorus was supplemented to 2300 mg/d. Throughout the final period (restricted), dietary phosphorus was restricted to 625 mg/d by replacing the sodium and potassium phosphate supplement with an equimolar amount of sodium and potassium chloride. Phosphorus intakes of 2300 and 625 mg/d are approximately the 75th and 25th percentile values, respectively, for healthy men 30–40 yr old ingesting typical diets (29). The intakes of sodium and potassium were constantly maintained throughout the study by administering additional sodium and potassium chloride during the control and phosphorus restricted periods, equal to the amount administered during the phosphorus-supplemented period. The basic diet provided, by calculation, 2000 kcal/d, 9% as protein, 34% as fat, and 57% as carbohydrate.

### *Biochemical assessment*

Blood was drawn in the morning fasting state during the last 2 d of each study period, and urine was collected throughout the last 24 h of each study period. Serum samples were analyzed for concentrations of $1,25(OH)_2D$, PTH, calcium, and phosphorus. Urine samples were analyzed for phosphorus, calcium, and creatinine concentrations. Serum samples also were frozen at −70 C for subsequent determination of FGF-23 concentrations.

Serum concentration of $1,25(OH)_2D$ was measured in duplicate using a competitive protein binding assay (30). Inter- and intraassay coefficients of variation of $1,25(OH)_2D$ in serum were 13.4 and 12.6%, respectively, at a serum concentration of 31 pg/ml. Serum concentrations of intact PTH were measured in duplicate by immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA); intraassay coefficient of variation was less than 6%. Serum concentrations of calcium were measured by atomic absorption spectrophotometry, serum and urinary concentrations of phosphorus by a modification of the Fiske-Subbarow method (31), and urinary concentration of creatinine by autoanalyzer. The threshold for renal tubular reabsorption of phosphate (TmPi/GFR) was calculated using the Bijvoet nomogram (32). Serum intact FGF-23 concentration was measured by sandwich ELISA (Kainos Laboratories, Inc., Tokyo, Japan) as previously described (33) using antibodies against the N- and C-terminal portions of human FGF-23 that detect full-length FGF-23.

### *Statistical analyses*

Data are presented as group means ± SD. The primary outcome of the effect of dietary phosphorus on serum concentrations of FGF-23 was analyzed with cross-sectional time-series generalized estimating equations. Using Fisher's least significant difference approach, we first tested the null hypothesis of homogeneity of the effect of different dietary phosphorus intake across the three study phases; when heterogeneity was detected at $P < 0.05$ in a two-sided test, we then carried out the pairwise comparisons of the treatment effects among the three study phases without adjustment of significance levels.

Correlation coefficients were calculated between the serum FGF-23 concentration and, sequentially, serum $1,25(OH)_2D$ concentration, serum PTH concentration, and urinary phosphorus excretion, considering $P < 0.05$ as significant. All analyses were performed using Intercooled Stata 8.0 (Stata Corp., College Station, TX).

## Results

### *FGF-23 and phosphorus intake*

Changes in dietary phosphorus intake had a significant effect on serum FGF-23 concentrations (Table 1 and Fig. 1). When dietary phosphorus was 1500 mg/d, the serum FGF-23 concentration was 28.9 ± 5.7 pg/ml. When dietary phosphorus was supplemented to 2300 mg/d, serum FGF-23 concentration increased slightly but not significantly to 30.7 ± 8.7 pg/ml. When dietary phosphorus was then restricted to 625 mg/d, serum FGF-23 concentrations decreased to 19.6 ± 7.0 pg/ml, a value significantly lower than that during the control or supplemented phosphorus periods ($P < 0.001$).

### *Serum $1,25(OH)_2D$*

Dietary phosphorus intake had a significant effect on serum $1,25(OH)_2D$ concentrations. Serum $1,25(OH)_2D$ concentrations were highest when phosphorus was restricted (40 ± 16 pg/ml, or 104 ± 42 pmol/liter) and lowest when phosphorus was supplemented (29 ± 10 pg/ml, or 75 ± 26 pmol/liter, $P < 0.001$) (Table 1 and Fig. 1). Across the range of dietary phosphorus, serum $1,25(OH)_2D$ concentrations varied inversely with those of serum FGF-23 ($r = -0.67$, $P < 0.001$) (Fig. 2).

**TABLE 1.** Effect of dietary phosphorus on serum concentrations in healthy men

| Dietary phosphorus | Phosphorus (mg/dl) | Total calcium (mg/dl) | FGF-23 (pg/ml) | $1,25(OH)_2D$ (pg/ml) | Intact PTH (pg/ml) |
|---|---|---|---|---|---|
| Control (1500 mg/d) | 3.6 ± 0.4 | 9.5 ± 0.3 | 28.9 ± 5.7 | 32 ± 9 | 21 ± 6 |
| Supplemented (2300 mg/d) | 3.7 ± 0.3 | 9.6 ± 0.5 | 30.7 ± 8.7 | 29 ± 10 | 18 ± 8 |
| Restricted (625 mg/d) | 3.8 ± 0.4 | 9.7 ± 0.6 | 19.6 ± 7.0[a,b] | 40 ± 16[b,c] | 14 ± 6[b,c] |
| Diet effect | $P = 0.13$ | $P = 0.07$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

Data are mean ± SD To convert serum phosphorus to millimoles per liter, multiply by 0.323; to convert serum total calcium to millimoles per liter, multiply by 0.25; to convert serum $1,25(OH)_2D$ to picomoles per liter, multiply by 2.6; to convert serum PTH to nanograms per liter, multiply by 1.0.

[a] $P < 0.001$. Mean value differs from that during 1500 mg/d dietary phosphorus.

[b] $P < 0.001$. Mean value differs from that during 2300 mg/d dietary phosphorus.

[c] $P < 0.05$. Mean value differs from that during 1500 mg/d dietary phosphorus.

### *Intact PTH*

Dietary phosphorus intake modestly affected serum intact PTH concentrations. When phosphorus was restricted, serum PTH decreased from 21.0 ± 6.3 to 14.4 ± 6.3 pg/ml ($P = 0.003$) (Table 1). When dietary phosphorus was then supplemented, serum PTH concentrations increased to 17.7 ± 8.0 pg/ml ($P < 0.001$). This value nevertheless remained lower than that during control phosphorus intake. There was no relationship between serum FGF-23 and intact PTH concentrations ($r = -0.19$, $P = 0.3$).

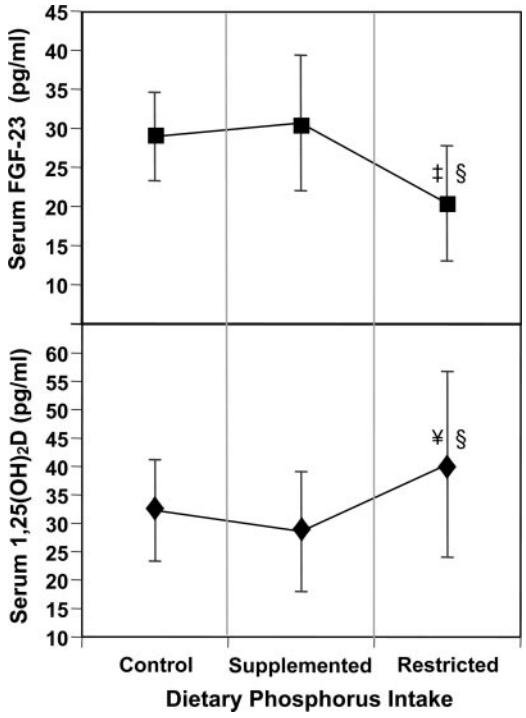


FIG. 1. Effects of restriction and supplementation of dietary phosphorus on serum concentrations of FGF-23 (*upper panel*) and $1,25(OH)_2D$ (*lower panel*). Depicted are mean ± SD. Subjects consumed a control phosphorus intake (1500 mg/d) during the first study period, a supplemented phosphorus intake (2300 mg/d) during the second period, and a restricted phosphorus intake (625 mg/d) during the last study period. When dietary phosphorus was restricted to 625 mg/d, serum FGF-23 concentrations were significantly lower than those during the control (‡, $P < 0.001$) or supplemented phosphorus intakes (§, $P < 0.001$). When dietary phosphorus was restricted to 625 mg/d, serum $1,25(OH)_2D$ concentrations were significantly higher than those during the control (¥, $P < 0.05$) or supplemented phosphorus intakes (§, $P < 0.001$). To convert serum $1,25(OH)_2D$ to picomoles per liter, multiply by 2.6.

### *Urinary and serum phosphorus*

When dietary phosphorus was supplemented, urinary phosphorus excretion increased by 78% ($P < 0.001$), whereas when dietary phosphorus was then restricted, urinary phosphorus decreased by 71% ($P < 0.001$) relative to values on the high phosphorus intake (Table 2). When dietary phosphorus was supplemented, the TmPi/GFR decreased by 28% ($P < 0.001$) relative to values on the control phosphorus intake (Table 2). Serum phosphorus concentrations, which were measured in the morning fasting state, did not change with alterations in dietary phosphorus intake (Table 1), nor did they correlate with serum FGF-23 concentrations ($r = 0.05$, $P = 0.7$).

### *Urinary and serum calcium*

Urinary calcium excretion varied directly with dietary phosphorus intake ($P < 0.001$) (Table 2). In contrast, serum calcium concentrations remained constant throughout the three study periods (Table 1).

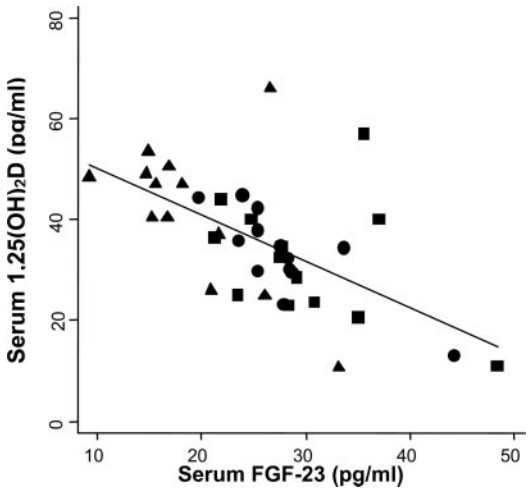


FIG. 2. Relationship between serum FGF-23 and $1,25(OH)_2D$ concentrations in healthy men during control (1500 mg/d) (●), supplemented (2300 mg/d) (■), or restricted phosphorus intake (625 mg/d) (▲). Serum $1,25(OH)_2D$ concentrations varied inversely with those of serum FGF-23 ($r = -0.067$, $P < 0.001$). To convert serum $1,25(OH)_2D$ to picomoles per liter, multiply by 2.6.

**TABLE 2.** Effect of dietary phosphorus on urinary parameters in healthy men

| Dietary phosphorus | Phosphorus (mg/d) | Calcium (mg/d) | FEPi (%) | TmPi/GFR (mg/liter) |
|---|---|---|---|---|
| Control (1500 mg/d) | 1165 ± 293 | 218 ± 86 | 18.6 ± 5.1 | 3.9 ± 0.9 |
| Supplemented (2300 mg/d) | 2069 ± 591[a] | 176 ± 110 | 30.4 ± 10.5[a] | 2.8 ± 0.7[a] |
| Restricted (625 mg/d) | 593 ± 199[a,b] | 321 ± 134[a,b] | 10.5 ± 6.4[a,b] | 3.9 ± 0.6[b] |
| Diet effect | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

Data are mean ± SD. FEPi, Fractional urinary excretion of phosphorus.
[a] $P < 0.001$. Mean value differs from that during 1500 mg/d dietary phosphorus.
[b] $P < 0.001$. Mean value differs from that during 2300 mg/d dietary phosphorus.

## Discussion

The present study demonstrates that in healthy men, manipulation of dietary phosphorus intake within a physiological range can regulate the serum concentration of intact FGF-23. When subjects ingested a high-normal phosphorus diet (2300 mg/d), serum FGF-23 concentrations were 57% higher than those observed when dietary phosphorus was low-normal (625 mg/d). Similarly, when phosphorus intake was 1500 mg/d, serum FGF-23 concentrations were 32% higher than those observed on the low-normal phosphorus intake.

Our findings are consistent with those in one (24) but not another (25) published report. Ferrari *et al.* (24) studied men as outpatients during a 5-d study period that included a restricted phosphorus and calcium intake, a 2-d washout period, and a high phosphorus and calcium intake (24). The investigators found that serum FGF-23 concentrations increased by 59% during the high dietary phosphorus and calcium period, compared with the restricted period. In that study, both phosphorus and calcium intakes were modified and other nutrients were not maintained constant throughout the study because participants prepared their own food at home. In contrast, in the present report we studied men as inpatients and provided them with a constant diet throughout the study except for alteration of phosphorus intake. The intakes of other nutrients that might have affected the calciotropic system, such as calcium, sodium, potassium, and magnesium, were unchanged throughout the study period. Thus, the results of the present study demonstrate that it is indeed dietary phosphorus that modulates serum FGF-23 concentration, rather than changes in the intake of calcium or other nutrients.

In the present study, we used an assay that measures only the intact FGF-23 protein (33), whereas Ferrari *et al.* (24) used an assay that measures both the intact and C-terminal portions of FGF-23. It has been shown that intact FGF-23 is the biologically active portion of the protein, responsible for inducing phosphaturia and hypophosphatemia (35). States in which only C-terminal FGF-23 and virtually no intact FGF-23 are present in the circulation are associated with impaired phosphate excretion and hyperphosphatemia, suggesting that the C-terminal portion of FGF-23 is inactive (18). It is reported that an assay that measures only the C-terminal portion of FGF-23 is less sensitive in detecting increases in serum FGF-23 concentration in patients with X-linked hypophosphatemia and tumor-induced osteomalacia (36). Thus, the present study demonstrates that alterations in dietary phosphorus induce changes in the concentration of biologically active FGF-23. Furthermore, the present data demonstrate that altering dietary phosphorus within a physiological range induced anticipated changes in serum $1,25(OH)_2D$ concentrations (37–40) and suggest that such changes are mediated by the alterations in FGF-23 concentrations. By contrast, Ferrari *et al.* did not observe significant differences in serum $1,25(OH)_2D$ concentrations among the three dietary phosphorus interventions used.

Our study design and findings also differ from those of Larsson *et al.* (25), who studied six healthy men as outpatients during a total period of 6 d. No intervention took place until study d 2 and 3, when subjects consumed a liquid diet along with a phosphate binder. During study d 4–6, participants consumed a normal diet supplemented with 2500 mg of phosphorus. Thus, the modifications in phosphorus intake were of shorter duration (2–3 d each) than in the present study (9 d) and resulted in no change in either serum FGF-23 or $1,25(OH)_2D$ concentration. The absence of change in serum $1,25(OH)_2D$ concentration in the Larsson study suggests that the alteration in phosphorus intake was not of sufficient magnitude or duration. Larsson *et al.* also used an assay that detects both intact and C-terminal portions of the protein, whereas we used an assay that detects only intact circulating FGF-23. It is not known whether the different findings in the two studies can be attributed to differences in the metabolism of intact compared with C-terminal FGF-23 in response to alterations in dietary phosphorus, or in the sensitivity of the two assays. Similarly, the different findings between the two studies might reflect differences in the duration of dietary intervention and in the sample size of six in the Larsson study, which might have been too small to detect a significant change in the parameters of interest.

The present study did not address the question of the mechanism by which changes in dietary phosphorus induce changes in circulating FGF-23. We and others have shown in normal rats and mice that FGF-23 concentrations vary directly with dietary phosphorus intake and with diet-induced changes in serum phosphorus concentrations (26, 27), suggesting that such changes in circulating FGF-23 are mediated by changes in the serum concentration of phosphorus. In those studies, dietary phosphorus was altered over an extreme range (from 0.02 to 1.6%) leading to significant changes in serum phosphorus concentrations. In humans, serum FGF-23 and phosphorus concentrations are found to correlate directly with each other, both in health and in disorders of phosphate homeostasis (12, 25, 41, 42). In the current study, we did not detect a significant change in morning fasting serum phosphorus concentrations when dietary phosphorus was altered. However, we previously showed that when dietary phosphorus is altered within a physio-

logical range from high-normal (2300 mg/d) to low-normal (625 mg/d) intakes, serum phosphorus concentrations in the afternoon and evening decrease significantly, whereas morning fasting phosphorus concentrations do not change (40).

It has long been known that dietary phosphorus intake regulates serum $1,25(OH)_2D$ concentration by regulating its renal production rate (38, 40). In the present study, we found that in healthy men, serum concentration of $1,25(OH)_2D$ increased significantly by 38% when phosphorus intake was decreased from high-normal to low-normal intakes. The serum concentrations of $1,25(OH)_2D$ varied inversely and significantly with those of FGF-23. These findings suggest that the regulation of $1,25(OH)_2D$ by dietary phosphorus in healthy men is mediated, at least in part, by diet-induced changes in circulating FGF-23. Our results are consistent with findings in experimental animals, which indicate that FGF-23 can regulate $1,25(OH)_2D$ production. Overexpression or infusion of hydrolysis-resistant FGF-23 into rats and mice was shown to decrease serum $1,25(OH)_2D$ concentrations and inhibit renal 1$\alpha$-hydroxylase gene expression (43–45). We have shown that in mice in which dietary phosphorus is manipulated over a broad range, renal 1$\alpha$-hydroxylase activity and mRNA abundance varied inversely and significantly with serum FGF-23 concentrations (26).

In the present study, a significant effect of dietary phosphorus on serum FGF-23 concentrations was observed when values on the control or high-normal intake were compared with those on the low-normal intake, whereas no significant change in serum FGF-23 concentrations was found between the control and high-normal intakes. It is possible that an increase in dietary phosphorus greater than 800 mg/d is necessary to induce a significant increase in serum FGF-23 concentrations. Alternatively, dietary phosphorus-induced changes in serum FGF-23 might not be linear, such that the increase in dietary phosphorus from a low to a moderate intake induced the largest increase in circulating FGF-23, a further increase in dietary phosphorus having little additional effect. The changes induced in serum $1,25(OH)_2D$ concentrations by dietary phosphorus followed a similar pattern.

In conclusion, our study demonstrates that in healthy men manipulation of dietary phosphorus within the physiological range of intakes regulates serum FGF-23 concentrations. Furthermore, the data suggest that regulation of $1,25(OH)_2D$ production by dietary phosphorus is mediated, at least in part, by changes in circulating FGF-23.

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