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Dietary and Serum Phosphorus Regulate Fibroblast Growth Factor 23 Expression and 1,25-Dihydroxyvitamin D Metabolism in Mice

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Fibroblast growth factor-23 (FGF-23) is a novel circulating peptide that regulates phosphorus (Pi) and vitamin D metabolism, but the mechanisms by which circulating FGF-23 itself is regulated are unknown. To determine whether the serum FGF-23 concentration is regulated by dietary intake of Pi, we fed wild-type (WT), *Npt2a* gene-ablated (*Npt2a*$^{-/-}$), and *Hyp* mice diets containing varying Pi contents (0.02–1.65%). In WT mice, increases in dietary Pi intake from 0.02–1.65% induced a 7-fold increase in serum FGF-23 and a 3-fold increase in serum Pi concentrations. Across the range of dietary Pi, serum FGF-23 concentrations varied directly with serum Pi concentrations ($r^2 = 0.72$; $P < 0.001$). In *Npt2a*$^{-/-}$ mice, serum FGF-23 concentrations were significantly lower than in WT mice, and these differences could be accounted for by the lower serum Pi levels in *Npt2a*$^{-/-}$ mice. The serum concentrations of FGF-23 in *Hyp* mice were 5- to 25-fold higher than values in WT mice, and the values varied with dietary Pi intake. *Fgf-23* mRNA abundance in calvaria was significantly higher in *Hyp* mice than in WT mice on the 1% Pi diet; in both groups of mice, *fgf-23* mRNA abundance in calvarial bone was suppressed by 85% on the low (0.02%) Pi diet. In WT mice fed the low (0.02%) Pi diet, renal mitochondrial 1α-hydroxylase activity and renal 1α-hydroxylase (P450c1α) mRNA abundance were significantly higher than in mice fed the higher Pi diets and varied inversely with serum FGF-23 concentrations ($r^2 = 0.86$ and $r^2 = 0.64$; $P < 0.001$, respectively). The present data demonstrate that dietary Pi regulates the serum FGF-23 concentration in mice, and such regulation is independent of *phex* function. The data suggest that genotype-dependent and dietary Pi-induced changes in the serum FGF-23 concentration reflect changes in *fgf-23* gene expression in bone. (*Endocrinology* 146: 5358–5364, 2005)

DIETARY PHOSPHORUS (Pi) intake and serum Pi concentration are critically important determinants of the renal metabolism of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. In animals, hypophosphatemia induced by dietary restriction of Pi stimulates the renal synthesis of 1,25$(OH)_2$D (1–7), independently of PTH (1, 2). Similarly, restriction of dietary Pi in healthy human subjects induces an increase (8–11), and supplementation of Pi induces a decrease, in the serum concentration of 1,25$(OH)_2$D and in its *in vivo* production rate (11–13).

We and others have shown in normal mice that dietary Pi restriction induces an increase in renal mitochondrial 1α-hydroxylase (P450c1α) activity and the renal abundance of 1α-hydroxylase (P450c1α, CYP27B1) mRNA and protein (14–16). The increase in P450c1α gene expression occurs in the proximal renal tubule and is due at least in part to an increase in P450c1α gene transcription. Renal P450c1α mRNA abundance and 1α-hydroxylase activity vary inversely and significantly with serum Pi concentrations (16).

Normal renal sodium (Na)/Pi cotransport is not necessary for the regulation of renal 1,25$(OH)_2$D production by dietary Pi. In mice in which the Na/Pi cotransporter gene, *Npt2a*, is ablated (*Npt2a*$^{-/-}$), restriction or supplementation of dietary Pi induces an appropriate increase or decrease, respectively, in serum 1,25$(OH)_2$D concentrations and renal 1α-hydroxylase activity and mRNA abundance (14). In *Npt2a*$^{-/-}$ mice, as in wild-type (WT) mice, values of P450c1α mRNA abundance vary inversely with serum Pi concentrations, suggesting that changes in serum Pi concentration *per se* are sufficient to initiate the signaling pathways involved in the regulation of P450c1α gene expression by manipulation of dietary Pi. By contrast, in X-linked hypophosphatemic (*Hyp*) mice, the murine homologue of human X-linked hypophosphatemia (XLH) (17, 18), regulation of P450c1α expression by dietary Pi is disordered; restriction or supplementation of dietary Pi induces a paradoxical decrease or increase, respectively, in serum 1,25$(OH)_2$D concentrations and renal 1α-hydroxylase activity and mRNA abundance, changes opposite those induced in WT mice (19). The precise mechanisms mediating the 1α-hydroxylase response in these two mouse models are unknown.

Fibroblast growth factor-23 (FGF-23) is a novel circulating peptide that is implicated in the pathogenesis of autosomal

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Abbreviations: Ct, Threshold cycle; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; FGF-23, fibroblast growth factor-23; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Phex, Pi-regulating gene with homologies to endopeptidases on the X-chromosome; Pi, phosphate; WT, wild type; XLH, X-linked hypophosphatemia.

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dominant hypophosphatemic rickets, tumor-induced osteomalacia, and XLH (20–24). These syndromes are characterized by hypophosphatemia due to renal Pi wasting, skeletal demineralization, and inappropriately low serum concentrations of 1,25(OH)$_2$D. That excess circulating FGF-23 contributes to the pathogenesis of these disorders is supported by observations that administration of recombinant FGF-23 or its overexpression in animals induces hypophosphatemia, inhibition of Na-dependent Pi transport in brush-border membrane vesicles, suppression of renal P450c1α mRNA and protein expression, and stimulation of P450c24 mRNA (21, 25, 26). The changes in P450c1α and P450c24 mRNA induced by the administration of FGF-23 are rapid, suggesting a direct action of FGF-23 on the expression of these genes (27). Conversely, in FGF-23-null mice, renal Pi reabsorption, serum Pi concentration, and P450c1α gene expression are abnormally increased, findings that demonstrate an essential role of FGF-23 in the regulation of Pi and vitamin D metabolism (28).

Because dietary Pi intake and circulating FGF-23 can regulate renal Pi transport and vitamin D metabolism, we sought to determine whether dietary Pi can regulate serum FGF-23 concentrations and, if so, whether such regulation plays a role in mediating the dietary Pi-induced changes in renal 1,25(OH)$_2$D production. We examined the effect of changing dietary Pi on serum FGF-23 concentrations in WT mice, *Npt2a*-null mice, and *Hyp* mice and on the abundance of *fgf-23* mRNA in bone in WT and *Hyp* mice. We also examined the effect of dietary Pi on renal mitochondrial 1α-hydroxylase activity and P450c1α mRNA abundance in WT mice.

Materials and Methods

Mice

C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). *Npt2a*-null mice were established by targeted mutagenesis as previously described (29) and were of mixed genetic background of 129Sv and C57BL/6J strains. These mice exhibit renal Pi wasting, an approximately 85% loss in renal brush-border membrane Na/Pi cotransport, hypophosphatemia, increased serum 1,25(OH)$_2$D concentrations, hypercalcemia, and hypercalciuria. *Hyp* mice were established by breeding C57BL/6J *Hyp*/+ females with C57BL/6J+/Y males. To determine the effect of dietary Pi intake on serum FGF-23 concentration, C57BL/6J mice (183 ± 13 d of age), *Npt2*a$^{-/-}$ mice, WT littermates (*Npt2*a$^{+/+}$; 82 ± 10 d of age), and mutant *Hyp*/Y mice (200 ± 5 d of age) initially were fed a vitamin D-replete diet containing 1.0% Pi and 1% calcium for 5 d (test diet TD 86129, Harlan Teklad, Madison, WI). Groups of mice then were fed otherwise identical diets containing 0.02% (TD 86128), 0.6% (TD 98243), or 1.65% (TD 88345) Pi for 5 more days. To determine the effect of dietary Pi intake on the abundance of FGF-23 mRNA in bone, C57BL6/J mice and *Hyp* mice (56–86 d of age) were fed the test diets containing 0.02% or 1.0% Pi for 5 d.

Animals were anesthetized with pentobarbital, and blood was drawn by cardiac puncture for determination of serum FGF-23 and Pi concentrations. The kidneys of WT mice were removed; one kidney was rapidly frozen in liquid nitrogen for subsequent extraction of RNA, and the other was placed in homogenizing medium at 4 C for isolation of renal mitochondria. The calvaria were removed from WT and *Hyp* mice and rapidly frozen in liquid nitrogen for subsequent extraction of RNA. All procedures were approved by the committee on animal research, University of California-San Francisco, and the Canadian Council on Animal Care.

Serum FGF-23 and Pi concentrations

Serum FGF-23 concentrations were measured by sandwich ELISA (Kainos Laboratories, Inc., Tokyo, Japan) as previously described (30), using antibodies against N- and C-terminal portions of human FGF-23 that detect full-length FGF-23. Mouse FGF-23 bears 72% amino acid homology to human FGF-23 and thus can be detected by the human anti-FGF-23 antibodies used in the ELISA. The serum Pi concentration was determined using a kit from Stanbio Laboratories (San Antonio, TX).

Bone fgf-23 mRNA abundance

Total RNA was isolated from the calvaria of WT and *Hyp* mice using TRIzol (Invitrogen Life Technologies, Carlsbad, CA). cDNA was synthesized using the Iscript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's protocol. Mouse *fgf-23* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes and primers were purchased from Applied Biosystems (Foster City, CA), and the abundance of *fgf-23* mRNA, relative to GAPDH mRNA, was quantitated by real-time PCR using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Five hundred nanograms of template cDNA were used per PCR, and the samples were amplified with an initial melt at 95 C for 12 min, followed by 45 cycles at 95 C for 15 sec and at 60 C for 1 min. The threshold cycle (Ct) at which a statistically significant increase in signal above background fluorescence was determined, and the Ct values for *fgf-23* product were normalized to Ct values for GAPDH. A passive reference dye, ROX (5-carboxy-x-rhodamine), was used to normalize for variations in volume or dye concentration between sample wells.

Renal mitochondrial 1α-hydroxylase activity

Renal mitochondrial 1α-hydroxylase activity was determined as previously described (16). Briefly, renal mitochondria were isolated (31), and duplicate 1-ml aliquots of mitochondrial protein (2.0–3.0 mg/ml) were suspended in buffer and incubated with 500 nM chromatographically purified 25-hydroxyvitamin D$_3$ at 24 C for 15 min. The reaction was stopped by the addition of acetonitrile, and lipid extraction of the sample was performed. 1,25(OH)$_2$D was isolated from the lipid extract by sequential C$_{18}$ and silica column chromatography and was quantitated in duplicate by radioreceptor assay.

Renal 1α- and 24-hydroxylase mRNA abundance

The abundance of renal P450c1α and P450c24 mRNA, relative to β-actin mRNA, was quantitated by ribonuclease protection assay using the HybSpeed ribonuclease protection assay kit (Ambion, Austin, TX). P450c1α and P450c24 riboprobes were derived from unique regions of their respective cDNA sequences as previously described (16). Total RNA isolated from kidneys using the TRIzol reagent (Invitrogen Life Technologies, Gaithersburg, MD) was hybridized with the appropriate riboprobes (5×10^5 cpm) at 68 C for 10 min and treated with ribonuclease A (5 U/ml) and T1 (200 U/ml) at 37 C for 30 min. The remaining protected RNA fragments were precipitated, denatured, and resolved on a denaturing 5% acrylamide/8 M urea gel. The gel was dried and exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA) for quantitation. Results are expressed as the ratio of P450c1α or P450c24 mRNA to β-actin mRNA.

Statistical analysis

Data are expressed as the mean ± SEM. The significance of differences between multiple groups was analyzed by ANOVA, and *post hoc* testing was performed using the Student-Newman-Keuls test. The relationship between the variables was assessed using the Pearson product-moment correlation coefficient or nonlinear regression analysis using the power function. $P < 0.05$ was considered statistically significant.

Results

Serum FGF-23 and phosphorus concentration

To determine the effect of dietary Pi on the serum concentration of FGF-23, four groups of normal mice were fed

diets containing 0.02%, 0.6%, 1.0%, or 1.65% Pi, each for 5 d. The serum concentrations of FGF-23 varied directly with dietary Pi intake. In mice fed the highest (1.65%) Pi intake, the mean serum FGF-23 concentration was 65 ± 6.5 pg/ml, a value 7-fold higher than that in mice fed the lowest (0.02%) Pi intake, 9.9 ± 1.0 pg/ml ($P < 0.05$; Fig. 1A). Diet-induced changes in serum FGF-23 concentrations occurred rapidly, with a 50% decrease occurring within 48 h of the change from high to low dietary Pi (93.5 ± 12.9 to 45 ± 15.9 pg/ml, respectively; $P < 0.01$). As expected, the serum concentrations of Pi varied directly with dietary Pi intake. Changes in dietary Pi from 1.65% to 0.02% elicited a nearly 3-fold decrease in serum Pi concentration from 3.4 ± 0.3 to 1.2 ± 0.2 mM ($P < 0.01$; Fig. 1B). Serum concentrations of FGF-23 varied directly and significantly with serum concentrations of Pi when values from the four diet groups were analyzed as a single set ($r^2 = 0.72$; $P < 0.001$; Fig. 1C).

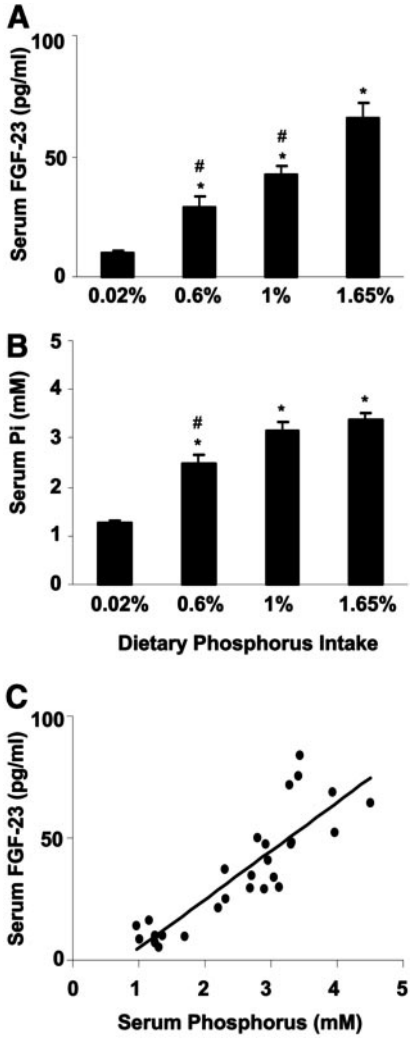

FIG. 1. Effect of dietary Pi on serum FGF-23 (A) and serum Pi (B) concentrations in normal mice. Mice were fed diets containing 1.65%, 1%, 0.6%, or 0.02% Pi for 5 d, then killed, and blood was obtained for determination of intact FGF-23 and Pi concentrations. *Bars* depict the mean ± SEM (n = 3–9 mice/diet group). The effect of dietary Pi was analyzed by one-way ANOVA and *post hoc* testing by the Student-Newman-Keuls test. *, $P < 0.05$ compared with the 0.02% Pi diet; #, $P < 0.05$ compared with the 1.65% Pi diet. C, The relationship between serum FGF-23 and serum Pi concentrations in normal mice fed the diets described. Each *point* depicts data from an individual mouse. Serum FGF-23 concentrations varied directly and significantly with serum Pi ($r^2 = 0.72$; $P < 0.001$).

Npt2a knockout mice

The serum concentration of FGF-23 might be regulated by the dietary intake of Pi *per se* or by its effect on the serum concentration of Pi. To distinguish between the effects of dietary intake of Pi and those of serum Pi, we fed *Npt2a*$^{-/-}$ mice and WT littermates diets containing 0.02%, 0.6%, 1%, and 1.65% Pi, each for 4–5 d. In both *Npt2a*$^{-/-}$ and WT mice, the increase in dietary Pi elicited a nearly 7-fold increase in serum FGF-23 concentrations (Fig. 2A). Serum FGF-23 concentrations were significantly lower in *Npt2a*$^{-/-}$ mice than in WT mice in each of the diet groups, except for the 0.02% Pi diet group (Fig. 2A). Serum Pi concentrations also were lower in the *Npt2a*$^{-/-}$mice than in WT mice in the 1.0% and 0.6% Pi diet groups. We then examined the relationship between serum FGF-23 and serum Pi concentrations in WT and *Npt2a*$^{-/-}$ mice fed the four different Pi intakes. Serum concentrations of FGF-23 varied directly and significantly with serum concentrations of Pi in both WT ($r^2 = 0.69$; $P < 0.001$) and *Npt2a*$^{-/-}$ ($r^2 = 0.62$; $P < 0.001$) mice (Fig. 2B), with

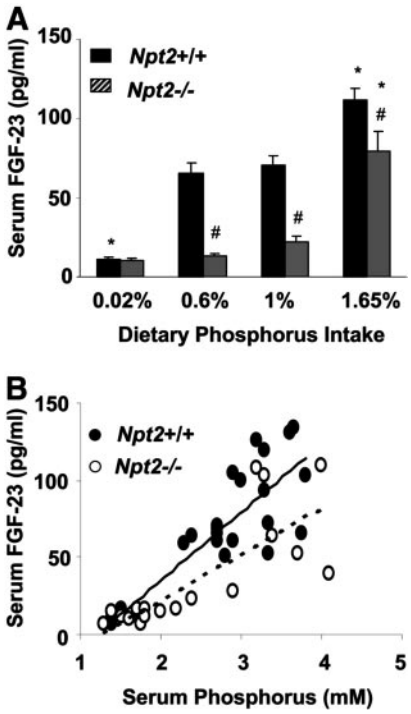

FIG. 2. A, Effect of dietary Pi on serum FGF-23 concentrations in WT and *Npt2*$^{-/-}$ mice. Mice were fed a 1.65%, 1%, 0.6%, or 0.02% Pi diet for 5 d. *Bars* depict the mean ± SEM (n = 3–6 mice/group). The effect of dietary Pi and genotype was analyzed by two-factor ANOVA and *post hoc* testing by the Student-Newman-Keuls test. *, $P < 0.05$ compared with the 1% Pi diet within each genotype; #, $P < 0.05$ compared with WT mice within each diet group. B, The relationship between serum FGF-23 and serum Pi concentrations in WT and *Npt2*$^{-/-}$ mice fed the diets described. Serum FGF-23 concentrations varied directly and significantly with serum Pi in both WT ($r^2 = 0.69$; $P < 0.001$) and *Npt2*$^{-/-}$ ($r^2 = 0.62$; $P < 0.001$) mice. The slopes of the regression lines were statistically different in the two mouse strains.

the concentrations of serum Pi being shifted to lower values in the *Npt2a*$^{-/-}$ mice. The slope of the relationship between serum FGF-23 and serum Pi in the WT mice was slightly lower, by 23% ($P < 0.05$), than the slope observed in *Npt2a*$^{-/-}$ mice.

Hyp mice

To determine whether normal Phex (Pi-regulating gene with homologies to endopeptidases on the X-chromosome) function is required for dietary Pi to regulate the serum FGF-23 concentration, we fed *Hyp* mice diets containing 0.02%, 0.6%, 1%, and 1.65% Pi, each for 5 d. The serum concentrations of FGF-23 in *Hyp* mice were 5- to 25-fold higher than values in WT mice, and the values varied directly with dietary Pi intake, as we observed in WT and *Npt2a*$^{-/-}$ mice. In *Hyp* mice fed the 1.65% Pi diet, the mean serum FGF-23 concentration was 600 ± 90 pg/ml, a value 3-fold higher than that in mice fed the 0.02% Pi diet (232 ± 30 pg/ml; $P < 0.05$; Fig. 3A). The serum Pi concentrations in *Hyp* mice also varied directly with Pi intake, as we previously reported (19), ranging from 2.25 ± 0.08 to 0.65 ± 0.08 mM ($P < 0.05$) on the 1.65% and 0.02% Pi diets, respectively. When the relationship between serum FGF-23 and serum Pi concentrations was examined, serum FGF-23 varied directly and significantly with serum Pi concentrations in *Hyp* mice ($r^2 = 0.36$; $P < 0.001$; Fig. 3B). The slope of this relationship in *Hyp* was significantly different ($P < 0.001$) from the slope observed in WT mice (Fig 1C).

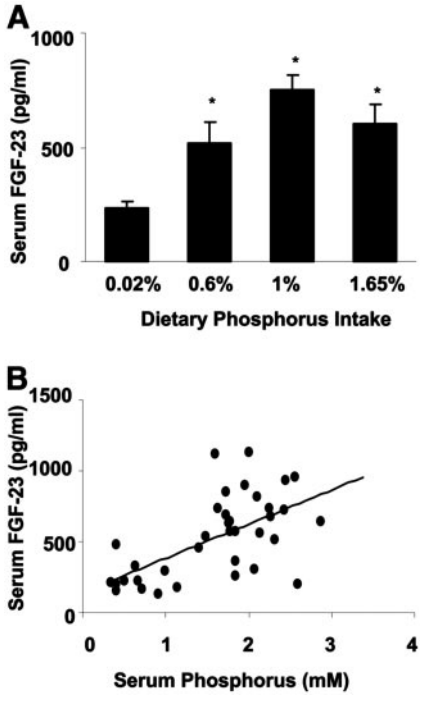

FIG. 3. A, Effect of dietary Pi on serum FGF-23 concentrations in *Hyp* mice. *Hyp* mice were fed a 1.65%, 1%, 0.6%, or 0.02% Pi diet for 5 d. *Bars* depict the mean ± SEM (n = 3–13 mice/diet group). The effect of dietary Pi was analyzed by one-way ANOVA and *post hoc* testing by the Student-Newman-Keuls test. *, $P < 0.05$ compared with the 0.02% Pi diet. B, The relationship between serum FGF-23 and serum Pi concentrations in *Hyp* mice fed the diets described. Each *point* depicts data from an individual mouse. The serum FGF-23 concentration varied directly and significantly with the serum Pi concentration in *Hyp* mice ($r^2 = 0.36$; $P < 0.001$).

Bone fgf-23 mRNA abundance

FGF-23 is predominantly expressed in bone (32), although the factors that regulate its expression are poorly understood. To examine whether restriction of dietary Pi might suppress *fgf-23* gene expression in bone, WT and *Hyp* mice were fed diets containing either 1% or 0.02% Pi for 5 d. In mice fed the 1% Pi diet, the abundance of *fgf-23* mRNA in calvaria from *Hyp* mice was 30-fold higher than that in WT mice (Fig. 4). Furthermore, in both WT and *Hyp* mice fed the low (0.02%) Pi diet, the abundance of *fgf-23* mRNA in calvaria was suppressed by 85% ($P < 0.001$) compared with the expression in each species fed the 1% Pi diet (Fig. 4).

Renal 1α-hydroxylase activity and P450c1α mRNA abundance

The effect of dietary Pi intake on renal mitochondrial 1α-hydroxylase activity and P450c1α mRNA abundance was examined in normal mice fed the 0.02%, 1%, or 1.65% Pi diet for 5 d. In mice fed the 0.02% Pi diet, renal 1α-hydroxylase activity was approximately 6-fold higher than values in mice fed the 1% Pi diet and 4-fold higher than values in mice fed the 1.65% Pi diet (Table 1). Similarly, in mice fed the 0.02% Pi diet, renal P450c1α mRNA abundance increased 3-fold compared with values in mice fed the 1% and 1.65% Pi diets. Values in mice fed the 1% and 1.65% Pi diets did not differ significantly from each other (Table 1). We examined the relationship between serum FGF-23 concentrations and renal P450c1α activity and mRNA abundance in mice fed the three different Pi intakes. Both 1α-hydroxylase activity ($r^2 = 0.86$; $P < 0.001$) (Fig. 5A) and P450c1α mRNA abundance ($r^2 = 0.64$; $P < 0.001$; Fig. 5B) varied inversely and significantly with serum FGF-23 concentrations.

Renal P450c24 mRNA abundance

The effect of dietary Pi on renal mRNA abundance of P450c24, the enzyme that initiates the catabolism of 1,25(OH)$_2$D, was examined in normal mice fed the 0.02%, 1%, and 1.65% Pi diets. In mice fed the 1.65% Pi diet, renal

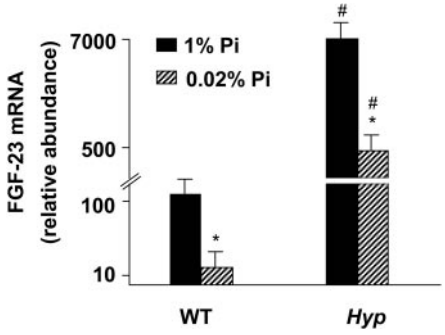

FIG. 4. Effect of dietary Pi intake on the abundance of *fgf-23* mRNA in calvaria of WT and *Hyp* mice. Bars depict *fgf-23* mRNA abundance normalized to that of GAPDH, expressed as a percentage relative to WT mice fed the 1% Pi diet. *Bars* depict the mean ± SEM (n = 5 mice/diet group). *, $P < 0.05$ compared with the 1% Pi diet within each genotype; #, $P < 0.05$ compared with WT mice within each diet group.

TABLE 1. Effect of dietary phosphorus on renal 1α-hydroxylase activity and P450c1α mRNA abundance in WT mice

Dietary Pi (%)	1α-Hydroxylase activity (pg/ml·15 min)	P450c1α mRNA % abundance (normalized to 1.0% Pi diet)	P450c24 mRNA % abundannce (normalized to 1.0% Pi diet)
0.02	80.9 ± 19.1	300 ± 42	70 ± 17
1.0	12.5 ± 1.5[a]	100 ± 7[a]	100 ± 9
1.65	20.8 ± 2.9[a]	110 ± 16[a]	165 ± 12[a]

Data are the mean ± SEM values for renal 1α-hydroxylase activity, P450c1α mRNA, and P450c24 mRNA abundance from six to nine mice per group. The effect of diet was determined by one-way ANOVA.
[a] $P < 0.05$ compared with the 0.02% Pi diet group.

P450c24 mRNA abundance was 1.5-fold higher than in mice fed the 1% Pi diet; with the low Pi diet, values did not change significantly (Table 1). Renal P450c24mRNA abundance varied directly and significantly with serum FGF-23 concentrations ($r^2 = 0.51$; $P < 0.01$; Fig. 6).

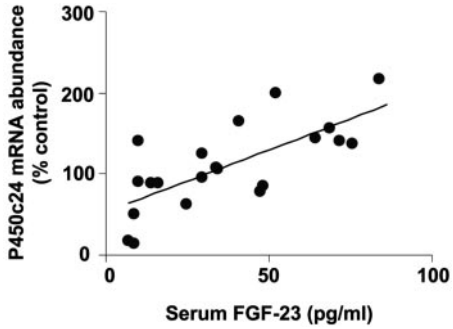

FIG. 6. Relationship between serum FGF-23 concentration and renal P450c24 mRNA abundance in normal mice. Mice were fed a 1.65%, 1%, or 0.02% Pi diet for 5 d. Serum FGF-23 concentrations and renal P450c24 mRNA relative to β-actin mRNA were determined as described in *Materials and Methods.* Renal P450c24 mRNA abundance varied directly with serum FGF-23 concentrations ($r^2 = 0.51$; $P < 0.01$). Each *point* depicts data from an individual mouse.

Discussion

In the present study we demonstrate that a decrease in the dietary intake of Pi induces a substantial decrease in the serum concentration of FGF-23 in normal mice. When mice were fed diets containing decreasing amounts of Pi from 1.65% to 0.02% for 5 d, serum concentrations of FGF-23 decreased over a 7-fold range in a linear, dose-dependent fashion. The change in serum FGF-23 was rapid, with a 50% decrease occurring within 48 h of feeding a low Pi diet. Decreasing the dietary intake of Pi also induced a 3-fold decrease in the serum concentration of Pi, as expected. Serum concentrations of FGF-23 varied directly and significantly with serum concentrations of Pi across the range of Pi intakes. These findings suggest that the circulating concentration of FGF-23 is regulated by some function of dietary Pi, possibly the serum concentration of Pi.

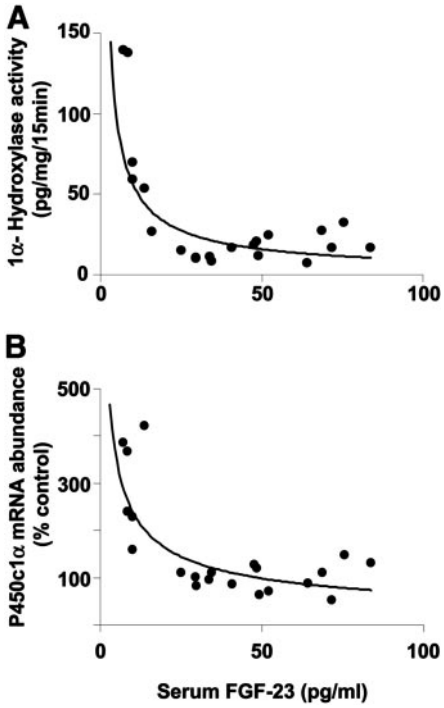

FIG. 5. Relationship between serum FGF-23 concentration and renal mitochondrial 1α-hydroxylase activity (A) and renal P450c1α mRNA abundance (B) in normal mice. Mice were fed a 1.65%, 1%, or 0.02% Pi diet for 5 d. Serum FGF-23 concentrations, renal 1α-hydroxylase activity, and P450c1α mRNA relative to β-actin mRNA were determined as described in *Materials and Methods.* Renal 1α-hydroxylase activity (A; $r^2 = 0.86$; $P < 0.001$) and P450c1α mRNA abundance (B; $r^2 = 0.64$; $P < 0.001$) varied inversely with serum FGF-23 concentrations. Each *point* depicts data from an individual mouse.

To address this question, we examined the effect of manipulating dietary Pi on serum FGF-23 and Pi concentrations in mice homozygous for *Npt2a* gene disruption. These mice exhibit hypophosphatemia due to an approximately 80% loss in renal brush-border membrane Na/Pi cotransport and increased serum concentrations of 1,25(OH)$_2$D (14, 29). In *Npt2a*$^{-/-}$ mice, serum FGF-23 concentrations were greatly suppressed by feeding a low Pi diet and increased substantially with the high Pi diet. In each of the diet groups except the 0.02% Pi group, serum FGF-23 concentrations were significantly lower in the *Npt2a*$^{-/-}$ mice than those in WT mice; serum Pi concentrations also were lower in the *Npt2a*$^{-/-}$ mice than in WT mice on the 0.6% and 1% Pi diets. Nevertheless, serum concentrations of FGF-23 varied directly and significantly with those of serum Pi in each group of mice. Our finding that the slopes of this relationship differed slightly, but significantly, between the two groups of mice suggests a genotype dependence in the regulation of serum FGF-23 by dietary Pi intake. These data suggest that the lower serum concentrations of FGF-23 in *Npt2a*$^{-/-}$ mice can be attributed to their lower serum concentrations of Pi. These data also suggest that diet-induced changes in the serum concentration of Pi *per se* are sufficient to initiate the signaling pathways involved in the regulation of circulating concentrations of FGF-23. Consistent with this formulation are recent findings that serum FGF-23 concentrations were suppressed by hypophosphatemia induced by Pi restriction in normal mice (33, 34) and rats with experimental renal insufficiency (35) or by secondary hyperparathyroidism in vitamin D receptor-null mice (33, 34). The latter finding provides evidence that regulation of circulating FGF-23 by dietary and serum Pi is independent of the genomic effects of vitamin D (33, 34). In rats with experimental renal insufficiency, serum FGF-23

concentrations were 6-fold higher than those in sham-operated animals on a control diet and increased additionally by 5-fold when dietary Pi was increased from 0.2% to 0.9% (35). It was also shown in rats with renal insufficiency that the serum Pi concentration can regulate serum FGF-23 independently of serum 1,25(OH)$_2$D, consistent with findings in vitamin D receptor-null mice (33, 34).

FGF-23 is a secreted peptide that is processed to amino- and carboxyl-terminal peptides at a consensus proprotein convertase (furin) site, RHTR (ArgHisThrArg) (20). In patients with autosomal dominant hypophosphatemic rickets (20), missense mutations involving the R residues at this cleavage site prevent processing (24, 36) of FGF-23 (24, 36, 37). XLH and *Hyp* are caused by loss of function mutations in *PHEX/Phex*, a Pi-regulating gene with homologies to endopeptidases on the X-chromosome (38–40). Circulating concentrations of FGF-23 are increased in some patients with XLH (23, 30, 41), and the abundance of FGF-23 mRNA in bone is increased in *Hyp* mice (32), suggesting that loss of PHEX function results in overexpression of FGF-23 in bone, leading to an increase in circulating FGF-23 (32). In the present study we observed that serum FGF-23 concentrations in *Hyp* mice were 5- to 25-fold higher than values in normal mice. Furthermore, with a decease in dietary Pi from high to low values, serum FGF-23 concentrations were induced to decrease by more than 3-fold, and the concentrations correlated directly with those of serum Pi. However, as observed in Npt2a$^{-/-}$ mice, the slopes of the relationship between serum FGF-23 and serum Pi were statistically different in the WT and *hyp* mice. This suggests a genotype-dependent difference in the regulation of serum FGF-23 in response to dietary Pi intake in the three mouse models. Our finding that serum FGF-23 concentrations can be regulated by dietary Pi in *Hyp* mice provides evidence that such regulation is independent of Phex function. The mechanism by which loss of Phex function gives rise to the increase in circulating FGF-23 is not addressed in the present study.

To investigate the mechanism of regulation of serum FGF-23 concentration by dietary Pi, we measured the abundance of *fgf-23* mRNA in calvaria in WT and *Hyp* mice fed 1.0% and 0.02% Pi diets. In *Hyp* mice, the abundance of *fgf-23* mRNA in calvaria was 30-fold higher than that in WT mice fed the 1% Pi diet, confirming previous reports (32). In both WT and *Hyp* mice, the abundance of *fgf-23* mRNA in calvaria was markedly suppressed on the low Pi diet. These findings provide evidence that *fgf-23* gene expression in bone is regulated by dietary Pi and also that such regulation is independent of Phex function. Thus, both dietary Pi-induced and Phex-dependent changes in serum FGF-23 concentrations appear to be mediated at least in part by changes in FGF-23 production by bone. Whether the action of dietary and serum Pi to regulate the expression of FGF-23 in bone is direct or is mediated by another regulatory factor remains to be determined.

We previously showed in normal mice that restriction of dietary Pi stimulates renal 1,25(OH)$_2$D production by increasing renal mitochondrial 1α-hydroxylase activity and P450c1α mRNA expression and decreasing P450c24 mRNA expression (14, 16, 19). In the present study of normal mice fed the lowest (0.02%) Pi diet, renal 1α-hydroxylase activity and mRNA abundance were approximately 6- and 3-fold higher, respectively, and P450c24 mRNA abundance was 1.5-fold lower than values in mice fed the highest (1.6%) Pi diet. When the relationship between renal 1α-hydroxylase expression and serum FGF-23 concentration was examined, both renal 1α-hydroxylase activity and its mRNA abundance varied inversely and significantly with serum FGF-23 concentrations. These data suggest that regulation of the renal metabolism of 1,25(OH)$_2$D by dietary Pi is mediated at least in part by dietary Pi-induced changes in circulating FGF-23. Of interest, we found that when dietary Pi was increased from 1% to 1.65%, renal 1α-hydroxylase activity and mRNA abundance did not decrease further, even though the serum FGF-23 concentration did increase by approximately 30%. This suggests that 1α-hydroxylase activity and mRNA abundance were maximally suppressed on the 1% Pi diet relative to the 0.02% diet, and additional increases in serum FGF-23 concentrations did not further suppress P450c1α gene expression or enzyme activity. Whether the increase in serum FGF-23 concentration in the 1.65% Pi diet group compared with that in the 1% Pi diet group is required for other physiological actions of FGF-23 is not known.

The present findings are consistent with previous studies in which overexpression or administration of FGF-23 in mice elicited a reduction in serum 1,25(OH)$_2$D concentrations, a corresponding decrease in renal abundance of 1α-hydroxylase mRNA, and a corresponding increase in 24-hydroxylase mRNA (21, 25, 27). Conversely, in FGF-23-null mice, serum Pi and 1,25(OH)$_2$D concentrations and renal P450c1α mRNA abundance were significantly increased (28). Although the latter studies demonstrated that FGF-23 plays a critical role in the regulation of Pi and vitamin D metabolism, the disruptions in Pi and vitamin D metabolism were achieved under nonphysiological conditions in which the circulating FGF-23 concentration was either extremely high or absent due to its gene ablation. Thus, it was not clear from these studies whether physiological changes in the serum FGF-23 concentration can play a role in the regulation of Pi and vitamin D metabolism. The present study of normal mice fed different Pi diets demonstrates that serum FGF-23 concentrations can be regulated within a physiological range by dietary Pi and suggest that such changes play a role in the regulation of vitamin D metabolism.

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