The pathogenesis of familial hypophosphatemic rickets (FHR) is incompletely understood. We therefore examined the effects of acute dietary phosphorus deprivation to see whether renal phosphate conservation and increased 1,25 dihydroxyvitamin D [1,25(OH)2D] plasma levels, which normally follow restriction of phosphorus intake, could be induced in patients with FHR. Six healthy male volunteers (age 26 +/- 3 yr) and seven male patients with FHR (age 24 +/- 3 yr) were placed on a low phosphorus diet supplemented with aluminum hydroxide and studied over a 4-d period. The patients with FHR excreted more than five times as much phosphorus per day at the conclusion of the study than did the controls (176 +/- 61 mg/24 h vs. 33 +/- 11 mg/h). In the normal subjects, maximum tubular reabsorptive capacity for phosphorus/glomerular filtration rate (TmP/GFR) rose progressively during phosphorus deprivation, and the rise from base line was more than two times greater than that seen in patients with FHR. Immunoreactive parathyroid hormone levels and nephrogenous cyclic AMP were initially normal in both groups and no change was seen in either group with phosphorus deprivation. In the normal subjects, 1,25(OH)2D levels rose progressively over the 96 h of the study (49 +/- 3 to 63 +/- 6 pg/ml, P less than 0.05), while mean circulating 1,25(OH)2D in the patients with FHR did not change [...]
Impaired Phosphorus Conservation and 1,25 Dihydroxyvitamin D Generation during Phosphorus Deprivation in Familial Hypophosphatemic Rickets

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ABSTRACT The pathogenesis of familial hypophosphatemic rickets (FHR) is incompletely understood. We therefore examined the effects of acute dietary phosphorus deprivation to see whether renal phosphate conservation and increased 1,25 dihydroxyvitamin D[1,25(OH)2D] plasma levels, which normally follow restriction of phosphorus intake, could be induced in patients with FHR.

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In the normal subjects, 1,25(OH)2D levels rose progressively over the 96 h of the study (49±3 to 63±6 pg/ml, P < 0.05), while mean circulating 1,25(OH)2D levels in the patients with FHR did not change (34±3 to 29±3 pg/ml). The changes in individual plasma 1,25(OH)2D levels correlated strongly with the change in individual nephrogenous cyclic AMP measurements in the patients with FHR (r = +0.93), while no such correlation was observed in the normal subjects. These data demonstrate a defective renal response to phosphorus deprivation in patients with FHR including a qualitatively abnormal response in 1,25(OH)2D generation.

INTRODUCTION

Familial X-linked hypophosphatemic rickets (FHR) is characterized by a low serum phosphorus concentration, an impaired ability of the kidney to reclaim filtered phosphate, decreased intestinal calcium and phosphate absorption, growth retardation, and rickets or osteomalacia of variable severity (1–5). The pathogenesis of this disorder is incompletely understood. A lowered renal threshold of phosphorus is invariably present in these patients, and current evidence supports the hypothesis that a defect in phosphate transport in the proximal renal tubule is of major importance in the genesis of FHR (6–8).

Despite the universal presence of phosphate wasting in patients with FHR, it is difficult to reconcile certain features of the disorder with the view that all aspects of its pathophysiology are simply due to the loss of

1 Abbreviations used in this paper: FHR, familial hypophosphatemic rickets; GF, glomerular filtrate; GFR, glomerular filtration rate; iPTH, immunoreactive PTH; NcAMP, nephrogenous cyclic AMP; 1,25(OH)2D, 1,25 dihydroxyvitamin D; PTH, parathyroid hormone; TmP, maximum tubular reabsorptive capacity for phosphorus.
phosphate into the urine. First, some of the cardinal features of phosphorus depletion, particularly myopathies and bone resorption with hypercalcemia, are not seen in FHR (4, 5). Second, most recent series have found concentrations of 1,25 dihydroxyvitamin D [1,25(OH)2D] to be normal or low-normal in FHR (9-12), even though hypophosphatemia is regularly associated with elevated circulating levels of 1,25(OH)2D.

In this study, the response to acute dietary phosphorus restriction was studied in normal subjects and patients with FHR to see whether renal phosphorus conservation and increased 1,25(OH)2D plasma levels, which normally follow dietary phosphorus restriction, could be induced by these means in patients with FHR. The results reveal that both renal phosphorus conservation and circulating 1,25(OH)2D respond abnormally in this disorder.

METHODS

Study subjects. Seven ambulatory male subjects (ages 30, 27, 25, 24, 23, 22, and 20) from five kindreds with FHR were selected for the study. There were three brothers from one kindred. Six of the seven had least two other affected family members. The seventh male subject, though hypophosphatemic from birth, was the only affected member in his family and represents a sporadic case of hypophosphatemic rickets. All seven had been under continuous treatment since infancy or early childhood with vitamin D or dihydrotachysterol and phosphorus. However, none of these subjects had taken any form of therapy for at least 2 yr before the present study. All had physical findings consistent with full phenotypic manifestations of their disease, including short stature, frontal bossing, poor dentition, and bowing of lower extremity long bones. All except two subjects had had corrective osteotomies in childhood. Only males were chosen to avoid the phenotypic variability often seen in heterozygous females. Six healthy male volunteers (ages 37, 31, 30, 21, 20, and 20) served as controls. All subjects were studied in the same manner. For the 3 d before study, the subjects followed dietary instructions to consume 1,000 mg of calcium/d. Daily dietary records were kept and reviewed by a dietitian to ensure compliance.

Study protocol. The study protocol was approved by the Yale University Human Investigations Committee. No subject was studied until informed, written consent was obtained. Studies were conducted on the Clinical Research Center of the Yale-New Haven Hospital.

During the 24 h before beginning the study all subjects collected a baseline 24-h urine for calcium, phosphorus, and creatinine determinations. On days 1 through 4 of the study, the subjects received a normal diet modified to contain 800 mg of calcium, 500 mg of phosphorus, and 100 meq of sodium/d. In addition, with each meal and at bedtime, they received 6 g of aluminum hydroxide liquid suspension, for a total of 24 g/24 h. The amount of aluminum hydroxide administered was derived from in vitro data (13) and was calculated to bind essentially all ingested phosphorus. All subjects successfully completed the study. The only side effects were mild nausea or constipation in five subjects.

Base-line blood determinations of electrolytes, creatinine, calcium, phosphorus, magnesium, total protein, albumin, and alkaline phosphatase were made. After 0, 24, 48, 72, and 96 h of phosphorus deprivation, fasting blood and urine samples were collected for measurement of calcium, phosphorus, creatinine, and 1,25(OH)2D concentrations and calculation of the renal tubular phosphate reabsorption threshold (TmP/GFR) and fasting calcium excretion. These samples were collected after the subjects had been supine for at least 30 min. From 24 to 48, 48 to 72, and 72 to 96 h after phosphorus deprivation was begun, 24-h urine collections for calcium, phosphorus, and creatinine were made. No urine collection was obtained between 0-24 h of phosphorus deprivation. Finally, after 0 and 96 h, measurements of fasting recumbent plasma and urinary cyclic AMP were made for calculation of nephrogenous cyclic AMP (NcAMP). Simultaneous samples were drawn for measurement of immunoreactive parathyroid hormone (iPTH).

Biochemical measurements. Serum and urinary calcium were determined by atomic absorption spectrophotometry (14). Serum and urinary phosphorus (15), serum creatinine (16), and alkaline phosphatase (17) were determined using standard methods. Plasma iPTH was measured by Dr. Constantine Anast (Boston Children’s Hospital, Boston, MA). The radioimmunoassay uses a carboxy-terminal antiserum supplied by Dr. Edwardo Slatopolsky of Washington University (18). The normal range for the assay is 2-25 μg/l. 1,25(OH)2D was determined by a modification of the method of Horst (19), using rachitic chick intestinal cytosol as a binding protein. Samples were purified on Sephadex LH20 and by high performance liquid chromatography. The tracer used was [25-263H] (OH)2D (110 Ci/M) obtained from New England Nuclear, Boston, MA. Plasma samples from 22 adult normal subjects have been cross-referenced in Dr. Horst’s laboratory and the mineral metabolism laboratories at Yale, with mean (±SD) values of 40.5±0.7 and 44.6±1.17 pg/ml, respectively.

Calculations. TmP/GRF was calculated using a nomogram (20) and results from fasting, hydrated 2-h urine specimens with midpoint blood specimens. NcAMP was calculated as previously reported (21).

Statistical analysis. Student’s t test for paired and unpaired observations was used for statistical analysis of the data.

RESULTS

Base-line studies. Serum electrolytes, creatinine, calcium, magnesium, total protein, albumin, and complete blood counts were normal in both groups. 24-h urinary calcium excretion and creatinine clearance were similar in both groups and were within the normal range. Serum phosphorus was significantly lower in the patients with FHR than in the control subjects (1.8±0.1 vs. 3.1±0.2 mg%, mean±SEM, P < 0.001). Alkaline phosphatase, although within the normal range (10-70 IU/dl) in both groups, was significantly higher in the patients with FHR (50±6 vs. 33±4 IU/ml, mean±SEM, P < 0.05).

Influence of phosphorus deprivation on serum and urinary phosphorus and TmP/GFR. Phosphorus deprivation caused a modest progressive fall in the serum phosphorus concentration in the normal subjects. Mean values fell from 3.1±0.3 to 2.6±0.2 mg% (P < 0.05) after 4 d of the diet. In the patients with FHR the
URINARY PHOSPHORUS (mg/24h)

HOURS OF PHOSPHORUS DEPRIVATION

FIGURE 1 Effect of phosphorus deprivation on urinary phosphorus excretion (left panel). 24-h urine specimens were collected for the 24 h just prior to beginning phosphorus deprivation and from 24 to 48, 48 to 72, and 72 to 96 h after beginning phosphorus deprivation (*P < 0.05). Effect of phosphorus deprivation on TmP/GFR (right panel) (**P < 0.001, §P < 0.01 compared with base line).

change was not significant (1.8±0.1 to 1.6±0.1 mg%). Urinary excretion before phosphorus deprivation was not statistically different in the two groups. However, from 72 to 96 h of phosphorus deprivation, the subjects with FHR excreted more than five times as much phosphorus as did the control subjects (176±61 vs. 33±11 mg/24 h, P < 0.05) (Fig. 1).

There were marked differences in the renal tubular response to phosphorus deprivation in the normal subjects and the patients with FHR (Table I). As expected, all the patients with FHR had abnormally low baseline values for TmP/GFR, with a mean value significantly below that of the normal subjects (1.7±0.2 vs. 2.8±0.2 mg/dl, P < 0.005). In response to phosphorus deprivation, the normal subjects showed a progressive rise in TmP/GFR, which was maximal after 72 h of phosphorus deprivation. In the patients with FHR, there was a rise in TmP/GFR which was maximal after 24 h of phosphorus deprivation and showed no further rise. After 96 h of phosphorus deprivation TmP/GFR remained significantly lower in the patients with FHR compared with controls 3.7±0.2 vs. 2.2±0.1 mg/dl, P < 0.001). The maximal rise in TmP/GFR was more than two times greater in the normal subjects than in the patients with FHR (1.3±0.2 vs. 0.5±0.2 mg/dl), and the difference was highly significant (P < 0.005).

Effects on calcium metabolism and parathyroid hormone (PTH) activity. Table II shows that there was no change in serum calcium during phosphorus deprivation in the control subjects. The patients with FHR, however, showed a small but significant rise in serum calcium during the study, from 9.1±0.1 mg% at base line to 9.7±0.2 mg% after 96 h of phosphorus deprivation (P < 0.05). There was no significant change in 24-h urinary calcium excretion in either group. Fasting calcium excretion doubled during the study.

<table>
<thead>
<tr>
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<th>TmP/GFR base line</th>
<th>TmP/GFR after 24 h</th>
<th>TmP/GFR after 48 h</th>
<th>TmP/GFR after 72 h</th>
<th>TmP/GFR after 96 h</th>
<th>Maximal change in TmP/GFR</th>
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<tr>
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<td>mg/dl</td>
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<td>Control subjects</td>
<td>2.8±0.2</td>
<td>3.5±0.4</td>
<td>4.0±0.2</td>
<td>4.1±0.2</td>
<td>3.7±0.2</td>
<td>1.3±0.2§</td>
</tr>
<tr>
<td>Patients with FHR</td>
<td>1.7±0.2</td>
<td>2.2±0.2</td>
<td>2.1±0.11</td>
<td>2.1±0.2</td>
<td>2.2±0.14</td>
<td>0.5±0.2</td>
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* All data are shown as mean±SEM.
† P < 0.01 compared with base line.
‡ P < 0.001 compared with base line.
§ P < 0.005 compared with subjects with FHR.

K. L. Insogna, A. E. Broadus, and J. M. Gertner
Calcium metabolism and PTH activity in response to phosphorus deprivation

<table>
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<tr>
<th>Control subjects</th>
<th>Patients with FHR</th>
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<tr>
<td></td>
<td>Base line</td>
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<tr>
<td>Serum Ca (mg/dl)</td>
<td>9.6±0.2</td>
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<tr>
<td>Urinary Ca (mg/24 h)</td>
<td>178±35</td>
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<tr>
<td>Fasting CaE (mg/100 ml GF)</td>
<td>0.06±0.01</td>
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<tr>
<td>NcAMP (nmol/100 ml GF)</td>
<td>0.86±0.18</td>
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<tr>
<td>iPTH (μg/100 ml)</td>
<td>16±2</td>
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* All data shown as mean±SEM. CaE, Ca excretion.
† P < 0.05 compared with base line.
‡ P < 0.01 compared with base line.

Period in the patients with FHR, rising from 0.04 to 0.08 mg/100 ml GF (P < 0.05), a value still within the normal range. The normal subjects also showed a rise in fasting calcium excretion from 0.06 to 0.08 mg/100 ml GF, although this change was not significant.

PTH activity was assessed by measurement of iPTH and NcAMP excretion. Both groups showed normal baseline values for NcAMP excretion, although the mean was higher in the patients with FHR, and one of these subjects had a frankly elevated value. In response to phosphorus deprivation, there was no significant change in NcAMP in either group (normal subjects 0.86±0.18 to 0.65±0.27 nmol/100 ml GF; patients with FHR 1.93±0.46 to 1.47±0.32 nmol/100 ml GF). Further, individual responses in each group were variable, with some subjects showing no change and others a small rise. Mean iPTH levels did not change significantly in either group, (normal subjects 16±2 to 10±3; patients with FHR 19±7 to 17±7).

Effects on 1,25(OH)₂D metabolism. There were marked differences between the two study groups in the changes in 1,25(OH)₂D levels in response to phosphorus deprivation (Fig. 2). The normal subjects showed a progressive rise in 1,25(OH)₂D levels over the course of the first 72 h of study, with a plateau thereafter. The mean value rose from 49±3 to 65±6 pg/ml after 96 h of phosphorus deprivation (P < 0.05). In contrast, the subjects with FHR showed no significant change in 1,25(OH)₂D levels (from 34±5 to 29 + 3 pg/ml, P = NS). The mean 1,25(OH)₂D values were statistically lower in the patients with FHR under both base-line conditions (P < 0.01) and at day 5 (P < 0.001).

There were individual differences in the 1,25(OH)₂D response in the subjects with FHR. Three showed small rises, the rest showing a fall. When the changes in 1,25(OH)₂D levels were analyzed as a function of the individual changes in NcAMP in this group, a highly significant positive correlation was observed (r = 0.93 P < 0.005, Fig. 3). However, when the changes in NcAMP and 1,25(OH)₂D levels were analyzed in the group of normal subjects, no correlation was observed (r = −0.04, P = NS). The change in PTH was not significantly correlated with the change in 1,25(OH)₂D in either group.

DISCUSSION

A reduced renal phosphate threshold is the phenotypic hallmark of FHR. Albright (1) originally attributed this renal phosphate wasting to secondary hyperparathyroidism. Recent evidence, however, would support the view that there is a primary defect in trans-epithelial renal phosphate transport in this syndrome. First, calculating PTH, which is the major hormonal influence on renal phosphate reabsorption, is normal in untreated subjects with FHR (5, 22, 23). Further, when parathyroid function is suppressed by calcium infusion, the renal phosphate threshold remains impaired (24). Partial parathyroidectomy has failed to improve the TmP/GFR (25), and a recently reported patient with idiopathic hypoparathyroidism and FHR had reduced renal phosphate threshold when the hypocalcemia was corrected (26). Thus, excessive PTH activity or enhanced sensitivity to normal circulating levels of this hormone do not seem to be present. Second, in the Hyp mouse model of FHR, parathyroidectomy does not correct the defect (27), and renal cor-
Figure 2 Effect of phosphorus deprivation on 1,25(OH)\textsubscript{2}D levels (*P < 0.05 compared with baseline and *P < 0.001 compared with the 96-h value in patients with FHR).

Figure 3 Change in 1,25(OH)\textsubscript{2}D levels vs. change in NcAMP in patients with FHR (*P = +0.93, *P < 0.005).

Despite the lack of compelling evidence that the activity of, or response to, PTH are important in the pathogenesis of FHR, most in vivo attempts to investigate the abnormality in renal phosphate reabsorption in these patients have used manipulation of parathyroid function. These studies have led to confusing and at times contradictory results. Glorieux and Scrivener (6) initially reported marked impairment in the phos-
phaturic response to PTH in three male patients with FHR. However, Hahn et al. (24) subsequently reported normal responses to PTH infusion in 14 patients with FHR. Finally, Short et al. (33) reported an exaggerated phaturic response to infused PTH in the posthypercalcemic state in two males with FHR.

Dietary phosphorus deprivation is a potent stimulus to renal phosphate conservation. Studies in animals (34) and man (35) have demonstrated that the capacity to reduce renal phosphate losses during phosphate deprivation is PTH independent. Further, phosphorus deprivation stimulates 1,25(OH)2D production in intact (36) and thyroparathyroidectomized animals (37), so that this response is also a PTH-independent effect. We therefore chose this stimulus as a probe to examine renal phosphate handling and circulating 1,25(OH)2D in patients with FHR.

We observed a defective renal tubular response to phosphorus deprivation in patients with FHR. The rise in TmP/GFR was significantly blunted in these patients, plateauing after only 24 h, while the normal subjects showed a progressive rise for 72 h, with more than a twofold greater maximal rise (Fig. 1). In keeping with the small mean rise in TmP/GFR observed in the patients with FHR, individual values for TmP/GFR remained well below the lower limit of normal at all times. Parathyroid activity was not elevated in the subjects with FHR and, in response to phosphorus deprivation, did not change. Despite this, phosphate resorption remained significantly impaired. These findings argue against a role for PTH in the pathogenesis of the renal tubular defect in patients with FHR.

To our knowledge, this is the first demonstration of a blunted renal response to phosphorus deprivation in subjects with FHR. However, similar observations have been made in the hypophosphatemic (Hyp) mouse model. Mulbauer et al. (38) demonstrated a markedly attenuated rise in TmP/GFR in Hyp as compared with control mice when the animals were placed on a low phosphorus diet. Studies using renal brush border membranes from Hyp mice have revealed subnormal sodium-dependent phosphate transport. Dietary phosphorus deprivation in vivo improved phosphate transport in these membranes but transport remained subnormal when compared with that seen in phosphate-depleted control animals (39).

Studies in animals (36) have demonstrated that phosphorus deprivation is a potent stimulus to 1,25(OH)2D production. The response of 1,25(OH)2D metabolism to phosphorus deprivation in man has not been extensively studied. Dominguez et al. (40) reported enhanced turnover of plasma 25-hydroxvitamin D pools in response to phosphorus deprivation in men and women. This correlated well with fractional calcium and phosphorus absorption, which the authors argued represented evidence of enhanced 1,25(OH)2D production. However, in a subsequent study (41), the same group directly measured 1,25(OH)2D levels in response to phosphorus deprivation and saw a marked sex difference, with levels rising in women and remaining unchanged in men. In contrast, we observed a rise in 1,25(OH)2D in normal males in response to phosphorus deprivation. Mean 1,25(OH)2D levels rose steadily and plateaued after 72 h of phosphorus deprivation in these subjects (Fig. 2). After 5 d of study, the mean plasma 1,25(OH)2D level (63±6 pl/ml) was close to the upper limit of normal (65 pg/ml), and it exceeded this value in three subjects.

The mean baseline plasma concentration of 1,25(OH)2D in the patients with FHR, although within the normal range, was significantly lower than that seen in normal subjects. Scriver et al. (42) initially reported mean values for circulating 1,25(OH)2D below the normal range, with more recent series (9–12) showing low-normal to normal mean values in additional patients with FHR. Our data corroborate these reports and underscore the inappropriateness of these values, given the ambient hypophosphatemia.

In contrast to the response observed in normal subjects, phosphorus deprivation was associated with no change in circulating 1,25(OH)2D in the subjects with FHR. Meyer et al. (43) have recently reported that 1,25(OH)2D levels fall dramatically in Hyp mice placed on a low phosphorus diet, in contrast to a rise seen in normal mice. Our studies document a qualitatively abnormal response in the human syndrome as well.

The mechanism responsible for this paradoxical response is not clear. PTH and serum phosphorus are two of the principal regulators of the plasma concentration of 1,25(OH)2D. In our normal subjects, phosphorus deprivation led to no change in PTH activity. Despite this, 1,25(OH)2D levels rose, suggesting that the phosphopenic stimulus may operate independently of any change in PTH activity in normal individuals. In contrast, in both the Hyp mouse and human FHR, circulating 1,25(OH)2D levels do not respond to the phosphopenic stimulus.

What role PTH has in regulating 1,25(OH)2D levels in this syndrome is not clear. Lyles and Dreznner (12) noted a blunted rise in circulating 1,25(OH)2D in response to exogenous PTH in patients with FHR when compared with control subjects (12). In response to phosphoenia, mean iPTH and NcAMP values did not change in our study. However, when individual changes in NcAMP were analyzed together with individual changes in plasma 1,25(OH)2D, a strong positive correlation was noted in the patients with FHR (Fig. 3). No such correlation was observed in normal subjects. Since the phosphopenic stimulus appears not to be operative in FHR, one interpretation of these data is
that PTH may be the principal regulator of circulating 1,25(OH)_{2}D levels in this syndrome, even given the quantitative abnormality noted previously (12). However, our data on this point are limited, and this supposition remains speculative.

The changes in blood and urinary calcium levels induced during this study were small, probably reflecting the short duration of the study (Table II). The rise in fasting calcium excretion would suggest that some, if not most, of this calcium is derived from bone, as has been observed in other studies of phosphorus depletion (35).

It is possible that the renal cortical intracellular phosphorus concentration is inappropriately normal or high in patients with FHR, explaining the lack of adaptive response in the present study. Data from studies in the Hyp mouse model, however, suggest that total intracellular phosphorus concentrations are normal (28), and nucleotide triphosphate concentrations may actually be low (44). Even if this were the case, however, one would expect the dietary phosphorus deprivation, superimposed on the phosphopenia of the disease, would eventually induce changes paralleling those seen in healthy subjects under similar circumstances. Such responses were not seen in our patients. There was a blunted rise in renal tubular phosphate threshold and a lack of response in circulating 1,25(OH)_{2}D. Additional carefully controlled in vivo studies of various potential regulators of 1,25(OH)_{2}D levels are needed in this syndrome.

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