Evaluation of a Role for 1,25-Dihydroxyvitamin D<sub>3</sub> in the Pathogenesis and Treatment of X-linked Hypophosphatemic Rickets and Osteomalacia

Marc K. Drezner, … , Mark R. Haussler, John M. Harrelson


Although a defect in renal transport of phosphate seems well established as the primary abnormality underlying the pathogenesis of X-linked hypophosphatemic rickets and osteomalacia, several observations indicate that renal phosphate wasting and hypophosphatemia cannot solely account for the spectrum of abnormalities characteristic of this disease. Thus, in the present study, we investigated the potential role of abnormal vitamin D metabolism in the pathogenesis of this disorder and the effect of 1,25-dihydroxyvitamin D<sub>3</sub> therapy on both the biochemical abnormalities characteristic of this disease and the osteomalacia. Four untreated patients, ages 14-30 yr, had normocalcemia (9.22±0.06 mg/dl); hypophosphatemia (2.25±0.11 mg/dl); a decreased renal tubular maximum for the reabsorption of phosphate per liter of glomerular filtrate (2.12±0.09 mg/dl); normal serum immunoreactive parathyroid hormone concentration; negative phosphate balance; and bone biopsy evidence of osteomalacia. The serum 25-hydroxyvitamin D<sub>3</sub> concentration was 33.9±7.2 ng/ml and, despite hypophosphatemia, the serum level of 1,25-dihydroxyvitamin D<sub>3</sub> was not increased, but was normal at 30.3±2.8 pg/ml. These data suggested that abnormal homeostasis of vitamin D metabolism might be a second defect central to the phenotypic expression of X-linked hypophosphatemic rickets/osteomalacia. This hypothesis was supported by evaluation of the long-term response to pharmacological amounts of 1,25-dihydroxyvitamin D<sub>3</sub> therapy in three subjects. The treatment regimen resulted in elevation of the serum 1,25-dihydroxyvitamin D levels to values in the supraphysiological range. Moreover, the […]

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Evaluation of a Role for 1,25-Dihydroxyvitamin D₃ in the Pathogenesis and Treatment of X-linked Hypophosphatemic Rickets and Osteomalacia

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A B S T R A C T Although a defect in renal transport of phosphate seems well established as the primary abnormality underlying the pathogenesis of X-linked hypophosphatemic rickets and osteomalacia, several observations indicate that renal phosphate wasting and hypophosphatemia cannot solely account for the spectrum of abnormalities characteristic of this disease. Thus, in the present study, we investigated the potential role of abnormal vitamin D metabolism in the pathogenesis of this disorder and the effect of 1,25-dihydroxyvitamin D₃ therapy on both the biochemical abnormalities characteristic of this disease and the osteomalacia. Four untreated patients, ages 14-30 yr, had normocalcemia (9.22+0.06 mg/dl); hypophosphatemia (2.25+0.11 mg/dl); a decreased renal tubular maximum for the reabsorption of phosphate per liter of glomerular filtrate (2.12+0.09 mg/dl); normal serum immunoreactive parathyroid hormone concentration; negative phosphate balance; and bone biopsy evidence of osteomalacia. The serum 25-hydroxyvitamin D₃ concentration was 33.9±7.2 ng/ml and, despite hypophosphatemia, the serum level of 1,25-dihydroxyvitamin D₃ was not increased, but was normal at 30.3±2.8 pg/ml. These data suggested that abnormal homeostasis of vitamin D metabolism might be a second defect central to the phenotypic expression of X-linked hypophosphatemic rickets/osteomalacia. This hypothesis was supported by evaluation of the long-term response to pharmacological amounts of 1,25-dihydroxyvitamin D₃ therapy in three subjects. The treatment regimen resulted in elevation of the serum 1,25-dihydroxyvitamin D levels to values in the supraphysiological range. Moreover, the serum phosphate and renal tubular maximum for the reabsorption of phosphate per liter of glomerular filtrate increased towards normal whereas the phosphate balance became markedly positive. Most importantly, however, repeat bone biopsies revealed that therapy had positively affected the osteomalacic component of the disease, resulting in normalization of the mineralization front activity. Indeed, a central role for 1,25-dihydroxyvitamin D₃ in the mineralization of the osteomalacic bone is suggested by the linear relationship between the serum level of this active vitamin D metabolite and the mineralization front activity. We, therefore, suggest that a relative deficiency of 1,25-dihydroxyvitamin D₃ is a factor in the pathogenesis of X-linked hypophosphatemic rickets and osteomalacia and may modulate the phenotypic expression of this disease.

INTRODUCTION

X-linked hypophosphatemic rickets and osteomalacia (XLH)¹ is a familial syndrome characterized by inadequate mineralization of cartilage and/or bone, conse-

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¹ Abbreviations used in this paper: 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 25(OH)D, 25-dihydroxyvitamin D; PTH, parathyroid hormone; TmP/GFR, renal tubular maximum for the reabsorption of Pi, normalized to glomerular filtration rate; XLH, X-linked hypophosphatemic rickets/osteomalacia.
quent skeletal deformities, and growth retardation. The hallmarks of this disease include a reduced serum concentration of inorganic phosphate (Pi) (1-3) and resistance to therapy with vitamin D (4-6). Current evidence suggests that the primary defect governing the genesis of XLH is an abnormality of Pi, transport in the renal tubule (7). This abnormal function results in Pi wasting and consequent hypophosphatemia, upon which all other components of the disorder apparently depend.

Despite the established primacy of renal Pi wasting in the pathogenesis of XLH, treatment aimed only at overcoming the resulting hypophosphatemia fails to normalize all of the manifestations of the disease (8,9), indicating that an additional factor(s), or an alternate physiological abnormality, may contribute to the pathogenesis of this disorder. Traditionally, an alteration of vitamin D-dependent calcium homeostasis has been suspected as such a potential abnormality (10). However, the presence and/or nature of this suspected defect remains undetermined. Nevertheless, the recent elucidation of the metabolic pathways for vitamin D activation (11,12) has focused interest on the possibility that an abnormality in this process may be operative in XLH.

Therefore, in the present investigation, we studied whether compromised availability of vitamin D or its active metabolite, 1,25(OH)2D3, is a factor in the pathogenesis of XLH. In these studies we performed detailed examination of vitamin D metabolism in untreated subjects with XLH and, in addition, evaluated the effects of long-term 1,25(OH)2D3 therapy on the biochemical and bone histomorphological abnormalities characteristic of this disease.

METHODS

Patient population. Four patients, a female aged 15 yr and three males, 14, 21, and 30 yr of age (cases 1-4, respectively) were selected for study. The adolescent subjects were probands from kindreds with an X-linked dominant transmission of the rachitic-osteomalacic disorder and the adult males were brothers in a third kindred in which XLH had been likewise documented.

At the initiation of the study the chronologically aged 15- and 14-yr-old subjects had a height age of 12 and 11 yr and a bone age (13) of 17 and 16 yr, respectively. In addition, premature fusion of the epiphyseal centers in the distal femur and proximal tibia had occurred in both subjects and consequently there was no evidence of active rickets. However, characteristic postrachitic skeletal deformities were present and there was radiographic evidence of active osteomalacia. At the completion of the 1-yr study, height age in these subjects was unchanged whereas bone age was 17 yr in both subjects. The adult subjects (cases 3 and 4) likewise presented with growth retardation (height 155 and 163 cm) and postrachitic deformities. In addition, active osteomalacia was similarly evidenced radiographically by pseudo-fractures, coarsened trabeculation, and riddled areas in the long bones.

Therapy with pharmacological amounts of vitamin D and/or vitamin D and oral phosphates had been previously administered to each subject. Initiation of treatment was at age 5-8 yr of age and adherence sporadic thereafter. However, no form of therapy with oral Pi or vitamin D and its metabolites had been administered to the patients for 4-9 yr before evaluation.

Complete metabolic studies and bone biopsies were performed in all four subjects in the untreated state on the Duke University Medical Center Clinical Research Unit. Subsequently, therapy with 1,25(OH)2D3 alone was initiated in cases 1-3. Daily treatment with 0.25 µg orally was begun and gradually modified by 0.25-µg increments to 2.25-3.00 µg/d (0.036-0.062 µg/kg per d), given in a split dose regimen at 9:00 a.m. and 9:00 p.m. Serial measurements of the serum calcium concentration and 24-h urinary calcium excretion were employed to monitor potential complications of therapy and 3.0 µg of 1,25(OH)2D3 per d arbitrarily established as the maximum amount of drug used. Ultimately, 1,25(OH)2D3 was continued at established maintenance levels for 12 mo. Complete metabolic studies were repeated at the 6-mo interval and bone biopsies after both 6 and 12 mo of therapy. In case 2 the dosage of 1,25(OH)2D3 was increased from 2.50 to 2.75 µg/d after 6 mo of therapy. All studies were performed with the informed consent of the patient, or the patient and his parents, and were approved by the Duke University Human Investigations Committee. The 1,25(OH)2D3 for oral administration was supplied by the Chemical Research Department, Hoffmann-La Roche Inc. (Nutley, N. J.).

Biochemical studies and radioimmunoassays. Serum calcium (normal 8.7-10.3 mg/dl) was measured by atomic absorption spectrophotometry and serum Pi (for age specific normals, see Table I) by the colorimetric method of Dryer et al. (14). Serum creatinine (normal 0.7-1.2 mg/dl) and alkaline phosphatase were determined on the Multichannel Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). Urine specimens were stored at -20°C before analysis of calcium (by atomic absorption spectrophotometry), Pi, (15), and creatinine (16). Fecal fat excretion was determined by the method of Van de Kamer et al. (17) on 72-h fecal collections marked by carmine red dye and collected during ingestion of 70 g fat/d.

Serum parathyroid hormone (PTH) concentration was measured by four separate radioimmunoassays. Carboxy-terminal-specific assays were purchased from the Mayo Medical Laboratory (Rochester, Minn.) and The Upjohn Laboratory (Kalamazoo, Mich.); normals were <40 µU eq/ml (18) and <150-375 pg eq/ml (19), respectively. An amino-terminal-specific assay (normal <0.125 ng/ml) employing a guinea pig antibody raised against the synthetic (1-34) amino-terminal peptide of human PTH was performed in the Duke University Laboratories (20, 21) and Dr. Leonard Deftos (University of California, La Jolla, Calif.) measured PTH (normal <300 pg/ml) by a predominantly carboxy-terminal immunnoassay employing previously published methods (22).

Competitive binding protein assays of Vitamin D metabolites. Serum 25-hydroxyvitamin D (25(OH)D) concentration was measured by a modification of the methods of Haddad and Chyu (20, 23). In each subject, in the base-line and treated state, the reported value is the mean±SEM of triplicate determinations made on three separate samples. The seasonally adjusted normal range (mean±2 SD) for this measurement in 60 normal subjects, aged 13-55 yr, is 15-80 ng/ml.

Serum concentration of 1,25(OH)2D was quantitated according to previously published methods (24-26). The reported value in each subject, in the base-line and treated state, is the mean±SEM of triplicate measurements made on two separate samples. When patients were treated with 1,25(OH)2D, samples for this determination were obtained.
at 11:00 a.m. This time of sampling was selected after pre-
liminary investigations of divided dose 1,25(OH)2D therapy
(9:00 a.m., 9:00 p.m.) indicated that circulating levels of this
metabolite, measured on single samples obtained at either
11:00 a.m., 3:00, 5:00, or 7:00 p.m., closely approximated
( 8.9%) the mean serum concentration maintained through-
out the day (26). Previous observations that serum 1,25-
(OH)2D concentration varies with chronological age (27, 28)
and presumably growth (29) necessitated that we establish
normal values for this measurement, appropriate for both the
adolescent and adult subjects whom we studied. In 79 adults,
aged 18-70 yr, the normal serum 1,25(OH)2D concentration
(mean ± 2 SD) is 20-45 pg/ml. In contrast, the normal range
in 20 normal adolescents, aged 13-17 yr, is 23-70 pg/ml,
levels comparable to values reported by Chesney et al. (27)
in a group of similarly aged subjects. However, in the eight
adolescents with a bone age > 16 yr the normal range is similar
to that in adults, 23-48 pg/ml.

Balance studies and assessment of renal phosphate handling. Calcium and phosphorus balance studies were
performed in the base-line and treated state in two 5-d periods
according to previously published methods (29). Gastro-
intestinal absorption of the minerals represents the net dif-
fERENCE BETWEEN measured dietary intake and fecal excretion
and mineral balance the difference between absorption and
urinary excretion. The results of balance studies performed in
12 normal adults, aged 20-40 yr, were used as the age and/or
growth rate matched control data for these studies.

The renal tubular maximum for the reabsorption of P,
normalized to glomerular filtration rate (TmP/GFR) was cal-
culated by the method of Bijvoet (29). For these studies
serial 2-h urine collections on selected mornings were pre-
ceded by a water load of 20 ml/kg body wt. The urine P, (15)
and creatinine (16) were determined in each specimen,
sodium determinations of P, (14) and creatinine at the mid-
point of each collection, and the TmP/GFR estimated (for
age-specific normals, see Table I) by appropriate calculations
and use of a published nomogram (30). Unpublished observa-
tions in our laboratory have confirmed that the estimated
value accurately approximates the value of TmP/GFR deter-
mined during P, infusion. The values reported are the mean±SEM
of six determinations.

Bone studies. Transcortical bone biopsies were obtained
from the anterior iliac crest under local anesthesia. Tetracy-
cline (250 mg orally every 6 h) was administered to each
subject over the 96- to 48-h period preceding the biopsy.
Bone specimens were fixed in glutaraldehyde. Sections were
made with a Buehler Isomet sectioning machine (Buehler Ltd.,
Evanston, Ill.), ground to a thickness of 40±2 μm and then mounted either in
the unstained state or after staining with toluidine blue and basic fuchsin.

Histomorphometric analysis of the trabecular bone in the
40-μm sections was accomplished by analysis of 50 micro-
scopic fields in a single plane of focus employing a Merz inte-
grated reticle (31). The results of such studies are no different
than those obtained by analysis of 5-μm thick Goldner stained
sections.7 Identification of mineralized bone-osteoid granular
interface by light microscopy and tetracycline label by fluores-
cent microscopy was employed to quantitate mineralization
front activity.

The following histological parameters were quantitated:
(a) Mineralization front activity (percent), the percentage of

7 Felsenfeld, A., R. Gutman, and J. M. Harrelson. Un-
published observations.

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tions.

osteoïd covered trabecular bone surface exhibiting a fluores-
cent tetracycline label and a bone-osteoid granular interface;
(b) Mean osteoid seam width (micrometers), the mean width
of 50 randomly selected osteoid seams which were measured
using a linear reticle calibrated with a stage micrometer;
(c) Osteoid surface (percent), the percentage of trabecular
bone surface covered by osteoid; and (d) Active resorption
(percent), the percentage of trabecular bone surface on which
Howship's lacunae containing multinucleated osteoclasts are
present. Normal values for these measurements were deter-
mined in bone biopsies from 10 normal subjects aged
15-35 yr.

Statistical analyses. Statistical evaluation of the data was
performed with Student's t test for unpaired observations
(32) with appropriate modification, when necessary, to ac-
commodate comparisons between an unequal number of data
points in the groups compared. Where necessary (Fig. 3) data
was fit to straight lines by the method of least squares (33).

RESULTS

Vitamin D metabolism in XLH. A history of normal
dietary intake in affected subjects and documented
normal fecal fat excretion (2.5±0.3 g/24 h; normal < 5)
provided no evidence for vitamin D deficiency. Normal liver
and renal function tests (creatinine clearance 120±5.1 ml/min [Table I]) and lack of drug exposure
(e.g., phenobarbital and dilantin) indicated no a priori
cause for altered vitamin D metabolism. Thus, we
measured the serum levels of 25(OH)D and 1,25(OH)2D
to assess whether the XLH mutation is associated with
decreased availability of either of these metabolites.
The serum 25(OH)D concentration averaged 33.9±7.2
ng/ml and was within the normal range in each subject
(Table I), indicating normal vitamin D stores and provi-
 ding evidence for normal vitamin D–25-hydroxylase
activity. However, despite the presence of hypophos-
phatemia, which is a known stimulus of 25-hydroxy-
vitamin D-1α-hydroxylase activity, the serum 1,25-
(OH)2D concentration averaged 30.3±2.8 pg/ml and
was marginally below or within the appropriate age-
matched normal range in each adolescent and adult
subject (Table I), not increased as expected. These
findings indicated that a relative deficiency of 1,25-
(OH)2D might contribute to the pathogenesis of XLH and,
consequently, we initiated therapy with this active
vitamin D metabolite.

Effects of 1,25(OH)2D therapy on the serum concentra-
tion of vitamin D metabolites. Therapy with
1,25(OH)2D3 (2.25-3.00 μg/d) was maintained for 6 mo,
after which we remeasured the serum concentration of
the vitamin D metabolites. The circulating level of
25(OH)D averaged 38.8±7.7 ng/ml, a value not signifi-
cantly different from that maintained in the base-line
period (Table I). In contrast, drug treatment resulted in
a significant elevation (P < 0.001) of the serum,
1,25(OH)2D concentration to levels averaging 51.3
± 1.2 pg/ml. Indeed, in each of the three treated subjects
the increase in the serum level of 1,25(OH)2D was

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significant ($P < 0.05$), resulting in maintenance of a supraphysiologically circulating concentration of this metabolite when values were compared to age- and bone age-matched controls (Table I).

**Effects of 1,25(OH)$_2$D$_3$ therapy on $P_1$ metabolism.** Each of the subjects with XLH presented initially with a characteristically decreased serum $P_1$ and evidence of renal $P_1$ wasting marked by a subnormal TmP/GFR (Table I). In response to therapy with 1,25(OH)$_2$D$_3$ the serum $P_1$ was significantly elevated in each treated subject ($P < 0.001$) and approached, or was within, the age-corrected normal range. Similarly, 1,25(OH)$_2$D$_3$ therapy resulted in a significant increase ($P < 0.01$) of the TmP/GFR (Table I). However, the TmP/GFR remained subnormal in each subject.

An additional abnormality of $P_1$ metabolism manifest at presentation was a negative $P_1$ balance, which ranged from $-3$ to $-60$ mg/dl (Table II). This abnormality resulted from apparent net gastrointestinal mal-absorption of $P_1$ (Table II) and renal $P_1$ wasting (TmP/GFR, 2.12±0.09 mg/dl) (Table I). Treatment with 1,25(OH)$_2$D$_3$ resulted in a remarkably positive $P_1$ balance, ranging from +144 to +264 mg/dl in the treated subjects (Table II). This alteration in $P_1$ balance was largely caused by a significant improvement in the net gastrointestinal absorption of $P_1$ in each subject (Table II) and renal $P_1$ wasting (TmP/GFR, 2.43±0.06) (Table I). Despite the improvement in renal $P_1$ wasting, however, 24-h urine $P_1$ increased significantly in response to therapy (Table II).

**Effects of 1,25(OH)$_2$D$_3$ therapy on calcium metabolism.** In the base-line period the serum calcium concentration averaged 9.22±0.06 mg/dl, a value well within normal limits. After treatment with 1,25(OH)$_2$D$_3$ the mean serum calcium remained normal, 9.27±0.23 mg/dl, and was not significantly different from the base-line level. However, in case 2, a significant increase, and in case 3, a significant decrease in the serum calcium did occur and persisted (Table I).

Further analysis of calcium homeostasis revealed that the patients maintained a marginally positive calcium balance, 83±30 mg/d, in the base-line period (Table III). This positive balance was maintained, despite a modest decrease in net gastrointestinal mal-absorption of calcium, by virtue of renal conservation of calcium (Table III). Treatment with 1,25(OH)$_2$D$_3$ resulted in markedly positive calcium balance of 438±30 mg/d (Table III). This calcium retention occurred in response to a significant increase in net gastrointestinal absorption of calcium in each treated subject and despite an increase in urinary calcium excretion (Table III). The urinary calcium excretion, however, remained within the appropriate normal range and was not significantly different from that in the untreated state.

**Effects of 1,25(OH)$_2$D$_3$ therapy on the serum PTH concentration.** Because many of the cardinal mani-

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**Table I**

**Metabolic Data in Four Patients with XLH**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>1,25(OH)$_2$D$_3$ Treatment</th>
<th>Serum</th>
<th>Renal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calcium</td>
<td>$P_1$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>µg/d</td>
<td>mg/dl</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>0</td>
<td>9.04±0.60*(16) (19)</td>
<td>2.11±0.05 (18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.00</td>
<td>9.20±0.07 (18)</td>
<td>2.73±0.06 (18)</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>0</td>
<td>9.21±0.09 (16)</td>
<td>2.12±0.05 (18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.50</td>
<td>9.69±0.07 (17)</td>
<td>2.87±0.06 (17)</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>0</td>
<td>9.33±0.06 (15)</td>
<td>2.18±0.06 (14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.25</td>
<td>8.92±0.10 (15)</td>
<td>2.71±0.06 (15)</td>
</tr>
<tr>
<td>Mean</td>
<td>10</td>
<td>0</td>
<td>9.29±0.11 (18)</td>
<td>2.59±0.07 (18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.25-3.00</td>
<td>9.27±0.23</td>
<td>2.77±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.001$</td>
</tr>
</tbody>
</table>

* Mean±SEM.
† Number of determinations.
‡ The normal values for serum $P_1$, serum alkaline phosphatase and TmP/GFR are age specific. In cases 1 and 2 the normal values for these measurements are 2.9–5.5 mg/dl, <170 IU, and 2.70–5.5 mg/dl, respectively. In cases 3 and 4 these normal values are 2.5–4.5 mg/dl, <110 IU, and 2.55–4.5 mg/dl.

* The serum 1,25(OH)$_2$D level varies with age and growth rate. In adults the normal range is 20–45 pg/ml. In contrast in adolescents (13–17), normal values range from 23 to 70 pg/ml. However, when bone age of adolescents is >16 yr and growth rate slowed the normal range is 23–48 pg/ml.
The data a mean±SEM.

**Significant difference from controls at P < 0.02.

† Significant difference from controls at P < 0.01.

§ Significant difference from controls at P < 0.05.

The data represent the results of two 5-d balance studies in each subject in the base-line state and, where possible, on treatment with 1,25(OH)₂D₃. Dietary intake, fecal excretion, gastrointestinal absorption, and urinary excretion and balance are expressed as a mean±SEM of the respective values in each balance period. The control data were obtained in 12 normal subjects, aged 20–40 yr, by performing two 5-d balance studies. (Although phosphate balance varies with age and is significantly greater in rapidly growing adolescents [by virtue of increased gastrointestinal absorption], the completed linear growth of the adolescent subjects in the present study makes subjects aged 20–40 yr appropriate growth rate-matched controls.) The data in these controls are expressed as the mean±SEM of the results in the 12 subjects. In addition the SD of these determinations is provided.

* Number of subjects studied.

† Mean±SEM.

‡ SD.

§ Significant difference from controls at P < 0.02.

** Significant difference from controls at P < 0.01.

** Significant difference from controls at P < 0.05.

festations of XLH, and the changes in biochemical abnormalities which we observed in response to 1,25(OH)₂D₃ therapy, might be related to abnormalities or alterations in the serum PTH concentration, we measured this variable in the untreated and treated periods. The serum PTH concentration, measured by four separate radioimmunoassays, with varying antigenic specificity, was normal in each subject in the base-line period, suggesting that altered parathyroid function was not central to the genesis of the syndrome. Nevertheless, 1,25(OH)₂D₃ treatment did result in a decrease in the circulating level of PTH, as indicated by the uniformly lowered posttreatment values measured by each assay (Table IV). Thus the effects of 1,25(OH)₂D₃ on the biochemical abnormalities of the syndrome may be modulated, in part, by suppression of PTH secretion.

**Effects of 1,25(OH)₂D₃ on bone histomorphology.**

To evaluate the effects of 1,25(OH)₂D₃ therapy on the osteomalacic bone lesions, we obtained bone biopsies in the base-line period and after 6 and 12 mo of therapy. Initial biopsies were marked by decreased mineralization front activity (17.7–29.2%; normal 65.5±7.8); osteoid seams of increased width (24.5–50.9 μm; normal 12.5±4.7); and the presence of increased amounts of osteoid-covered trabecular bone surface (50.2–81.4%; normal 15.3±5.5). These abnormalities are indicative of osteomalacia (Fig. 1; Table V). Moreover, typical of the bone abnormality in XLH, osteocytic osteolysis was uniformly present (Fig. 1). In contrast, after 6 and 12 mo of therapy an apparent resolution of the mineralization defect had occurred. Bone biopsies from each treated subject were marked by a significant increase in tetracycline-labeled mineralization front activity (Fig. 2) and an apparent decrease in the osteocytic osteolysis. This apparent bone healing was accompanied by a significant decline in the serum alkaline phosphatase activity in each treated patient (Table I). Quantitative histomorphological analysis of the bone biopsies confirmed...
TABLE III

Calcium Balance Studies in Subjects with XLH

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Dietary intake</th>
<th>Fecal excretion</th>
<th>Gastrointestinal absorption</th>
<th>Urinary excretion</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (12)*</td>
<td>20–40</td>
<td>0 935 ± 45</td>
<td>(156)§</td>
<td>605 ± 22 (76)</td>
<td>330 ± 33 (114)</td>
</tr>
<tr>
<td>Case 1</td>
<td>15</td>
<td>0 947 ± 42</td>
<td>662 ± 28</td>
<td>285 ± 70</td>
<td>127 ± 13</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>0 909 ± 26</td>
<td>194 ± 23</td>
<td>715 ± 49</td>
<td>223 ± 28</td>
</tr>
<tr>
<td>Case 2</td>
<td>14</td>
<td>0 876 ± 20</td>
<td>787 ± 39</td>
<td>89 ± 19</td>
<td>71 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>0 928 ± 33</td>
<td>360 ± 45</td>
<td>568 ± 12</td>
<td>178 ± 14</td>
</tr>
<tr>
<td>Case 3</td>
<td>21</td>
<td>0 894 ± 27</td>
<td>745 ± 19</td>
<td>149 ± 33</td>
<td>89 ± 16</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>0 890 ± 17</td>
<td>225 ± 21</td>
<td>665 ± 30</td>
<td>233 ± 12</td>
</tr>
<tr>
<td>Case 4</td>
<td>30</td>
<td>0 898 ± 26</td>
<td>675 ± 24</td>
<td>223 ± 35</td>
<td>127 ± 19</td>
</tr>
<tr>
<td>Mean</td>
<td>—</td>
<td>0 904 ± 15</td>
<td>717 ± 21</td>
<td>187 ± 43</td>
<td>104 ± 14</td>
</tr>
<tr>
<td>Cases 1–4</td>
<td>2.25–3.00</td>
<td>0 909 ± 11</td>
<td>260 ± 51**</td>
<td>649 ± 42**</td>
<td>211 ± 17</td>
</tr>
<tr>
<td>NS</td>
<td></td>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

The data represent the results of two 5-d balance studies in each subject in the base-line state and, where possible, on treatment with 1,25(OH)2D3. Dietary intake, fecal excretion, gastrointestinal absorption, and urinary excretion and balance are expressed as mean ± SEM of the respective values in each balance period. Control data were obtained in 12 normal subjects, aged 20–40 yr by performing two 5-d balance studies. (Although calcium balance varies with age and is significantly greater in rapidly growing adolescents [by virtue of increased gastrointestinal absorption], the completed linear growth of the adolescent subjects in the present study makes subjects aged 20–40 yr appropriate growth rate matched controls.) The data in these controls are expressed as the mean ± SEM of the results in the 12 subjects. In addition the SD of these determinations is provided.

- Number of subjects studied.
- Mean ± SEM.
- Significant difference from controls at P < 0.02.
- Significant difference from controls at P < 0.05.
- Significant difference from controls at P < 0.001.

that 1,25(OH)2D3 therapy resulted in normalization of the decreased mineralization front activity present in each biopsy in the base-line state (Table V). Further, a central role for the 1,25(OH)2D3 in the induction of mineralization in the osteomalacic bone is suggested by the linear relationship between the serum level of this metabolite and mineralization front activity (Fig. 3). In both the untreated and treated state, mineraliza-

TABLE IV

Concentration of Immunoreactive Serum PTH in Patients with XLH

<table>
<thead>
<tr>
<th>Case</th>
<th>1,25(OH)2D3 treatment</th>
<th>Serum PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/d</td>
<td>µ eq/ml</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2.25</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;40</td>
<td>&lt;150–375</td>
</tr>
</tbody>
</table>

* ND, nondetectable.

1,25-Dihydroxycitamin D3 and X-linked Hypophosphatemic Rickets and Osteomalacia 1025
tion front activity closely correlated with the serum levels of 1,25(OH)₂D circulating at the time of biopsy.

Despite the normalization of mineralization front activity, treatment with 1,25(OH)₂D₃ had variable effects on the wide osteoid seams and the excess osteoid covered trabecular bone surface present on initial biopsies. Although osteoid seam width and the extent of osteoid covered trabecular bone surface progressively diminished over the 12 mo of treatment in case 2, the biopsies from cases 1 and 3 showed no change in the extent of osteoid and variable changes in osteoid seam width (Table V).

**DISCUSSION**

Of the several theories for the pathogenesis of XLH which have evolved, no single explanation has gained universal acceptance and the metabolic basis for this disease remains unknown. Nevertheless, cumulative data from several studies (7, 34, 35) indicate that a defect in transepithelial transport of P₂ in kidney (resulting in partial impairment in net P₁ reabsorption) and perhaps also in intestine and bone is likely the primary determinant of the XLH phenotype in man. This proposal is supported by several lines of evidence: (a) treatment of affected subjects with orthophosphate, in doses sufficient to restore the serum P₁ to normal, results in radiographically evident healing of rickets and promotes "catch-up" linear growth in childhood (36, 37); (b) subtotal parathyroidectomy does not change renal P₁ handling, suggesting that the renal tubular defect is innate (38, 39); and (c) animals rendered hypophosphatemic by P₁ depletion develop abnormalities in bone mineralization similar to those of XLH (40). Moreover, Tenenhouse et al. (41) have reported that purified renal cortical brush membranes from the murine homologue of XLH, the X-linked hyp-mouse, exhibit a partial loss of the sodium-dependent phosphate transport process. This finding in the hyp-mouse is compatible with a partial loss of renal phosphate transport in XLH. Nevertheless this hypothetical P₂ leak and resultant hypophosphatemia do not account for all of the abnormalities of XLH, especially the characteristic decrease in intestinal absorption of calcium and P₁, or its normalization after therapy with 1α(OH)D₃ (42, 43). In addition, the variable presence of the bone lesion and the lack of correlation between the magnitude of the hypophosphatemia and the severity of the rickets or osteomalacia is enigmatic (1). Finally, treatment with P₁ alone or in combination with pharmacological amounts

**FIGURE 1** (A) Microradiograph showing both hypomineraledized "splits" (S) and patchy osteocytic osteolysis (O). (B) Unstained section demonstrating extensive osteoid seams (arrows) with minimal mineralization.

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of vitamin D does not restore bone mineralization to normal (44) refuting the hypothesis that all the component abnormalities are solely dependent upon hypophosphatemia.

The results of the present study indicate an abnormality in vitamin D metabolism as an additional factor in the phenotypic expression of XLH. A disordered regulation of vitamin D metabolism is suggested by the serum levels of 1,25(OH)2D in affected subjects in the base-line state (Table I). Recent studies in both man (45) and various animals (46–48) indicate an inverse relationship between serum levels of P1 and production rate or serum concentration of 1,25(OH)2D. Therefore, it follows that patients with XLH should manifest both an increased production rate for and an increased circulating level of 1,25(OH)2D. However, we found that three affected subjects had only a ‘normal’ circulating level and one a marginally decreased serum concentration of 1,25(OH)2D. These data indicate a functional impairment in the regulation of 1,25(OH)2D biosynthesis which may contribute to the clinical expression of XLH.

The therapeutic response to pharmacological amounts of 1,25(OH)2D3 substantiates a role for altered vitamin D homeostasis in the pathogenesis of XLH. Therapy raised the serum 1,25(OH)2D level and partially restored P1 homeostasis; long-term treatment normalized the mineralization defect in bone. The effects of therapy on P1 homeostasis were apparently modulated by the direct action of 1,25(OH)2D3 on a variety of tissues. These included a marked increase in the net gastrointestinal absorption of P1 (Table II), an apparent suppression of PTH secretion (Table IV); and an amelioration of renal P1 wasting, indicated by a significant increase in TmP/GFR (Table I).

However, the concomitant increase in serum P1 (Table I) and consequently in the filtered load of P1, significantly increased the 24-h urine Pi excretion (Table II) obscuring any effects on renal Pi reabsorption. In any case, the TmPi/GFR remained below the normal range and the 24-h urine Pi, significantly above that in the base-line state, indicating persistent and inappropriate P1 wasting. Thus, 1,25(OH)2D3 treatment did not correct the basic renal defect, but did alter P1 homeostasis to circumvent the abnormality and to achieve a positive P1 balance (Table II) and a significant increase in the serum P1.

Most importantly, however, 1,25(OH)2D3 therapy repaired the osteomalacic bone lesion. Treatment restored normal mineralization front activity to the endosteal bone surface, reversing a primary abnormality of the XLH osteomalacic disorder. Moreover, the linear correlation between mineralization front activity and the serum level of 1,25(OH)2D3 (Fig. 3) indicates that this metabolite may directly affect deposition of calcium in bone. Although this relationship may be indirect and modulated by other factors, the lack of correlation between serum P1 at the time of bone biopsy and mineralization front activity (data not shown) and the failure of alternate therapeutic means to cause this response (44), despite similar effects on the characteristic biochemical abnormalities of the syndrome, suggest that the action on bone is direct.

Moreover, the apparent need to achieve supraphysiological serum levels of 1,25(OH)2D to effect bone healing reaffirms the hypothesis that an abnormality of vitamin D metabolism contributes to the manifestations of XLH. Several lines of evidence confirm that pharmacological amounts of 1,25(OH)2D3 are needed to induce the observed changes in bone

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**Table V**

Quantitative Histomorphology of Bone Biopsies

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Normals (10)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mineralization front activity, %</strong></td>
<td>17.7</td>
<td>72.5</td>
<td>73.6</td>
<td>23.0</td>
<td>65.5±7.8$</td>
</tr>
<tr>
<td><strong>Mean osteoid seam width, μm</strong></td>
<td>24.5</td>
<td>18.7</td>
<td>34.1</td>
<td>28.6</td>
<td>12.5±4.7</td>
</tr>
<tr>
<td><strong>Osteoid surface, %</strong></td>
<td>50.2</td>
<td>57.0</td>
<td>61.5</td>
<td>74.2</td>
<td>13.5±5.5</td>
</tr>
<tr>
<td><strong>Active resorption, %</strong></td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Number of normal controls.
† Mineralization front activity—the percent of osteoid covered trabecular bone surface over which mineralization is occurring.
§ Mean±1 SD.
¶ Mean osteoid seam width—mean width of osteoid seams determined by measurement of 50 seams using a calibrated reticle.
‡ Osteoid surface—the percentage of trabecular bone surface covered by osteoid.
** Active resorption—the percentage of total trabecular bone surface occupied by Howship's lacunae containing multinucleated osteoclasts.
These data indicate that 1028 M. doses of normal maintain to comparable activity despite those employed and photomicrograph underlying bone, improvement, lamellar bone.

First, mineralization. First, Glorieux et al. recently reported (49) the treatment of XLH with near physiological doses of 1,25(OH)$_2$D$_3$ (1 µg/d), but amounts less than those employed in the present study. They found improvement, but not normalization, of mineralization front activity despite concomitant therapy with oral P$_1$ (1.2–3.3 g/d) which increased the serum P$_1$ values comparable to those achieved in the present study. These data indicate that doses of 1,25(OH)$_2$D$_3$ must be significantly greater than physiological requirements to maintain normal mineralization front activity in XLH. Secondly, previous trials of 1,25(OH)$_2$D$_3$ therapy at doses of 1.0 and 1.3 µg/d and 1α(OH)D$_3$ therapy at 1.0 µg/d (50–53) have failed to improve net gastrointestinal absorption of P$_1$, and/or calcium and P$_1$ balance or have resulted in insignificant changes in tubular reabsorption of P$_1$ and/or TmP/GFR. These observations are similar to our own which we noted during the course of drug titration in the present study (data not shown) and contrast to the response seen (Tables I–III) when we used pharmacological amounts of 1,25(OH)$_2$D$_3$.

Further, in each treated subject in the present study the circulating concentration of 1,25(OH)$_2$D$_3$ increased significantly in response to pharmacological doses of this metabolite, attaining supraphysiological levels (Table I). While the values in the adolescent patients remained within the chronologically age-matched normal range (23–70 pg/ml), they were above values (23–48 pg/ml) maintained in normals matched for bone mineralization. First, Glorieux et al. recently reported (49) the treatment of XLH with near physiological doses of 1,25(OH)$_2$D$_3$ (1 µg/d), but amounts less than those employed in the present study. They found improvement, but not normalization, of mineralization front activity despite concomitant therapy with oral P$_1$ (1.2–3.3 g/d) which increased the serum P$_1$ values comparable to those achieved in the present study. These data indicate that doses of 1,25(OH)$_2$D$_3$ must be significantly greater than physiological requirements to maintain normal mineralization front activity in XLH. Secondly, previous trials of 1,25(OH)$_2$D$_3$ therapy at doses of 1.0 and 1.3 µg/d and 1α(OH)D$_3$ therapy at 1.0 µg/d (50–53) have failed to improve net gastrointestinal absorption of P$_1$, and/or calcium and P$_1$ balance or have resulted in insignificant changes in tubular reabsorption of P$_1$ and/or TmP/GFR. These observations are similar to our own which we noted during the course of drug titration in the present study (data not shown) and contrast to the response seen (Tables I–III) when we used pharmacological amounts of 1,25(OH)$_2$D$_3$.

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age (and presumably growth rate). In early childhood and adolescence serum levels of 1,25(OH)₂D are normally higher than at other periods of life, probably caused by rapid growth in early childhood and teenage years (28). This relationship to growth is substantiated in the present study by the internal disparity of the values seen in normal adolescents when segregated by bone age rather than chronological age. Thus, commensurate with plateau of growth and a bone age of 16 yr or greater, the normal range for serum 1,25(OH)₂D₃ is lower than that in the more rapidly growing adolescents who have a bone age ranging from 12 to 15 yr. The adolescent subjects with XLH whom we treated in the present study had a bone age of 16 yr or greater and the attenuated growth characteristic of the disease. Therefore, we used both chronological and bone age to select appropriate controls for serum 1,25(OH)₂D₃ concentration.

Our findings regarding serum concentration of 1,25(OH)₂D are consistent with previous observations. Meyer et al. (54) reported that the serum 1,25(OH)₂D levels in the X-linked hyp-mouse model are normal and not elevated as expected and as, in fact, are found in phosphate depleted normal mice of the same strain (55). Scriver et al. (55) reported serum levels of 1,25(OH)₂D₃ in human patients with XLH which were considerably lower (15.6±7.8 pg/ml) than those obtained in the present study. However, the patients studied by Scriver et al. were younger than ours and the majority were already being treated with oral P₃ and vitamin D. This therapeutic regimen may lower serum levels of 1,25(OH)₂D₃ and account for the noted differences (56). In any case, the observations of Scriver et al. substantiate that there is no increase in serum 1,25(OH)₂D₃. Moreover, previous studies in the X-linked hyp-mouse, provide data which may explain the relative deficiency of 1,25(OH)₂D. Despite renal P₃ wasting, the hyp-mouse has a normal intracellular P₃ content in kidney (41). This finding implies an intact component of P₃ transport (perhaps influx at the basolateral membrane) which maintains intracellular renal P₃ concentrations and masks the intracellular hypophosphatemia precluding the anticipated increase in 25(OH)D-1α-hydroxylase activity. Such a compensatory mechanism, if isolated to the kidney, may protect the renal cell from mineral disequilibrium while subjecting other tissues particularly bone to the effects of hypophosphatemia in the absence of an increased serum 1,25(OH)₂D₃ concentration.

In any case a relative deficiency of 1,25(OH)₂D₃ may explain the enigmatic findings which have precluded universal acceptance of a model for the pathogenesis of XLH. Birge and Miller (57) recently reported data which indicates that the calcium and P₃ malabsorption, characteristic of XLH, may result from a blunted effect of 1,25(OH)₂D₃ on gastrointestinal absorption in the presence of inadequate P₃. In their studies, 1,25(OH)₂D₃ had a diminished effect on radio-labeled calcium and P₃ transport across inverted gut sacs from rats in the presence of low medium P₃. Thus, the abnormal gastrointestinal absorption in XLH may reflect an inability of normal levels of 1,25(OH)₂D₃ to overcome the suppression of absorption induced by hypophosphatemia. Further, it is likely that the effects of therapy on calcium and P₃ absorption result from the cumulative effects of both 1,25(OH)₂D₃ itself and the increased serum P₃. In addition, the absence of hypercalcuria in the presence of hypophosphatemia is no doubt caused by the relative deficiency of 1,25(OH)₂D with consequent calcium malabsorption. Finally, the hypothesis that at least two factors underlie XLH, may account in part for the variable severity of bone involvement in affected subjects. For example, if some hypophosphatemic subjects retain a capacity to increase the synthesis of 1,25(OH)₂D₃, any resulting bone lesion may be remarkably less severe. Indeed, in subjects with hypophosphatemic bone disease, Scriver et al. (55) proposed that a retained capacity to synthesize 1,25(OH)₂D₃ explains the disparity between the severity of the hypophosphatemia and the severity of the expressed bone disease.

Despite the data supporting our hypothesis that a relative deficiency of 1,25(OH)₂D₃ is a factor in the genesis of XLH, we cannot exclude a potential role for alternate or additional abnormalities. We did not measure other vitamin D metabolites, including 24,25(OH)₂D and 1,24,25(OH)₃D, thus precluding determination of whether the apparent aberration in vitamin D metabolism is more widespread than appreciated. However, the relatively unknown physiological role of these metabolites makes speculation concerning their importance in the genesis of XLH difficult. Secondly, it is possible that target organ resistance to the effects of 1,25(OH)₂D may account for the beneficial responses which we noted in response to pharmacological therapy. However, in those disease states marked by a resistance to 1,25(OH)₂D₃, affected subjects have hypocalcemia, hypophosphatemia, secondary hyperparathyroidism and, most importantly, a markedly elevated serum level of 1,25(OH)₂D (58, 59). Moreover, recent studies (60) in the X-linked hyp-mouse model indicate that transport of 1,25(OH)₂D₃ into cytoplasm and subsequently to the nucleus of intestinal mucosal cells is normal, eliminating the usual mechanisms of end organ resistance. Thus end organ resistance apparently is not a major factor in genesis of XLH. Finally, we cannot completely exclude the possibility that the changes which we observed in response to 1,25(OH)₂D₃ therapy may simply represent pharmacological effects of 1,25(OH)₂D₃ in a group of patients who have preserved the ability to respond to this hormone. However, the coupling of an inappropriately low serum
1,25(OH)\(_2\)D level, suggesting inadequate homeostatic regulation, and the beneficial responses to therapy with 1,25(OH)\(_2\)D\(_3\) support the presence of an underlying defect in vitamin D metabolism.

Nevertheless, several observations in the present study are apparently at variance with establishing a relative deficiency of 1,25(OH)\(_2\)D as central to the pathogenesis of XLH. First, in all of the subjects studied bone age exceeded 16 yr, longitudinal growth had ceased, and there was no evidence of rickets. Thus, we cannot determine whether the rachitic lesions, characteristic of XLH, respond to 1,25(OH)\(_2\)D\(_3\) therapy nor whether treatment promotes catch up growth in affected subjects. However, a requirement for 1,25(OH)\(_2\)D\(_3\) in the healing of the rachitic disease is questionable because there is ample radiographic evidence that the rickets heals in response to a variety of treatment regimens. Moreover, a potential diversity of response is not surprising because there may be differential regulation of the mineralization of epiphyseal and diaphyseal bone. Nevertheless, further studies in young children will be necessary to examine the effects of 1,25(OH)\(_2\)D on the rachitic lesions and statural growth. Secondly, and most importantly, the efficacy of 1,25(OH)\(_2\)D\(_3\) therapy on the osteomalacic lesions may be limited because posttreatment bone biopsies show excess osteoid despite normalized mineralization front activity. The persistence of unmineralized osteoid is most likely secondary to an increased rate of osteoid synthesis or an unappreciated defect in mineralization. Increased osteoid synthesis is a reversible and self-limited cause of excess osteoid associated with disorders of accelerated bone turnover such as hyperparathyroidism and with bone healing (e.g., fractures). At present we cannot determine if the reparative process going on in the treated patients has increased osteoid synthetic rate and hence resulted in a time-limited excess of osteoid. Similarly, our data are insufficient to determine if mineral appositional rate is abnormal. Appositional rate is a second factor, in addition to mineralization front activity, which controls the rate of bone mineralization (61) and is likely abnormal in XLH (62). A persistent defect in this function would result in incomplete healing of the osteomalacia. Thus, at present we cannot predict whether 1,25(OH)\(_2\)D\(_3\) therapy resolves the osteomalacic disorder of XLH. Nevertheless, our data do illustrate that 1,25(OH)\(_2\)D\(_3\) therapy normalizes mineralization front activity which is a primary defect underlying the bone abnormalities in XLH.

Thus, our studies suggest that altered regulation of vitamin D metabolism is a fundamental abnormality in XLH. Whether the resulting ‘deficiency’ of 1,25(OH)\(_2\)D is secondary to decreased biosynthesis or increased degradation remains to be determined. However, at the present time we propose that the sequence of metabolic events underlying this disorder includes: (a) a genetic lesion in the renal tubule results in Pi wasting and consequent hypophosphatemia; (b) the hypophosphatemia fails to elicit the anticipated increase in 1,25(OH)\(_2\)D\(_3\) biosynthesis; and (c) relative deficiency of 1,25(OH)\(_2\)D and associated absolute deficiency of Pi result in gastrointestinal malabsorption of calcium and Pi and, most importantly, in the characteristic defect in mineralization front activity in XLH bone. Although additional defects are likely to be revealed with further studies, our findings indicate that therapeutic regimens which include 1,25(OH)\(_2\)D\(_3\) may be beneficial in this refractory disorder.

**ACKNOWLEDGMENTS**

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