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Research Article

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Healing of Bone Disease in X-linked Hypophosphatemic Rickets/Osteomalacia

Induction and Maintenance with Phosphorus and Calcitriol

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Abstract

Although conventional therapy (pharmacologic doses of vitamin D and phosphorus supplementation) is usually successful in healing the rachitic bone lesion in patients with X-linked hypophosphatemic rickets, it does not heal the coexistent osteomalacia. Because serum 1,25-dihydroxyvitamin D levels are inappropriately low in these patients and high calcitriol concentrations may be required to heal the osteomalacia, we chose to treat five affected subjects with high doses of calcitriol (68.2±10.0 ng/kg total body weight/d) and supplemental phosphorus (1–2 g/d) performing metabolic studies and bone biopsies before and after 5–8 mo of this therapy in each individual.

Of these five patients, three (aged 13, 13, and 19 yr) were receiving conventional treatment at the inception of the study and therefore showed base-line serum phosphorus concentrations within the normal range. The remaining two untreated patients (aged 2 and 37 yr) displayed characteristic hypophosphatemia before calcitriol therapy. All five patients demonstrated serum calcitriol levels in the low normal range (22.5±3.2 pg/ml), impaired renal phosphorus conservation (tubular maximum for the reabsorption of phosphate per deciliter of glomerular filtrate, 2.13±0.20 mg/dl), and osteomalacia on bone biopsy (relative osteoid volume, 14.4±1.7%; mean osteoid seam width, 27.7±3.7 μm; mineral apposition rate, 0.46±0.12 μm/d).

On high doses of calcitriol, serum 1,25-dihydroxyvitamin D levels rose into the supraphysiologic range (74.1±3.8 pg/ml) with an associated increment in the serum phosphorus concentration (2.82±0.19 to 3.78±0.32 mg/dl) and improvement of the renal tubular maximum for phosphate reabsorption (3.17±0.22 mg/dl). The serum calcium rose in each patient while the immunoactive parathyroid hormone concentration measured by three different assays remained within the normal range. Most importantly, repeat bone biopsies showed that high doses of calcitriol and phosphorus supplements had reversed the mineralization defect in all patients (mineral apposition rate, 0.88±0.04 μm/d) and consequently reduced parameters of bone osteoid content to normal (relative osteoid volume, 4.1±0.7%; mean osteoid seam width, 11.0±1.0 μm). Complications (hypercalcemia and hypercalciuria) ensued in four of these five patients within 1–17 mo of documented bone healing, necessitating reduction of calcitriol doses to a mean of 1.6±0.2 μg/d (28±4 ng/kg ideal body weight per day). At follow-up bone biopsy, these four subjects continued to manifest normal bone mineralization dynamics (mineral apposition rate, 0.88±0.10 μm/d) on reduced doses of 1,25-dihydroxyvitamin D with phosphorus supplements (2 g/d) for a mean of 21.3±1.3 mo after bone healing was first documented. Static histomorphometric parameters also remained normal (relative osteoid volume, 1.5±0.4%; mean osteoid seam width, 13.5±0.8 μm).

These data indicate that administration of supraphysiologic amounts of calcitriol, in conjunction with oral phosphorus, results in complete healing of vitamin D resistant osteomalacia in patients with X-linked hypophosphatemic rickets. Although complications predictably require calcitriol dose reductions once healing is achieved, continued bone healing can be maintained for up to 1 yr with lower doses of 1,25-dihydroxyvitamin D and continued phosphorus supplementation.

Introduction

X-linked hypophosphatemic rickets/osteomalacia (XLH)[1] is a disorder characterized by renal phosphate wasting and abnormal mineralization of bone. The defective bone mineralization results in rachitic changes at the growth plate and osteomalacia in trabecular and cortical bone. Traditional therapy with phosphorus supplementation and pharmacologic doses of vitamin D often reverses the roentgenographic signs of active rickets in XLH-affected children without healing their coexistent osteomalacia (1).

Recent work by several groups suggests that vitamin D metabolism is also deranged in patients with XLH (2, 3, 4). While hypophosphatemia is a potent stimulus for calcitriol production and increases plasma levels of this metabolite in several mammalian species (5, 6, 7), patients with XLH maintain a plasma 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] concentration only within the normal range. This observation has led investigators to use low doses of this vitamin D metabolite (up to 1 μg/d) in conjunction with phosphorus

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1. *Abbreviations used in this paper:* 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D; 25$(OH)D_3$, 25-hydroxyvitamin D_3; AR, active bone resorbing surface; IBW, ideal body weight; MFA, mineralization front activity; MiAR, mineral apposition rate; MLT, mineralization lag time; MOSW, mean osteoid seam width; ObS, osteoblast covered osteoid surface; OS, osteoid-covered trabecular bone surface; Pi, phosphorous; PTH, parathyroid hormone; ROV, relative osteoid volume; TmP/GFR, renal tubular maximum for the reabsorption of phosphate per deciliter of glomerular filtrate; XLH, X-linked hypophosphatemic rickets/osteomalacia.

supplementation, in an attempt to correct the XLH-associated metabolic defect(s) more directly (8, 9). While roentgenographic cure of the rachitic lesion in XLH is effected with this therapy, bone biopsies show persistent osteomalacia in these patients (1). The failure of this regimen may be due to the fact that low doses of calcitriol do not elevate plasma $1,25(OH)_2D$ levels into the supraphysiologic range.

In this study we chose to treat affected patients with high doses (>1 μg/d or >55 ng/kg ideal body weight [IBW] per day) of $1,25(OH)_2D_3$ (in conjunction with phosphorus supplementation) in an effort to more closely simulate the normal response to low serum phosphorus. We evaluated the therapeutic response by comparing metabolic and bone biopsy data in five patients before and after high dose calcitriol therapy. We continued to follow four of these patients for a mean of nearly 2 yr after the second bone biopsy. During this follow-up period all subjects required calcitriol dose reductions because of persistent hypercalciuria or hypercalcemia. Metabolic and bone biopsy data were obtained on each patient after 11.4±0.9 mo of reduced dose therapy.

Methods

Patient population. We studied five patients with hypophosphatemia and osteomalacia from four families in which an inheritance pattern compatible with X-linked dominant transmission was demonstrated. Two females (one, aged 2 yr, 2 mo; and the other, 37 yr) were untreated on entry into the study. Two males and a female (aged 13 yr, 8 mo; 19 yr, 8 mo; and 13 yr, 10 mo, respectively) had been treated with vitamin D (20,000–75,000 IU/d) and phosphorus (2 g/d) for more than 6 mo. Of the previously untreated patients, the 2 yr, 2 mo-old child had roentgenographic signs of active rickets at inception of the study, while the 37-yr-old woman exhibited a femoral pseudofracture and short stature. All patients previously treated with conventional therapy had characteristic postrachitic deformities, but no evidence of active rickets.

High dose calcitriol and phosphorus regimen. After an average vitamin D free interval of 4 wk in previously treated patients, all subjects received calcitriol for a mean of 10 mo (range, 8–11 mo). Over the first 3–4 mo of therapy, doses were titrated to a peak that was maintained for an average of 6.4±0.6 mo (range, 5–8 mo). The maximum dose employed was 3.0 μg/d (divided into two daily doses) in adolescents and adults, while the youngest child (2 yr, 2 mo-old) received 1.5 μg/d on a similar schedule (arbitrarily chosen to yield a dose of 55–70 ng/kg IBW per d). The maximum dose of calcitriol averaged 68.2±10.0 ng/kg IBW per d.

The patients received phosphorus supplementation (given as K-phos Neutral) for 9.0±0.9 mo (range, 6–11 mo). During this period, a peak dose of 2 g/d (given in four doses) was maintained for 8.3±0.3 mo (range, 8–9 mo) in all patients except the 2 yr, 2 mo-old child, who received a maximum dose of 1 g/d for 5 mo.

Reduced dose calcitriol and phosphorus regimen. Continuation of the high dose calcitriol and phosphorus regimen resulted in the development of a complication of therapy (hypercalcemia or hypercalciuria) in four of the five patients (the fifth patient was lost to follow-up). Calcitriol doses were subsequently reduced to a level at which hypercalcemia and/or hypercalciuria were abolished (1.6±0.2 μg/d or 28±4 ng/kg IBW per d). Serum calcium, phosphorus, and creatinine levels obtained during the complication period and before $1,25(OH)_2D_3$ dose reduction have been excluded from analysis. The reduced dose regimen was maintained for 11.4±0.9 mo.

Experimental. We performed metabolic studies and bone biopsies in each subject before and after high dose calcitriol and phosphorus therapy. Subsequently, we completed similar studies in four of the five subjects after they were switched to and maintained on low dose calcitriol and phosphorus therapy. The base-line (precalcitriol) studies in subjects previously treated with vitamin D and phosphorus were conducted while they were taking these medications. The patient or the patient and his/her parents gave informed consent for all studies. These investigations were approved by the Duke University Human Investigations Committee and were performed on the Duke University Clinical Research Unit.

Biochemical studies. Serum calcium (normal, 8.7–10.2 mg/dl) was measured by atomic absorption spectrophotometry and serum phosphorus (for age specific normals, see Table I) was determined by the colorimetric method of Dryer et al. (10). Serum creatinine (normal, 0.7–1.2 mg/dl) and alkaline phosphatase (for age specific normals, see Table I) were determined on the Multichannel Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, NY). Urine specimens were stored at −20°C before analysis of calcium (by atomic absorption spectrophotometry), phosphorus (11), and creatinine (12).

The renal tubular maximum for the reabsorption of phosphorous (Pi) normalized according to the glomerular filtration rate was calculated by the method of Bijvoet and Walton (13). Studies were performed as previously described (14) and age specific normals are shown in Table I. Medicines (Pi and vitamin D or $1,25(OH)_2D_3$) were not withheld on the day of the study.

The serum parathyroid hormone (PTH) concentration was measured by three radioimmunoassays with antigenic specificity to the intact molecule, the midregion, and its carboxyterminal fragment, respectively. The intact molecule assay was obtained from the Upjohn Laboratory (Kalamazoo, MI); normal values were <150–375 pg-eq/ml (15). We performed the midmolecule PTH assay at Duke employing a kit obtained from the Cambridge Laboratories (Billerica, MA); the normal range in 85 normals was 0.4–1.5 ng-eq/ml. The Mayo Medical Laboratory (Rochester, MN) performed a carboxyterminal assay with a normal range of <70 μl-eq/ml (16).

We measured the serum 25-hydroxyvitamin D concentration by a modification of the methods of Haddad and Chyu (14, 17). Each sample was assayed in triplicate and at least two samples were obtained from every patient before and after calcitriol therapy was initiated. Results are reported as mean±SEM for all samples drawn during a single period (before or after calcitriol). The seasonally adjusted normal range for 25(OH)D in 50 normal subjects, aged 5–55 yr, is 12–80 ng/ml. Values in males and females were not different.

Serum $1,25(OH)_2D$ levels were assayed in 3–4 ml samples by a modification of the methods of Eisman et al. (18) as previously described (19). Since the serum $1,25(OH)_2D$ concentration varies with chronological age (20), we established normal values appropriate for youth and adult subjects. In 38 adults, aged 20–72 yr, the serum $1,25(OH)_2D$ concentration (mean±2 SD) was 19–50 pg/ml. In contrast, the normal range in 15 youths, aged 5–18 yr, was 21–75 pg/ml, a range comparable to that reported by Chesney et al. (20) in a similar group. No seasonal variation was observed and values were similar in both sexes. Individual samples were assayed in triplicate. Before inception of calcitriol therapy we measured from two to four fasting calcitriol levels and these data are reported as the mean±SEM. To determine the mean $1,25(OH)_2D$ levels during experimental therapy, serum was obtained on five to eight occasions (typically at 2-h intervals) on a randomly chosen day. Patients took their medications in such a way as to simulate the home situation on this day. The values reported represent the mean±SEM for these samples.

Bone studies. Transcortical bone biopsies were obtained from the anterior iliac crest under local anesthesia. First and second biopsies were obtained 11 mo apart in patient 1, and 10, 11, 10, and 8 mo apart in patients 2–5. Second and third biopsies were separated by 19, 24, 19, and 23 mo in patients 2–5, respectively. Declomycin (300 mg, orally, three times daily) was administered to each subject over a 3-d period from day 26 to day 24 before biopsy and chlortetracycline (250 mg, orally, four times daily) was administered over a 3-d period from day 6 to day 3 before biopsy. Bone specimens, unstained or stained by the methods of Villanueva (21), were fixed in ethanol and embedded in methylmethacrylate. 20-μm and 5-μm sections were made and prepared according to previously published methods (22).

Table I. Metabolic Data in Patients with XLH Before and During Calcitriol/Pi Therapy (High and Reduced Dose)

		Treatment			Plasma					Renal	
Patient	Age at entry	D_2	$1,25(OH)_2D_3$	Pi	Calcium	Fasting Pi	Alkaline P'tase	25(OH)D	$1,25(OH)_2D$	Creat Cl	TmP/GFR
		IU/d	*μg/d*	*g/d*	*mg/dl*	*mg/dl*	*IU/liter*	*ng/ml*	*pg/ml*	*ml/min*	*mg/dl*
1	2 yr, 2 mo	0	0	0	9.0±0.1 (9)*	3.00±0.13 (10)	602±235 (2)	23.3±0.2 (2)	34.0±1.1 (2)	95±7 (5)‡	2.80±0.02 (2)
		0	1.5	1.0	9.2±0.2 (7)	5.02±0.48 (7)	180±1 (2)	12.1±1.6 (2)	88.7±7.5 (7)	121±12 (13)‡	3.79±0.15 (2)
2	37	0	0	0	8.6±0.2 (11)	2.30±0.10 (8)	96±1 (2)	18.0±1.8 (4)	21.6±1.5 (4)	109±6 (2)	1.95±0.15 (4)
		0	3.0	2.0	8.8±0.1 (21)	3.53±0.15 (6)	79±7 (4)	20.1±5.2 (2)	71.9±6.4 (5)	102±19 (6)	2.56±0.14 (4)
		0	1.5	2.0	9.2±0.2 (15)	2.63±0.15 (6)	54±2 (3)	36.9±9.2 (2)	49.1±5.6 (8)	81±4 (5)	1.70±0.07 (4)
3	13 yr, 8 mo	20,000	0	2.0	9.1±0.1 (15)	3.38±0.14 (15)	417±21 (4)	88.4±4.4 (4)	23.0±1.6 (4)	95±6 (3)	2.35±0.07 (6)
		0	3.0	2.0	9.3±0.1 (18)	3.71±0.11 (6)	126±40 (3)	71.9±3.9 (2)	72.6±7.8 (7)	141±6 (15)	3.50±0.22 (4)
		0	2.0	2.0	9.9±0.1 (11)	2.72±0.10 (6)	64±5 (3)	51.9±3.3 (2)	40.5±5.3 (5)	106±7 (5)	1.81±0.13 (4)
4	13 yr, 10 mo	75,000	0	2.0	8.9±0.1 (12)	2.91±0.13 (13)	414±18 (4)	143.0±8.8 (4)	20.0±1.8 (4)	117±6 (6)	1.76±0.07 (6)
		0	3.0	2.0	9.0±0.1 (18)	3.43±0.25 (6)	86±20 (3)	44.8±12.5 (2)	66.3±4.8 (7)	152±15 (10)	2.80±0.14 (4)
		0	2.0	2.0	9.4±0.1 (19)	2.29±0.12 (5)	46±3 (3)	39.9±2.4 (2)	43.3±5.1 (8)	74±7 (8)	1.61±0.04 (4)
5	19 yr, 8 mo	75,000	0	2.0	9.2±0.1 (14)	2.51±0.08 (14)	219±9 (3)	227.7±15.4 (4)	14.0±2.2 (4)	117±7 (4)	1.79±0.08 (5)
		0	3.0	2.0	9.5±0.1 (19)	3.22±0.13 (6)	109±35 (3)	34.7±0.8 (2)	71.0±6.4 (8)	114±5 (10)	3.20±0.06 (4)
		0	1.0	2.0	10.4±0.1 (13)	2.34±0.22 (5)	66±2 (3)	50.1±7.6 (2)	59.6±11.5 (10)	81±6 (2)	1.82±0.63 (2)
Mean±SEM		Before $1,25(OH)_2D_3$Rx			9.0±0.1	2.82±0.19	350±88	100.1±39.2	22.5±3.2	107±5	2.13±0.20
		High dose $1,25(OH)_2D_3$Rx			9.2±0.1	3.78±0.32‖	116±18‖	36.7±10.4	74.1±3.8‖	126±9	3.17±0.22‖
		Reduced dose $1,25(OH)_2D_3$Rx			9.7±0.3‖	2.50±0.11¶	58±5‖	44.7±3.7	48.1±4.2‖¶	86±7¶	1.74±0.05¶
Normal range					8.7–10.2	2.5–4.5 4.5–6.7§	<110 <170§	15–80	19–50 21–75§	90–120 85–125§	2.5–4.5 4.2–5.9§

* No. of determinations. ‡ Values given as creatinine clearance per 1.73 square meters of body surface. § Normal values for a child aged 2–4 yr. ‖ Significantly different from precalcitriol treatment data by analysis of variance with Tukey's studentized range testing at $P < 0.05$. ¶ Significantly different from high dose calcitriol treatment data by analysis of variance with Tukey's studentized range testing at $P < 0.05$.

Histomorphometric analysis of the trabecular bone in the section was accomplished by examining 25–50 microscopic fields with a Merz integrated reticle (23). The following histological functions were quantitated: (*a*) mineralization front activity, the percentage of osteoid-covered trabecular bone surface (OS) exhibiting a fluorescent chlortetracycline second (distinguished by color from the first label—declomycin) label with a minimum width of 3 μm; (*b*) mineral apposition rate, the average distance (in micrometers) between declomycin and chlortetracycline fluorescent labels normalized by the days elapsed between the administration of the labels; (*c*) mean osteoid seam width, the mean width (in micrometers), of 25–50 randomly selected osteoid seams, each measured at four points equidistant from each other using a linear reticle calibrated with a stage micrometer; (*d*) osteoid surface, the percentage of trabecular bone surface covered by osteoid; (*e*) active resorption surface, the percentage of trabecular bone surface on which Howship's lacunae containing multinucleated osteoclasts are present; (*f*) osteoblastic surface, the percentage of trabecular bone surface on which osteoid covered by osteoblasts is present; (*g*) relative osteoid volume, the percentage of trabecular bone volume composed of unmineralized bone; and (*h*) bone volume, the percentage of the sample composed of trabecular bone. In addition, mineralization lag time, a measure of the time (in days) that newly formed osteoid remains unmineralized, was calculated by dividing the mean osteoid seam width by the corrected mineral apposition rate (24). Normal values for these measurements (apart from mineralization dynamics) were determined in bone biopsies from 12 normal subjects, aged 5–35 yr. Normal values for mineralization dynamic measurements were obtained from the bone biopsies of six normal subjects kindly provided by Dr. Michael Parfitt (Detroit, MI) and from normal ranges in the medical literature (25, 26).

Statistical analyses. Statistical evaluation of the data was performed using analysis of variance with Tukey's studentized range testing (27).

Materials. The $1,25(OH)_2D_3$ administered to the patients was provided by Hoffmann-LaRoche Drug Co. (Nutley, NJ). Oral Pi supplements were a gift from Beach Pharmaceuticals (Tampa, FL). The authentic $1,25(OH)_2D_3$, used in the assay for this metabolite, was a gift from Dr. Milan Uskokovic, Hoffmann-LaRoche Drug Co. The 25(OH)D was obtained from Upjohn Drug Co. (Kalamazoo, MI). [^{3}H]$1,25(OH)_2D$ (92 ci/mmol) and [^{3}H]25(OH)D (90 ci/mmol) were purchased from Amersham Co (Arlington Heights, IL).

Results

Metabolic studies before calcitriol therapy. At the start of the study the two untreated patients exhibited characteristic biochemical abnormalities of XLH (patients 1 and 2, Table I). Both demonstrated unequivocal fasting hypophosphatemia and diminished renal tubular maximum for the reabsorption of phosphate per deciliter of glomerular filtrate (TmP/GFR) in the presence of normocalcemia. The three patients treated with Pi and vitamin D_2 on entry into the study were normocalcemic with fasting serum phosphorus levels that fell within the normal range, but manifested continued depression of renal tubular phosphate reabsorption. Alkaline phosphatase blood levels were high in all patients with open epiphyses (patients 1, 3, and 4) despite treatment with conventional drugs (patients 3 and 4). High doses of vitamin D_2 and Pi supplementation eliminated all roentgenographic signs of active rickets in patients 3, 4, and 5.

Renal glomerular function was normal in all patients, as documented by creatinine clearance determinations (Table I). Vitamin D deficiency was ruled out by normal or supranormal (in vitamin D treated patients) pretreatment serum 25(OH)D levels and normal serum calcitriol concentrations in all patients

Table II. Serum Concentration of Immunoreactive PTH in Patients with XLH Before and During Calcitriol/Pi Therapy (High and Reduced Dose)

Patient	Treatment			COOH-terminal assay	Midmolecule assay	Intact assay
	D_2	$1,25(OH)_2D$	Pi			
	IU/d	*μg/d*	*g/d*	*μl eq/ml*	*ng eq/ml*	*pg eq/ml*
1	0	0	0	20	0.5	290
	0	1.5	1.0	20	1.05	214
2	0	0	0	40	0.45	307
	0	3.0	2.0	34	0.65	421
	0	1.5	2.0	33	0.85	247
3	20,000	0	2.0	18	0.20	156
	0	3.0	2.0	23	0.25	301
	0	2.0	2.0	32	0.40	309
4	75,000	0	2.0	30	0.45	297
	0	3.0	2.0	35	0.65	347
	0	2.0	2.0	28	0.60	308
5	75,000	0	2.0	38	0.55	279
	0	3.0	2.0	51	0.85	242
	0	1.0	2.0	62	1.35	297
Mean±SEM	Before $1,25(OH)_2D_3$Rx			29±4	0.43±0.06	266±28
	High dose $1,25(OH)_2D_3$Rx			33±6	0.69±0.13	305±37
	Reduced dose $1,25(OH)_2D_3$Rx			36±9	0.80±0.20*	290±15
Normal range				20–70	0.4–1.5	150–375

* Significantly different from precalcitriol treatment data by analysis of variance with Tukey's studentized range testing at $P < 0.05$.

(Table I). Normal serum PTH levels by three different assays (Table II) indicated that hyperparathyroidism did not cause the renal phosphate wasting observed in these patients.

Bone histomorphometry precalcitriol therapy. Bone biopsies from all subjects demonstrated excessive osteoid surface and volume, changes characteristic of the osteomalacic state (Fig. 1 *A*, and Table III). The normal number of active resorption surfaces confirmed the absence of parathyroid hormone hypersecretion. Treatment with vitamin D and Pi in patients 3, 4, and 5 had only a minimal effect on static histomorphometric parameters (Fig. 1 *B*, and Table III). In untreated patients mineralization dynamics assessed by measurement of mineralization front activity (MFA) and mineral apposition rate (MiAR) were characteristically abnormal (Fig. 1 *C*, and Table III). In contrast, although MFA was quantitatively normalized by conventional therapy in patients 3–5, the incorporated label appeared qualitatively abnormal, as evidenced by smudged, nondiscrete chlortetracycline bands (Fig. 1 *D*). In any case, the continued presence of a subnormal MiAR confirmed the persistence of a disturbance in bone mineralization (Table III). Additionally, all patients displayed a markedly prolonged mineralization lag time (MLT) averaging 98 d (normal range, 12–22 d).

Metabolic studies on high dose calcitriol therapy. Treatment with high doses of calcitriol resulted in a dramatic increment in serum $1,25(OH)_2D$ levels (Table I). To eliminate erratic intestinal absorption of calcitriol and time of blood drawing as confounding variables, we obtained all treatment levels of $1,25(OH)_2D$ by averaging the results of five to eight nyctohemeral determinations drawn while the patient took medications according to his/her usual schedule. In concert with the elevation of the serum $1,25(OH)_2D$ concentration into the supraphysiologic range, we noted a significant increase of the fasting serum phosphorus levels, explained in part by improved renal tubular phosphorus conservation (Table I). Similarly, serum calcium concentrations increased in each patient, resulting in a small but significant difference between average pre-treatment and post-treatment levels. Despite this increment, no significant change in serum PTH occurred with calcitriol therapy (Table II). Glomerular filtration rates, as reflected by creatinine clearance determinations, rose significantly upon calcitriol administration; in the instance of the 2 yr, 2 mo-old child (patient 1), the increase was in accord with normal expectations (28).

Bone histomorphometry on high dose calcitriol therapy. High doses of calcitriol and phosphorus therapy resulted in changes of both static and dynamic bone histomorphometric parameters indicative of bone healing (Table III, Fig. 2 *A* and *C*). Bone mineralization was normalized in each patient as evidenced by second labeling frequency (mean MFA, 81.6±2.9%, Table III) and interlabel distance (MiAR, 0.88±0.04 μm/d). The qualitative appearance of the fluorescent bands was normal, as evidenced by a discrete, double linear pattern (Fig. 2 *C*). In addition, the mean osteoid seam width (MOSW) was normalized in all patients (11.0±1.0 μm, Table III) with the consequence that MLT fell into the normal range in each individual (16±2 d; normal, 12–22 d). Likewise, relative osteoid volume (ROV) was reduced in all patients, although patient 1 continued to show a mild excess at biopsy (Table III). Concomitant improvements in OS were also observed, with only patients 1 and 5 exhibiting values above the normal range. Active resorption surfaces were significantly increased by calcitriol therapy but to values that remained within the normal range. Osteoblastic surface was increased in patients 1 and 5, both of whom had been on high dose calcitriol for relatively short intervals when biopsies were taken (patient 1, 5 mo at 1.5 μg/d; patient 5, 5 mo at 3 μg/d). However, alkaline phosphatase values were significantly reduced in all subjects (Table I), despite the increased osteoblast-covered osteoid surface (ObS) present in the bones of the aforementioned patients.

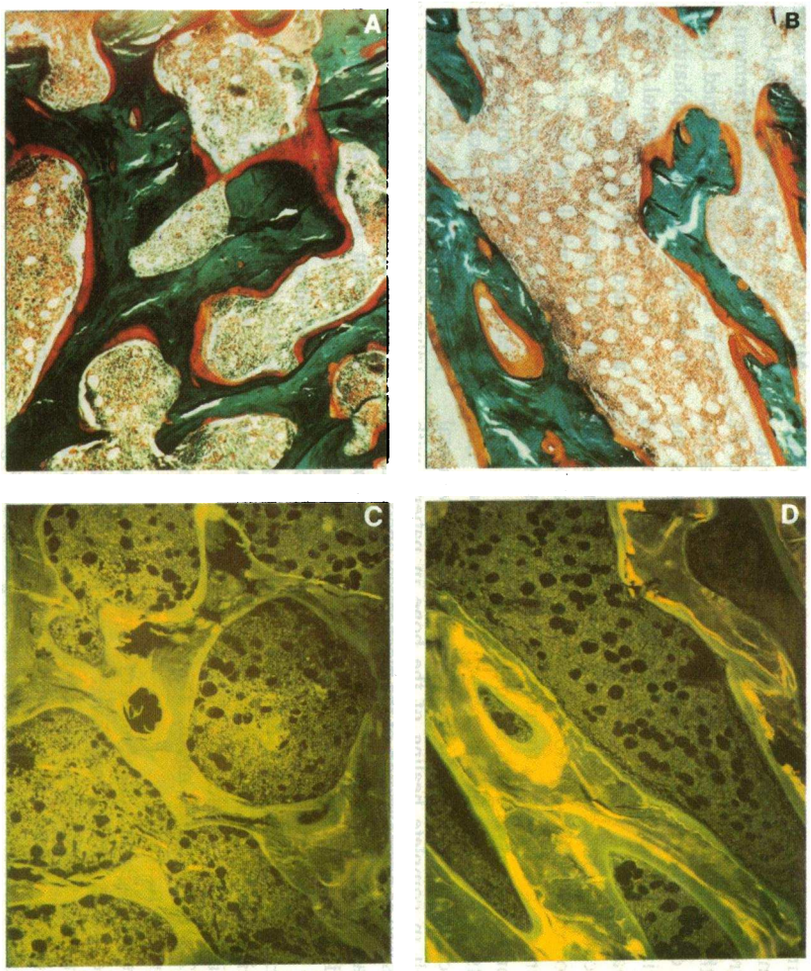
Complications on high dose calcitriol therapy. Patients 2–5 remained in the study after documentation of bone healing and experienced complications of therapy at 1, 17, 1, and 3 mo, respectively, after the second biopsy. Specifically, patients 2–4 became hypercalciuric on the high dose regimen (maximal 24-h urinary calcium: patient 2, 603 mg; patient 3, 367 mg; patient 4, 368 mg) while remaining normocalcemic (maximum serum calcium concentration, 9.3, 9.4, and 9.5 mg/dl, respectively). Patient 5 manifested only hypercalcemia (serum calcium level, 10.6 mg/dl with a maximum 24-h urinary calcium of 209 mg). All patients exhibited a decrement in glomerular filtration (nadir of creatinine clearance, 60, 78, 62, and 62 ml/min, respectively) during the complication period.

Metabolic studies on reduced doses of calcitriol therapy. After $1,25(OH)_2D_3$ dose reduction was necessitated by hypercalcemia or hypercalciuria in the four subjects remaining in the study, each experienced a significant fall in fasting plasma Pi concentrations in conjunction with a marked decrement of TmP/GFR into the subnormal range (Table I). As expected, integrated plasma calcitriol levels (obtained from nyctohemerally collected blood samples) fell into the normal range (48.1±4.2 pg/ml) and were significantly lower than levels obtained in similar fashion on the high dose regimen (Table

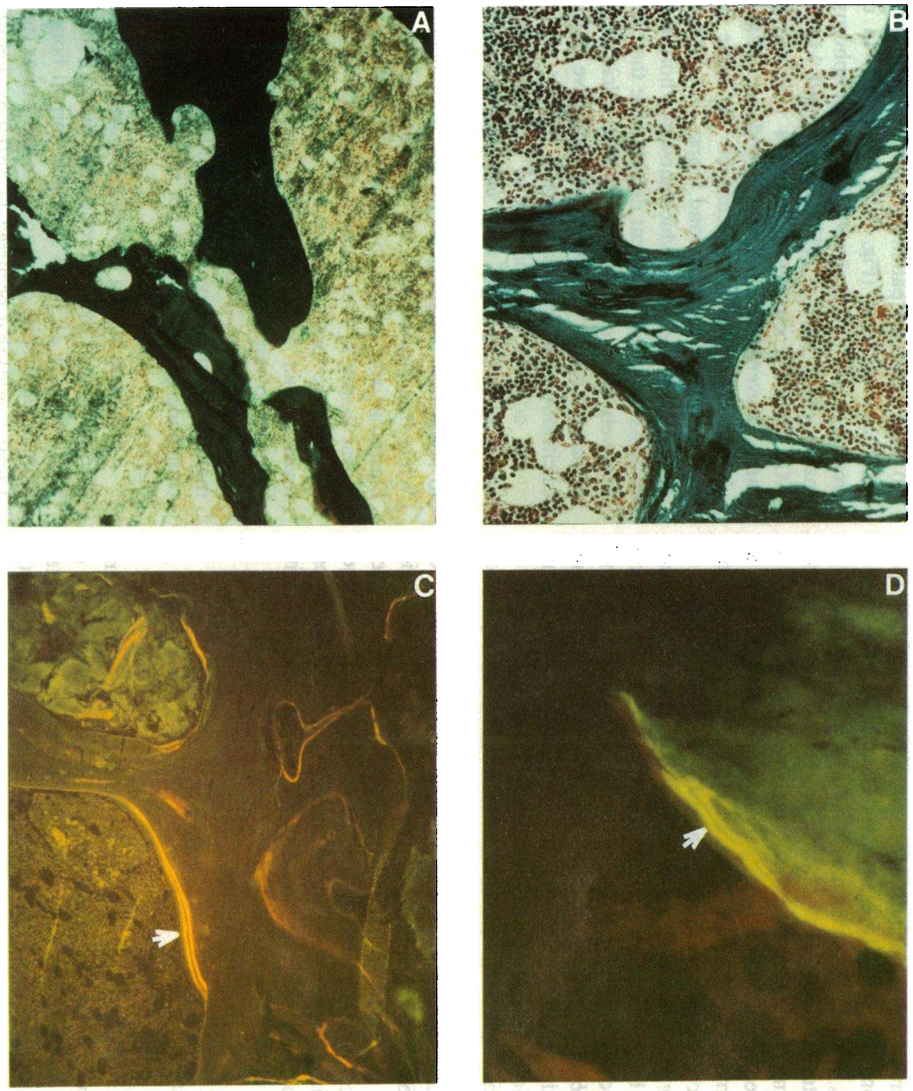
Figure 1. Microscopic appearances of bone biopsies from patients with XLH before and after therapy. (*A*) Goldner stained specimen from an untreated patient showing an excessive amount of red osteoid on green stained mineralized bone surfaces. (*B*) Goldner stained specimen from a patient treated conventionally with pharmacologic doses of vitamin D and phosphorus supplements. Note the continued presence of excess osteoid. (*C*) Unstained specimen from an untreated patient viewed under fluorescent light. The absence of yellow-orange fluorescent bands in the osteoid seams signifies a lack of active mineralization during the labeling period. (*D*) Unstained specimen from a conventionally treated patient viewed under fluorescent light. Note the diffuse, smudged appearance of the yellow label, suggesting a persistent mineralization defect.

Figure 2. Microscopic appearance of bone biopsies from patients with XLH on high and reduced dose calcitriol regimens. (*A*) Goldner stained specimen from a patient treated with high doses of calcitriol and phosphorus supplements. Red staining osteoid seams are scarce, consistent with normal histomorphology. (*B*) Goldner stained specimen from a patient treated with reduced doses of calcitriol and supplemental phosphorus. Red staining osteoid seams are again scarce. (*C*) Unstained specimen from a patient treated with high dose calcitriol and phosphorus supplement viewed under fluorescent light. Note the discrete double linear yellow-orange labeling pointed out by the arrow. Mineralization dynamics were normal in this patient. (*D*) Villanueva stained specimen from a patient treated with reduced doses of calcitriol and supplemental phosphorus viewed under fluorescent light. Once again, the fluorescent labels are deposited in a discrete double linear pattern as indicated by the arrow. Histomorphometric analysis revealed normal mineralization dynamics in the bone biopsy of which this specimen is a part.

1



2



I). Serum calcium concentrations rose in each subject despite reductions in calcitriol doses and plasma levels (Table I). In contrast, serum PTH determinations remained unchanged by COOH-terminal and intact hormone specific assays but rose significantly within the normal range when measured by a midmolecule specific antibody. Glomerular filtration rates, as approximated by creatinine clearance data, fell in all patients on reduced doses of calcitriol (Table I).

Bone histomorphometry on reduced dose calcitriol therapy. All four bone biopsies procured from previously healed patients on reduced doses of $1,25(OH)_2D_3$ were qualitatively normal (Fig. 2 *B* and *D*). Static histomorphometric parameters confirmed the subjective impression of normality obtained from histologic inspection. MOSW and ROV remained normal in each patient (13.5±0.8 μm and 1.5±0.4%, respectively), while the previously demonstrated persistent elevation of OS in patient 5 disappeared. Indices of bone cellular activity (AR and ObS) were diminished by the lowered plasma calcitriol levels in these patients (Table III). Finally, bone mineralization dynamics, as evidenced by the MFA and MiAR, remained normal in all four subjects, suggesting that a delayed decrement in osteoid mineralizing capability had not occurred with reduced doses of calcitriol (Table III).

Discussion

XLH is the prototypic vitamin D resistant bone disease and until recently was treated as such with pharmacologic doses of vitamin D (29). However, long-term observations indicated that this therapy failed to heal the attendant bone disease (30). With the recognition that the manifestations of XLH might be due to Pi depletion, phosphorus supplementation was added to this traditional therapeutic regimen in an attempt to raise the serum Pi concentration (31, 32). Pharmacologic amounts of vitamin D were employed to prevent the secondary hyperparathyroidism that may be induced by administration of Pi. While this combination therapy results in roentgenographic healing of the physeal rachitic lesions in affected subjects, the mineralization of endosteal and cortical bone surfaces remains abnormal (33).

Recently, the recognition that a defect in the homeostatic regulation of $1,25(OH)_2D_3$ biosynthesis may contribute to the pathogenesis of XLH has prompted therapeutic trials of calcitriol or 1α-hydroxyvitamin D_3 in conjunction with Pi supplements (1, 34–36). In this report we have shown that pharmacologic doses of $1,25(OH)_2D_3$, administered with oral Pi, result in complete healing of the bone in patients with XLH, as well as normalization of the biochemical abnormalities that characterize the disease. Employing a calcitriol dose of 3.0 μg/d in two adolescents and two adults and 1.5 μg/d in a 2-yr-old child, the serum $1,25(OH)_2D$ level rose from 22.5±3.2 to 74.1±3.8 pg/ml (Table I). In association with this supraphysiologic circulating $1,25(OH)_2D$ concentration, the renal handling of Pi improved significantly (TmP/GFR increased from 2.13±0.20 to 3.17±0.22 mg/dl, Table I) and the serum Pi concentration increased.

Moreover, with the achievement of elevated calcitriol levels, all of the treated patients manifested normal bone mineralization dynamics (Table III). At the inception of the study, conventionally treated patients (numbers 3–5) had some smudged and nondiscrete fluorescent labeling of bone (Fig. 1 *D*) while untreated patients (numbers 1 and 2) had little or no label incorporation (Fig. 1 *C*, Table III). In contrast, after treatment with high dose calcitriol/phosphorus, all subjects manifested a normal MFA, MiAR, and MLT. In addition, ROV and OS improved to values well within the normal range. Nevertheless, patients 1 and 5 continued to exhibit abnormal amounts of osteoid on the bone surface and/or increased ROV at the second biopsy. A subsequent biopsy in patient 5 (23 mo later), which showed a normal ROV and OS, suggests that these apparent defects were the consequence of a relatively short duration of full dose calcitriol therapy (5 mo in patients 1 and 5) rather than indicative of insurmountable calcitriol/phosphorus resistance.

In addition to evidence of healing, the bone biopsies obtained during high dose calcitriol treatment showed an apparent increase in bone resorption and formation surfaces to levels that remained within the normal range (Table III). While it is possible that the increased osteoclastic and osteoblastic activity is due to $1,25(OH)_2D_3$ effects on bone (37), repeat biopsy of four patients (numbers 2–5) 19–24 mo later (while these patients were on reduced doses of calcitriol) revealed that bone resorption and formation indices had returned to values indistinguishable from those present before calcitriol therapy. Thus, it is likely that these transient abnormalities are indicative of the rapid bone turnover associated with bone healing and do not represent permanent effects of therapy.

Once bone healing was achieved with high doses of calcitriol (as documented by the second bone biopsy), this state persisted for a mean of 21.3±1.3 mo. The normal bone histology was maintained despite calcitriol dose reductions (to an average of 1.6±0.2 μg/d) that occurred 11.4±0.9 mo before the third and final biopsy. While bone mineralization parameters remained normal on reduced doses of $1,25(OH)_2D_3$ (MFA, 69.1±4.6%; MiAR, 0.88±0.10 μm/d), the serum Pi concentration fell to the precalcitriol treatment level (2.50±0.11 mg/dl) in conjunction with a TmP/GFR decrement to 1.74±0.05 mg/dl. Thus, bone healing was maintained for nearly a year in the setting of recrudescent hyperphosphaturia and hypophosphatemia.

The use of supplemental Pi in patients with XLH has been associated with an increased incidence of secondary hyperparathyroidism (32). Using two PTH assays that recognize COOH-terminal and intact molecule determinants, we found no significant change of the circulating PTH level in the pretreatment and post-treatment periods (Table II). A third assay with midmolecule specificity showed a progressive increase of parathyroid hormone levels across the three periods, but all values remained within the normal range. Thus, the concurrent administration of $1,25(OH)_2D_3$ effectively prevented PTH hypersecretion often seen in patients given supplemental Pi. Other potential side effects of calcitriol/Pi therapy include hypercalcemia, hypercalciuria, and renal dysfunction (38). During the induction of bone healing, we did not encounter any of these complications. Indeed, creatinine clearance determinations actually rose during high dose calcitriol therapy in three of five patients. However, within 1–17 mo of bone healing (as documented by the second bone biopsy), all four patients remaining in the study developed hypercalciuria or hypercalcemia, which necessitated calcitriol dose reductions. Subsequently, although the serum calcium concentration and urinary calcium excretion decreased remarkably, creatinine clearances, which had fallen to a nadir of 66±4 ml/min during

Table III. Quantitative Histomorphometry of Bone Biopsies in Patients with XLH Before and During Calcitriol/Pi Therapy

	Treatment			Volume		Surface				Mineralization		
Patient	D_2	$1,25(OH)_2D$	Pi	VO	ROV	MOSW	OS	AR	ObS	MFA	MiAR	MLT
	IU/d	*μg/d*	*g/d*	%	%	*μm*	%	%	%	%	*μm/d*	*d*
1	0	0	0	19.9	18.6	30.0	65.6	1.0	9.0	0	0	∞
	0	1.5	1.0	10.3	7.0	12.6	31.8	1.1	24.3	82.6	0.89	17
2	0	0	0	13.8	18.8	17.0	65.3	0	3.3	16.5	0.54	189
	0	3.0	2.0	23.4	4.1	7.4	24.2	0.2	4.8	80.7	0.99	9
	0	1.5	2.0	34.8	2.2	12.9	13.1	0.6	3.1	69.8	0.71	26
3	20,000	0	2.0	30.3	11.7	21.1	56.9	0	1.5	84.6	0.64	39
	0	3.0	2.0	30.2	3.2	11.9	19.0	1.2	7.1	73.6	0.93	18
	0	2.0	2.0	31.1	1.9	14.5	13.3	0	5.8	74.7	1.11	17
4	75,000	0	2.0	20.7	11.6	35.9	77.9	0	8.3	70.5	0.54	94
	0	3.0	2.0	10.3	2.8	10.1	9.2	0.5	2.1	79.5	0.74	17
	0	2.0	2.0	39.1	0.3	11.5	13.6	0.1	0	56.0	0.70	29
5	75,000	0	2.0	46.0	11.4	34.3	63.8	0	0	84.1	0.60	69
	0	3.0	2.0	48.1	3.6	13.2	29.5	1.0	11.8	91.6	0.87	17
	0	1.0	2.0	35.6	1.7	15.0	15.3	0.4	1.8	76.0	0.99	20
Mean±SEM	Before $1,25(OH)_2D_3Rx$			26.1±5.6	14.4±1.7	27.7±3.7	65.9±3.4	0.2±0.2	4.4±1.8	51.1±17.9	0.46±0.12	98±32
	High dose $1,25(OH)_2D_3Rx$			24.5±7.0	4.1±0.7‡	11.0±1.0‡	22.7±4.0‡	0.8±0.2	10.0±3.9	81.6±2.9	0.88±0.04‡	16±2‡
	Reduced dose $1,25(OH)_2D_3Rx$			35.2±1.6	1.5±0.4‡	13.5±0.8‡	13.8±0.5†	0.3±0.1	2.7±1.2	69.1±4.6	0.88±0.10‡	23±3‡
Normal range				24.5±10.0*	2.5±1.6	13.0±4.0	14.6±6.2	4.4±2.9	4.3±3.4	68.2±8.3	0.7–1.2	22±4

* Mean±1 SD. ‡ Significantly different from precalcitriol data by analysis of variance with Tukey's studentized range testing at $P < 0.05$.

the complication period, remained depressed at 86±7 ml/min (Table I). While this persistent abnormality probably does not present a significant consequence to the patient, it is conceivable that a shorter course of high dose therapy might circumvent this and other complications.

The bone healing and normalization of biochemistries that we observed in response to therapy are in marked contrast to the previously reported effects of similar combination drug regimens. Several investigators have shown that treatment employing relatively low doses of $1,25(OH)_2D_3$ (mean dose of 32 ng/kg per day or 1.0 μg/d) or $1\alpha(OH)D$ (mean dose, 1.27 μg/d) and Pi raised the serum phosphorus concentration in the majority of patients without altering the renal phosphate threshold (1, 36, 39). Although bone mineralization improved somewhat in treated subjects, complete healing of the osteomalacia was not achieved, as shown by persistent osteoid excess and/or disturbed mineralization dynamics. While the failure to reduce osteoid surface/volume to normal amounts may have been related to a relatively short course of therapy, in 21 of 24 treated patients mineralization dynamics remained distinctly abnormal (1, 36, 39). One patient treated with $1\alpha(OH)D_3$ showed a normal MFA, but the MiAR was not given (39). In the remaining two subjects, aged 8 and 9 yr, indices of bone mineralization improved to values that were comparable to adult reference normals (1). Since normal mineralization dynamics are requisite for bone healing and should be apparent shortly after initiation of successful therapy, osteomalacia persisted in the vast majority of treated subjects. Indeed, in the setting of persistent abnormal mineralization dynamics, there is little reason to believe that any of the aforementioned 21 patients would ever heal their osteomalacia, no matter what the duration of low dose therapy.

The continued presence of abnormal bone mineralization and renal phosphate handling observed in these studies may be due to the relatively small doses of $1,25(OH)_2D_3$ (or $1\alpha(OH)D_3$) employed. Since recent studies indicate that patients with XLH do not manifest an appropriately elevated serum $1,25(OH)_2D$ level (2, 3, 4), a pharmacologic dose of this active vitamin D metabolite may be necessary to restore normal Pi homeostasis and induce complete healing of the osteomalacia. Glorieux and associates (1, 32) could not conclusively show that the $1,25(OH)_2D_3$ dose that they administered is more than physiologic replacement. In one study, measurement of the serum $1,25(OH)_2D$ concentration after low dose calcitriol therapy revealed no increment of the circulating level above that in the pre-treatment period (36). In a second study they reported a significant elevation above pre-treatment levels (41±4 vs. 61±7 pg/ml; normal, 28–58) (1). However, recording such an increase was highly dependent on the time elapsed between drug administration and blood sampling. In contrast, by performing multiple measurements throughout a 24-h period, we have shown that treated patients in our study maintained a pharmacologic circulating $1,25(OH)_2D$ level throughout most of the day. Thus, the complete bone healing and normalization of renal phosphate handling that we uniformly observed may represent the effects of larger calcitriol doses in conjunction with the Pi supplements.

The mechanism(s) by which the pharmacologic amounts of calcitriol effect bone healing remain uncertain. While the response may be secondary to increasing the serum calcium and/or phosphorus concentration, the failure of other regimens (e.g., vitamin D and Pi) to initiate healing despite similar effects on calcium and phosphorus makes this unlikely. Alternatively, the salutary effect of calcitriol may be mediated by activation of bone matrix synthesis and stimulation of calcium/phosphorus transfer from the osteoblast to the matrix. Indeed, Meunier et al. (40) have shown that calcitriol promotes mineralization only at osteoid surfaces that are covered by osteoblasts. Thus, the mineralization defect in XLH may be due, in part, to the failure of the osteoblast to respond adequately to normal circulating $1,25(OH)_2D$ concentrations. It is possible that sustained supraphysiologic levels of calcitriol serve to overcome this putative osteoblast resistance and induce normal mineralization. Furthermore, our data indicate that once this initial calcitriol resistance is overcome by high doses of the hormone, lesser doses of $1,25(OH)_2D_3$ are capable of maintaining bone healing. Although it is possible that the 11-mo interval between dose reduction and third biopsy was insufficient to allow for the reappearance of hyperosteoidosis, a mineralization defect should have been apparent on the third biopsy if a recurrence of osteomalacia was impending. In fact, the MiAR and MFA remained normal in all four patients who underwent third biopsies. Surprisingly, normal mineralization is maintained despite recrudescent hypophosphatemia, a finding that implies that multiple factors are involved in the genesis of the XLH-associated mineralization defect. Perhaps in the setting of a high normal plasma calcitriol level, hypophosphatemia becomes a less potent inducer of impaired mineralization in these patients.

We also observed a significant improvement in TmP/GFR with high doses of calcitriol, which was abolished after $1,25(OH)_2D_3$ dose reduction. Present evidence favors the hypothesis that the defective renal phosphate transport typical of XLH is due to an intrinsic defect of the proximal tubule (41). However, several studies of the Hyp-mouse (the murine homologue of XLH) suggest that the abnormal circulating levels of vitamin D metabolites may play a direct or complementary role in the genesis of the decreased renal tubular Pi transport (42, 43). These studies show that D metabolites can alter phosphate transport in isolated renal tubules. Similar effects of $1,25(OH)_2D_3$ on the sodium-dependent phosphate transport of isolated chick renal cells and of rat brush border membranes have been reported (44, 45). The fact that TmP/GFR remains subnormal (although improved from pre-treatment values) in humans with XLH who are treated with pharmacologic doses of $1,25(OH)_2D_3$ alone, however, does not substantiate this hypothesis (19). In addition, the failure of low dose $1,25(OH)_2D_3$ and Pi regimens to effect a similar directional change of TmP/GFR to that which we observed is unexplained (1, 36, 46). However, methodologic differences may account for this disparity. Glorieux and associates (1, 36) collected blood and urine for estimation of TmP/GFR on a morning when all medicines were withheld. Thus, the beneficial effects of supraphysiologic calcitriol levels or Pi on renal phosphorus handling may have been overlooked. In contrast, we administered medication ~1 h before initiation of the first 2-h urine collection and 2 h before the first blood collection. Administering the drug allowed us to determine with greater certainty whether the treatment regimen affected the TmP/GFR. Alon and Chan (47) have reported beneficial effects of calcitriol/Pi therapy on renal phosphorus threshold using similar methods. Therefore, it is possible that high doses of $1,25(OH)_2D_3$ and Pi favorably affect renal phosphorus handling when measured under appropriate circumstances.

In conclusion, our data support the view that combined Pi and high-dose calcitriol (55–70 ng/kg IBW per day) therapy is the initial treatment of choice in patients with XLH. Such therapy results in complete healing of the bone disease and amelioration of the characteristic abnormal biochemistries. On this regimen, hypercalcemia or hypercalciuria inevitably occur, necessitating careful follow-up and eventual calcitriol dose reduction. Early detection of these complications is essential since reduced renal glomerular function may quickly ensue and persist after dose reduction. Although reduced doses of $1,25(OH)_2D_3$ do not maintain normophosphatemia in these patients, bone healing is persistent for at least 11 mo. Further studies are necessary to determine whether shorter courses of high dose therapy can promote bone healing while avoiding complications, and to resolve whether treatment with low doses of calcitriol can maintain normal bone mineralization for even longer periods of time.

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