DISORDERS OF VITAMIN D EXCESS

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"Some circumstantial evidence is very strong, as when you find a trout in the milk."

Henry David Thoreau

Internal Medicine Grand Rounds

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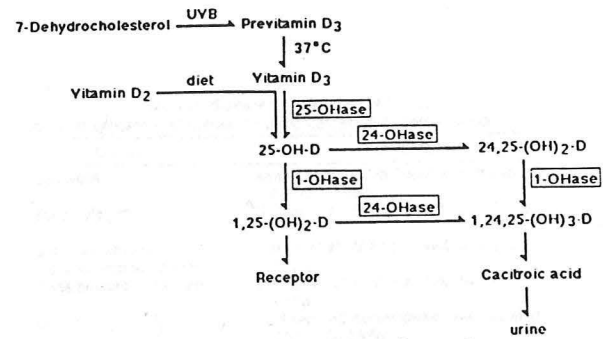
1. Introduction

Excessive endogenous production of vitamin D may cause hypercalcemia, hypercalciuria and/or bone loss. The three most common causes of human hypercalcemia are primary hyperparathyroidism, malignancy and granulomatous diseases (such as sarcoidosis). A disturbance in vitamin D metabolism may contribute to a state of disordered calcium homeostasis in each of these categories of disease. Moreover, there is mounting evidence that excess production of or sensitivity to $1,25\text{-}(OH)_2D$ may be causally related to absorptive hypercalciuria, the single major cause of kidney stones. Evidence is also beginning to emerge linking $1,25\text{-}(OH)_2D$ to the bone loss of patients with idiopathic osteoporosis. Exogenous administration of vitamin D metabolites, as in the treatment of hypoparathyroidism or renal failure, or drinking milk that is excessively fortified with vitamin D, may result in vitamin D intoxication, which includes elements of hypercalcemia, hypercalciuria and increased bone resorption. This review will focus on the following proposed disorders of vitamin D excess:

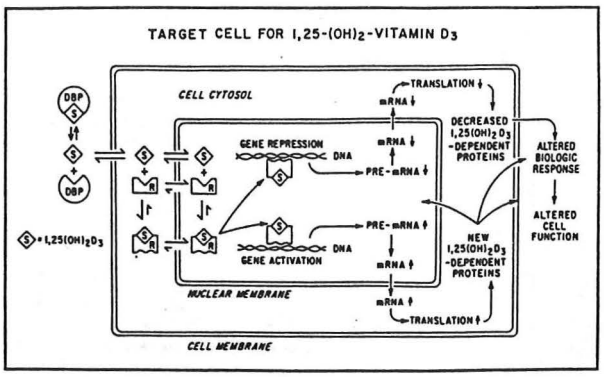
- Granulomatous Disorders/Lymphoma
- Absorptive Hypercalciuria
- Idiopathic Osteoporosis
- Vitamin D Intoxication

2. Synthesis and Metabolism of Vitamin D (Ref.1)



Synthesis and metabolism of vitamin D.

3. Proposed Genomic Mode of Action of 1,25-$(OH)_2D$ (Ref.2)



Proposed Genomic Mode of Action of $1,25(OH)_2D_3$.
Target organs and cells for $1,25(OH)_2D_3$ by definition contain receptors for this seco-steroid that enable them to modulate genomic events. The interaction of $1,25(OH)_2D_3$ with genomic material is thought to be analogous to the mode of action of other steroid hormones. S denotes steroid hormone, R receptor protein, DBP vitamin D–serum binding protein, and P RNA polymerase.

4. Granulomatous Disorders/Lymphoma

A. Granulomatous diseases associated with 1,25-$(OH)_2D$-mediated hypercalcemia/hypercalciuria (Ref.3)

Human diseases associated with 1,25-dihydroxyvitamin D mediated hypercalcemia/hypercalciuria

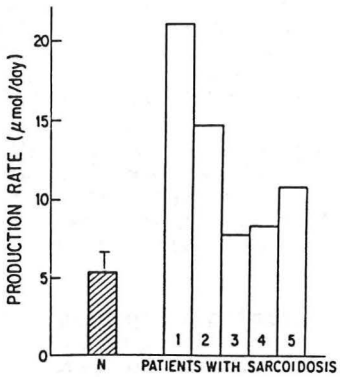
Disease	Reference
Sarcoidosis	Adams et al. *J Clin Endocrinol Metab.* 60:960, 1985
Tuberculosis	Gkonos et al. *N Engl J Med.* 311:1683, 1984
Silicone-induced granulomatous disease	Kozeny et al. *N Engl J Med.* 311:1103, 1984
Disseminated candidiasis	Kantarjian et al. *Am J Med.* 74:721, 1983
Leprosy	Hoffman and Korzeniowski. *Ann Int Med.* 105:890, 1986
Lymphoma	Breslau et al. *Ann Int Med.* 100:1, 1984

B. Increased production of 1,25-$(OH)_2D$ in sarcoidosis (Ref.4)

1,25-$(OH)_2D$ kinetic parameters in normal subjects, patients with absorptive hypercalciuria (AH), and patients with sarcoidosis

	n	MCR (mL/min)	1,25-$(OH)_2D$ (pmol/L)	Production rate (μmol/day)
Normal subjects	13	37 ± 6	101 ± 14.4	5.4 ± 1.2
Patients with AH	9	35 ± 4	163 ± 14.4	8.2 ± 1.2
Patients with sarcoidosis				
Patient	1	53	274	20.9
Patient	2	42	238	14.4
Patient	3	44	121	7.7
Patient	4	31	185	8.3
Patient	5	31	238	10.7
Mean		40 ± 9	211 ± 60*	12.4 ± 5.3*

*$P < 0.001$ compared with normal individuals and $P < 0.05$ compared with patients with absorptive hypercalciuria.



1,25-$(OH)_2D$ production rate data for the normal subjects and the five patients with sarcoidosis. The *hatched bar* represents the mean ± SD in the normal subjects. The *open numbered bars* represent the values in the individual patients with sarcoidosis.

C. Cellular Source of Active Vitamin D Metabolites

The report of Barbour et al. proved the source of 1,25-$(OH)_2D$ to be extrarenal in sarcoidosis(5). These investigators described an anephric patient with sarcoidosis, hypercalcemia and a high serum 1,25-$(OH)_2D$ concentration. The elevated 1,25-$(OH)_2D$ concentration in patients with sarcoidosis is now known to result from increased production of 1,25-$(OH)_2D$ by the macrophage, a prominent constituent of the sarcoid granuloma(6). Synthesis of 1,25-$(OH)_2D$ from 25-$(OH)D_3$ has been demonstrated in vitro by alveolar macrophages from hypercalcemic patients with sarcoidosis and by granulomatous tissue.

D. Salient properties of the macrophage 25-(OH)D 1-hydroxylation reaction (Ref.7)

Characteristics of the sarcoid macrophage 25-hydroxyvitamin D-1-hydroxylation reaction *in vitro*

- Side chain-substituted substrates preferred*
- High affinity for substrate (84 ± 24 nM)*
- Little or no inhibition by product
- Not accompanied by 24-hydroxylase
- Most potent stimulator is interferon-gamma
- Most potent inhibitor is glucocorticoid

* Similar to the renal 1-hydroxylase

E. Why does the macrophage produce 1,25-$(OH)_2D$?

1,25-$(OH)_2D$ is known to exert a potent immunoinhibitory effect on activated human lymphocytes in vitro. These actions include inhibition of lymphocyte proliferation, lymphokine production, and immunoglobulin synthesis(8). It has been suggested that 1,25-$(OH)_2D$ produced by the macrophage in granulomatous diseases exerts a paracrine immunoinhibitory effect on neighboring active lymphocytes which express receptors for the hormone and that this acts to slow an otherwise "overzealous" immune response that may be detrimental to the host. Recently, high free 1,25-$(OH)_2D$ concentrations were found in tuberculous pleural effusions (but not other pleural effusions) at a level capable of inhibiting the cellular immune response in vitro(9).

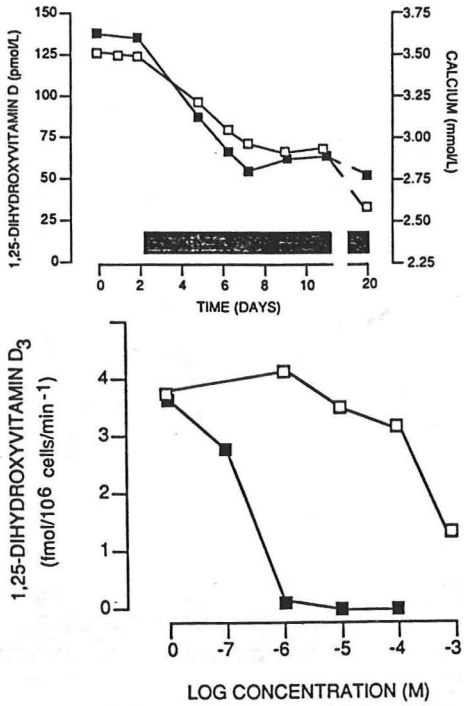
F. Treatment of hypercalcemia/hypercalciuria of sarcoidosis

1. Prednisone. Glucocorticoids (40-60 mg prednisone or equivalent daily) are the mainstay of therapy of disordered calcium homeostasis resulting from endogenous overproduction of active vitamin D metabolites. Institution of glucocorticoid therapy results in a prompt decrease in the circulating 1,25-$(OH)_2D$ concentration (within 3 days) presumably through a direct action of the steroid on the macrophage hydroxylation reaction. Normalization of the serum calcium usually occurs within a matter of days, although normalization of urine Ca secretion may take weeks. Failure to normalize the serum Ca after 10 days of therapy suggests the coexistence of another hypercalcemic process (i.e. hyperparathyroidism, humoral hypercalcemia of malignancy). The responsiveness of patients with sarcoidosis to prednisone 50 mg daily is contrasted with other groups in the table below(10,11). Glucocorticoids also appear to be effective in the management of vitamin D-mediated hypercalcemia or hypercalciuria in other granulomatous diseases and lymphoma(12).

SHORT-TERM EFFECTS OF GLUCOCORTICOID ADMINISTRATION IN VARIOUS GROUPS

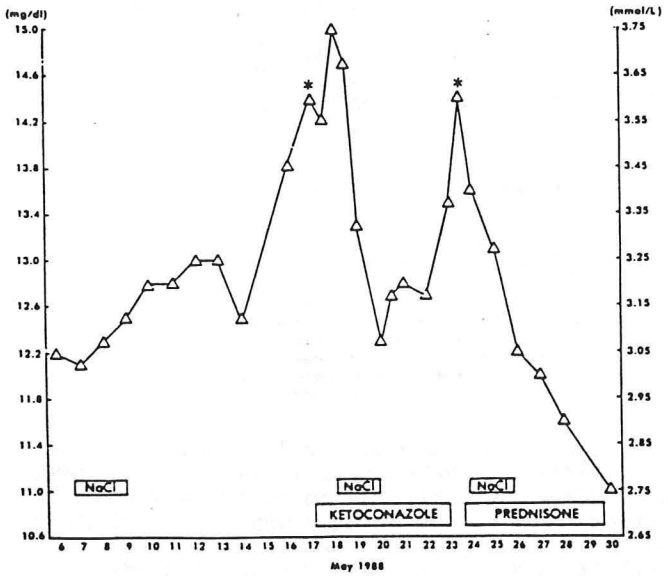
	NORMAL (N=7)	ABSORPTIVE HYPERCALCIURIA (N=6)	SARCOIDOSIS (N=8)	PHPT (N=8)
24-H URINARY Ca, MG/DAY	130→228	230→343	181→174	393→529
SERUM 1,25-$(OH)_2D$, PG/ML	41→47	36→44	48→33	75→77
INTES ^{47}Ca ABS. FRACTION	.44→.43	.73→.70	.58→.46	.77→.75

2. Chloroquine. Chloroquine and its hydroxy-analogue (hydroxy-chloroquine) are also capable of reducing serum $1,25\text{-}(OH)_2D$ and calcium concentration in patients with sarcoidosis(13-15). Because of the limited experience with these drugs as anti-hypercalcemic agents, they should be limited to patients in whom steroid therapy is unsuccessful or contraindicated.



Top. Serial serum 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2\text{-}D$, *solid squares*) and total calcium (*open squares*) concentrations in a hypercalcemic patient with sarcoidosis compared with the oral administration of chloroquine, 250 mg orally twice daily (*stippled bar*). The normal range for $1,25\text{-}(OH)_2\text{-}D$ and total calcium is 39 to 156 pmol/L and 2.12 to 2.62 mmol/L, respectively. *Bottom.* The effect of incubation with increasing concentrations of chloroquine (*solid squares*) and ammonium chloride (*open squares*) on the conversion of 5 nM $[^3H]25\text{-}OH\text{-}D_3$ to $[^3H]1,25\text{-}(OH)_2\text{-}D_3$ by primary cultures of pulmonary alveolar macrophages from the same host. The specific activity of the $25\text{-}OH\text{-}D_3$-1-hydroxylation reaction is expressed in terms of femtomoles $[^3H]1,25\text{-}(OH)_2\text{-}D_3$ synthesized per 10^6 cells per minute^{-1}. Each data point is the mean of duplicate values.

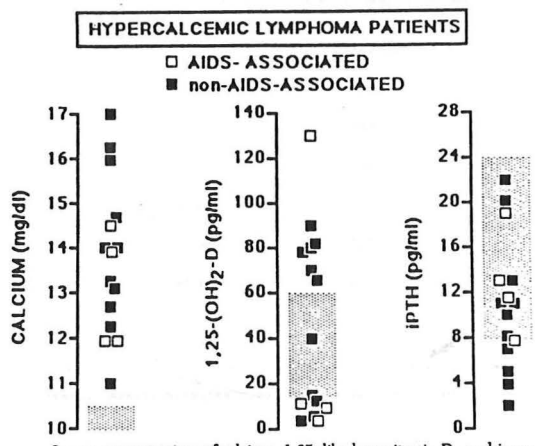
3. Ketoconazole. Ketoconazole has also been reported to lower serum $1,25\text{-}(OH)_2D$ levels in a couple of patients with sarcodosis, but hypercalcemia was not very well controlled(16). Moreover, it is unlikely that agents such as ketoconazole would destroy the granulamotous deposits in various organs as effectively as prednisone. The action of ketoconazole appears limited to inhibition of the 25-(OH)D 1-hydroxylase. Ketoconazole may also adversely affect renal function(16).



Serum values of total serum calcium (autoanalyzer) in a 27-year-old man with previously untreated sarcoidosis (Case 1). Asterisks indicate times when the patient became symptomatic from hypercalcemia. NaCl indicates periods of administration of intravenous saline and furosemide. KETOCONAZOLE indicates period of administration of ketoconazole 200 mg every 8 h. PREDNISONE indicates period of administration of prednisone 30 mg/day

G. Incidence of increased 1,25-$(OH)_2D$ among unselected lymphoma patients

Our initial observation of elevated levels of 1,25-$(OH)_2D$ in hypercalcemic lymphoma patients(12) has been confirmed by numerous laboratories(17-25). It has been suggested that ectopic expression of 1α-hydroxylase activity in malignant lymphocytes or other cells in lymphoma patients may not be infrequent(22).



Serum concentrations of calcium, 1,25-dihydroxyvitamin D_3, and immunoreactive parathyroid hormone in 15 patients with lymphoma prior to institution of antitumor or specific antihypercalcemic chemotherapy. The shaded areas represent the range of normal values. (*From* Adams JS, Fernandez M, Gacad MA, et al: Vitamin D metabolite–mediated hypercalcemia and hypercalciuria patients with AIDS- and non-AIDS-associated lymphoma. Blood 73:235–239, 1989; with permission.)

The source of the excess 1,25-$(OH)_2D$ in these patients has not been conclusively demonstrated. However, 1α-hydroxylase activity was reported to be present in lymph node homogenate from a patient with B-cell lymphoma(20). Thus, neoplastic or reactive lymphoid tissue may be capable of metabolizing 25(OH)D to 1,25-$(OH)_2D$.

The prospective study of Adams et al.(22) suggested that 1,25-$(OH)_2D$ levels were elevated in a significant proportion (47%) of hypercalcemic lymphoma patients. A recently completed study by Charles Eil's group(25) suggests that ectopic expression of 1α-hydroxylase activity in malignant lymphocytes or other cells in lymphoma patients may be even more frequent. Using 25-(OH)D loading (200 mcg/d x 4 days), Eil's group demonstrated abnormal regulation of 1α-hydroxylase in 6 out of 6 normocalcemic lymphoma patients. Normal subjects and normocalcemic patients with other types of cancer do not show an increase in serum 1,25-$(OH)_2D$ in response to challenge with 25(OH)D. Using similar vitamin D loading studies, other investigators have documented abnormal regulation of the 1α-hydroxylase enzyme in patients with primary hyperparathyroidism(26,27) and in normocalcemic patients with sarcoidosis(28).

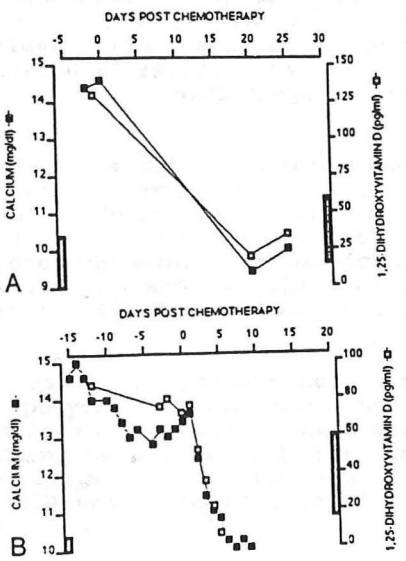
These results suggest that it might not be uncommon for lymphomatous tissue to have the capability of expressing the 1α-hydroxylase enzyme. The degree of activity of this extra-renal 1α-hydroxylase may depend on the availability of substrate (25-(OH)D), on tumor bulk, or both. It also seems to correlate directly with serum LDH levels(25).

Serum 1,25(OH)D Levels (pmol/L) before and after 25(OH)D loading For 6 Lymphoma Patients.

Patient	Baseline	Peak-Post
1	79.9	110
2	72.8	109.7
3	59.2	88.8
4	94.4	131.1
5	127.9	236.7
6	65.9	238.6
Mean ± SEM	83.4 ± 10.2	*152.5 ± 27.4

*p<.025 by paired t-test. Normal baseline 1,25-(OH)$_2$D = 48.5-97.1 pmol/L

H. Response of 1,25-(OH)$_2$D and serum Ca to anti-tumor therapy in lymphoma



Temporal changes in the serum calcium and 1,25-dihydroxyvitamin D concentrations in two lymphoma patients, with (*A*) and without AIDS (*B*), in relation to an effective course of antitumor chemotherapy.

5. Absorptive Hypercalciuria

A. Definition. Absorptive hypercalciuria (AH) is the most common cause of kidney stones, accounting for over half the cases(29). The basic abnormality in AH is the intestinal hyperabsorption of calcium(30). A patient with calcareous calculi may be diagnosed as having AH if the following features are present(31): normocalcemia, hypercalciuria (urinary Ca >200 mg/dl on a restricted diet), normal fasting urinary Ca (< .11 mg/dl GF), exaggerated urinary Ca following an oral Ca load (>.20 mg/dl GF) and normal or suppressed parathyroid function (normal serum immunoreactive PTH). The diagnosis of AH may be confirmed by more direct isotopic techniques such as fractional absorption of ^{47}Ca.

B. Case Reports Illustrating Overlap Between Sarcoidosis and AH.

Case 1 - JL

A 27 year-old white male was first seen in 1985 because of a two year history of passing mixed Ca oxalate/Ca phosphate kidney stones. He had passed three stones spontaneously and required one left ureterolithotomy. Abdominal x-ray revealed two small 2-3 mm residual stones in the lower pole of his left kidney. There was no history of bone fractures and no family history of stones. Initial evaluation revealed serum Ca 10.4, P 3.2, PTH_m-175 pg/ml (normal 100-400), alkaline phosphatase 176 (normal <115), Cr 1.2, C_{Cr}89 ml/min. Random 24-hr urine Ca levels were 271 and 442, decreasing to 193 after a week of restricted diet. A fast and load test revealed values of .19 mg/dl GF and .39 mg/mg cr, respectively. Following an SCP trial, fasting U_{Ca} decreased to .08 (PTH_m-200). Bone density was low normal at the radius, femoral neck and lumbar spine. The patient was believed to have absorptive hypercalciuria and was started on thiazide diuretics. Over the ensuing years, serum Ca remained in the 10.0 mg/dl range and 24-hr U_{Ca} was 190 mg.

In August, 1988, the patient complained of some left flank pain and hematuria. Serum Ca was 11.6, P 3.2, Cr 1.8, PTH_m-195, 24-hr U_{Ca} 351. In September, an IVP showed the left kidney stones had migrated and were now blocking the left ureter, causing hydronephrosis. Ultrasonic lithotripsy was performed via ureteroscopy and IVP obtained afterwards was normal. By February, 1989, Ca was 10.2, P 3.2, Cr 1.3, Hgb 15.7, Hct 47.

In late June, 1989, the patient complained of several weeks of fatigue, polyuria, itchy eyes, nausea, decreased appetite and weight loss of 5 pounds. There was no cough, SOB or nightsweats. Serum Ca was 14.2, P 2.8, Cr 2.4, PTH_{IRMA}-2 pg/ml (normal 10-65 pg/ml), alk phos 138. 24-hr U_{Ca} was 377. Serum 25-(OH)D was 17 ng/ml (normal 7-42) and 1,25-

$(OH)_2D$ was 65 pg/ml (normal 20-50). Physical exam did not reveal any adenopathy or splenomegaly and lungs were clear. Thiazides were discontinued for two weeks, but repeat blood test revealed Ca 15.4, Cr 3.7, Hgb 10.1, Hct 31, TP 6.5, Alb 4.2. A CXR revealed left hilar adenopathy and bilateral upper lobe nodular infiltrates. The patient was admitted to an isolation room on the pulmonary service to rule out tuberculosis. The workup revealed anergy, ↓DLCO, and bronchoscopic biopsy revealed non-caseating granulomas. Sputa for AFB were negative. A diagnosis of sarcoidosis was made and the patient was started on Prednisone 60 mg daily.

Within one week of starting Prednisone, serum Ca had decreased to 10.0, Cr 2.2 and PTH_{IRMA} was 8. After a month on Prednisone, serum Ca was 9.2, 24-hr U_{Ca} 235, Cr 1.5 and PTH_{IRMA}-23. Over the ensuing five months, Prednisone was gradually tapered off. At the conclusion of glucocorticoid therapy, serum Ca was 9.8, 24-hr U_{Ca} 119, Cr 1.5 and PTH_{IRMA}-30. Hgb was 15.7 and Hct 46. During the course of treatment, the patient noticed increased appetite, gained 10 pounds, and developed some facial rounding and acne, but did not develop any hypertension or other stigmata of Cushing's syndrome. Steroids were discontinued in December 1989. In the four months after steroid therapy was discontinued, serum Ca gradually rose to 10.4, 24-hr U_{Ca} increased to 274 mg and serum PTH_{IRMA} became suppressed at 4 pg/ml. The patients sarcoidosis appeared to be re-activating. At this point, the patient moved to Minnesota, where his medical records were sent.

Case 2 - NS

The patient presented as a 49 year old white female with nephrolithiasis since age 35. She had passed 3 Ca oxalate stones spontaneously and two were removed surgically. There was a strong family history of kidney stones. Outpatient metabolic evaluation revealed serum Ca 9.8, PTH_m-270 pg/ml, random 24-hr urine Ca levels of 260, 275 and level after one week of restricted diet of 254. Fasting urinary Ca was normal at .10 mg/dlGF, and there was an exaggerated load response of .62 mg/mg Cr, consistent with absorptive hypercalciuria.

Interestingly, at age 32, the patient had seen her physician because of cough and shortness of breath. She was found to have diffuse lymphadenopathy and hilar adenopathy on chest radiograph. A supraclavicular node biopsy revealed non-caseating granulomas, consistent with sarcoidosis. She was treated with glucocorticoids for two years. Her symptoms gradually improved and she no longer had cough, shortness of breath or lymphadenopathy. Her kidney stone problems began about three years after sarcoidosis was treated.

At the time of her current assessment for stone disease, CXR was normal and ACE level was 33 U/L (normal 8-52), total

protein 6.4, albumin 4.1. The patient underwent both Ketoconazole and Prednisone suppression testing with the following results:

Case 2 (NS) - Response to Ketoconazole and Prednisone

	Control	Ketoconazole	Prednisone
$1,25\text{-}(OH)_2D$	26	10	34
Intes ^{47}Ca Abs	.67	.51	.71
24-hr U_{Ca}	290	180	410

Case 3 - BP

A 35 year-old Chilean male had been previously healthy except for spontaneous passage of a kidney stone while in college in 1978. He developed recurrent nephrolithiasis in April and June, 1990, involving the left and right side, respectively. Serum Ca was normal (9 to 10 mg/dl), but he was hypercalciuric and was initially assumed to have absorptive hypercalciuria. The patient had no chest pain, cough, fever or shortness of breath. Nor did he have any bone or joint pain. However, later that summer, he began to develop diffuse lymphadenopathy involving the cervical, axillary and inguinal nodes. Serum Ca remained normal, but there was reversal of the albumin/globulin ratio and an increased ACE level. CXR revealed coarse interstitial markings and scattered nodules. Chest CT confirmed hilar and paratracheal lymphadenopathy in addition to multiple parenchymal nodules. In August, 1990, axillary lymph node biopsy disclosed non-caseating granulomas. No treatment was given. Over the next few months, the patient remained asymptomatic, but lost about 5-10 pounds and noted a progressive increase in the number and size of his lymph nodes.

Beginning in May or June, 1991, he began to experience malaise, fatigue, loss of appetite, nausea, pruritus which caused insomnia, polyuria, dehydration, decreased ability to concentrate and memory loss. In July, 1991, serum Ca was 14.3, P 4.2 and creatinine 2.8. The patient was referred to Dr. Robert Johnson and to me.

The patient still did not complain of cough or shortness of breath, and pulmonary function tests were nearly normal. The patient provided an interesting family history in that his 38 year-old sister was recently found to have sarcoidosis and uveitis. She responded well to a 6 month course of steroids. There was no family history of kidney stones. On physical exam, the patient had multiple, large (lemon-sized) rubbery, non-tender lymph nodes in the cervical, axillary and inguinal area. A KUB revealed several small stones in the lower pole

of the left kidney. In late July, 1991, a baseline evaluation was performed at the GCRC, followed by a trial of Ketoconazole, and then Prednisone (see below). At baseline, Hgb was 11.3, Hct 32, wbc 5000 with 80 pmn and 14 lymphs, Cr 2.8, C_{Cr} 40 ml/min, ESR 103, ACE level 115 (normal <50), total protein 8.2, albumin 3.6, and the patient was anergic.

Calcium Metabolism Parameters

	Control	Ketoconazole	Prednisone
Serum Ca	14.6	12.2	10.2
P	3.1	2.7	3.2
Alk Phos	101	110	125
PTH_{IRMA}	5	4	45
$1,25\text{-}(OH)_2D$	74	52	34
Intes ^{47}Ca Abs	.85	.74	- -
24-Hr U_{Ca}	612	565	93

Other Parameters

	Control	Ketoconazole	Prednisone
Serum Creatinine	3.1	2.4	1.2
Creatinine Clearance	41	46	129
Hemoglobin	11.3	9.8	14.1
Hematocrit	32.4	28.3	39.4
ESR	103	- -	17
TP/Alb	8.2/3.6	7.5/3.2	7.9/4.1
Glucose	105	98	127
Cholesterol	188	169	345
Triglycerides	203	226	310
Weight (pounds)	158	161	188
Blood Pressure	110/70	124/78	130/100

C. Pathogenesis. The exact cause for the increased intestinal absorption of calcium in AH is not known. On the one hand, there is evidence that the hyperabsorption of calcium by the intestine may be "primary" and independent of vitamin D. Thus, studies in Dallas indicated that over 2/3 of these patients did not have increased 1,25-$(OH)_2D$ levels, and that fractional Ca absorption did not correlate with the serum concentration of 1,25-$(OH)_2D$ for the group as a whole(32). Moreover, intestinal perfusion studies disclosed an absorption profile (selective jejunal hyperabsorption of Ca) which was different from 1,25-$(OH)_2D$ action(33). Recently, a colony of genetically hypercalciuric rats has been established through selective inbreeding in order to explore the pathogenetic role of intestinal Ca absorption and 1,25-$(OH)_2D$ in hypercalciuria(34). Hypercalciuria in these rats was associated with intestinal overabsorption of dietary Ca, but serum 1,25-$(OH)_2D$ levels were normal.

On the other hand, there is evidence that the increased intestinal absorption of Ca in AH may be due to an exaggerated renal synthesis of 1,25-$(OH)_2D$. Increased serum 1,25-$(OH)_2D$ levels have been reported in up to 80% of patients with AH in some series(35). In a study of 1,25-$(OH)_2D$ kinetics by the infusion equilibrium technique, the basis for this elevation in circulating 1,25-$(OH)_2D$ concentration was shown to be an increased production rate, presumably by the kidney(36). Administration of 1,25-$(OH)_2D$ to normal subjects induces a condition with most of the essential features of AH(37). The cause for the exaggerated renal synthesis of 1,25-$(OH)_2D$ in most patients with AH has not been apparent, since no perturbations were found in the usual stimuli for the renal 1α-hydroxylase including PTH, serum phosphate and nephrogenous cyclic AMP(35,36).

D. Use of Ketoconazole to Probe the Pathogenetic Importance of 1,25-$(OH)_2D$ in AH(38).

Recent reports have indicated that ketoconazole, an antimycotic agent, is capable of lowering serum 1,25-$(OH)_2D$ levels(39,40). Because of its well-known ability to block cytochrome P450-dependent enzymes(41), ketoconazole was able to reduce the serum concentration of 1,25-$(OH)_2D$ in a dose-dependent fashion, when given to normal volunteers(39). At a dosage of 600 mg daily, which had minimal side effects, ketoconazole was able to reduce the serum 1,25-$(OH)_2D$ level by 40% in normal subjects and patients with primary hyperparathyroidism after one week of therapy(39,40). It therefore appeared that ketoconazole could serve as a useful probe to investigate the pathogenetic importance of 1,25-$(OH)_2D$ in patients with AH. In those patients whose intestinal hyperabsorption of Ca was vitamin D-dependent, restoration of normal serum 1,25-$(OH)_2D$ concentration by ketoconazole should lower Ca absorption and urinary Ca excretion back toward the normal range. Patients with a "primary" intestinal hyperabsorption of Ca (vitamin D-independent) might be

unaffected by the ketoconazole-induced reduction in serum $1,25\text{-}(OH)_2D$ concentration.

E. Results of Ketoconazole Study(38).

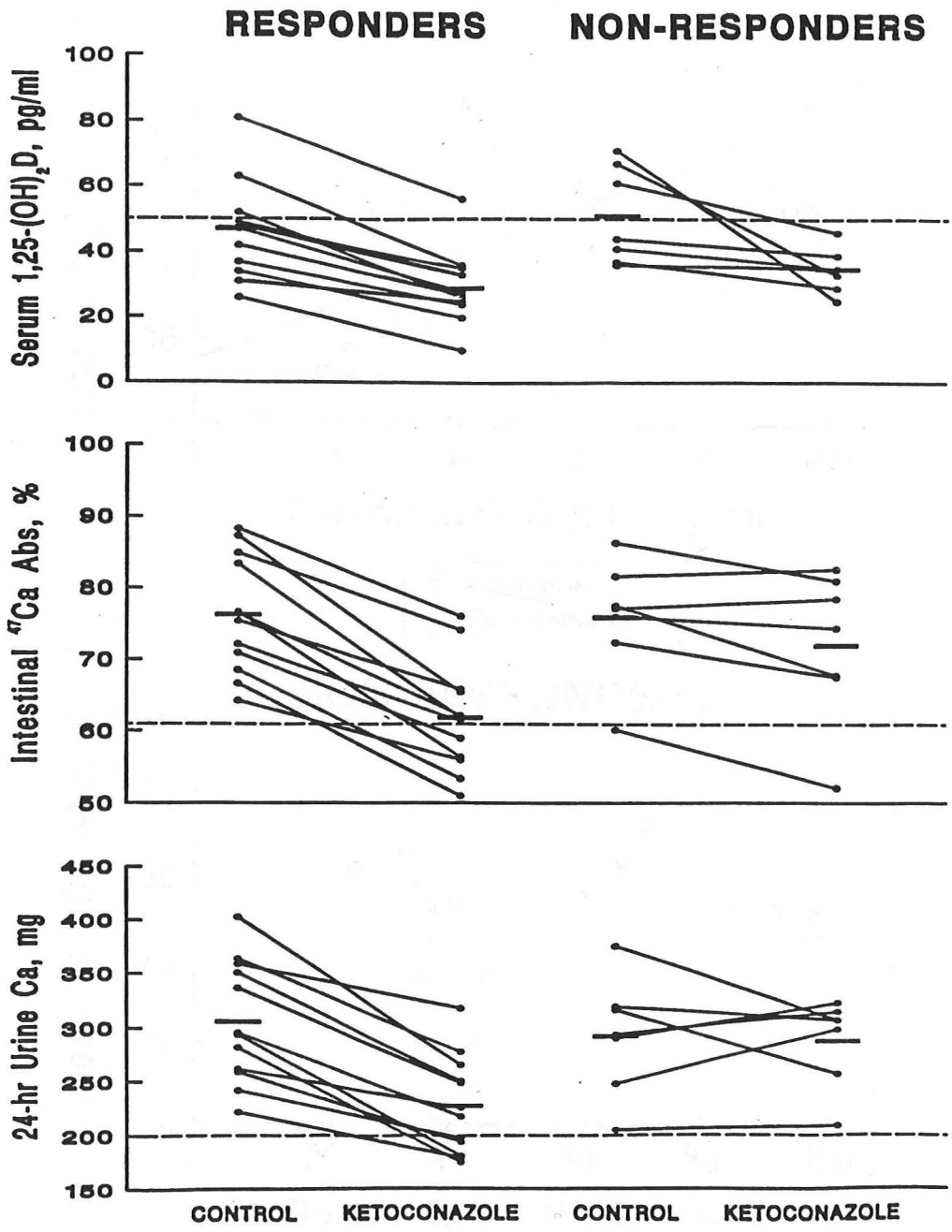
Does $1,25\text{-}(OH)_2D$ Play a Role in Absorptive Hypercalciuria?

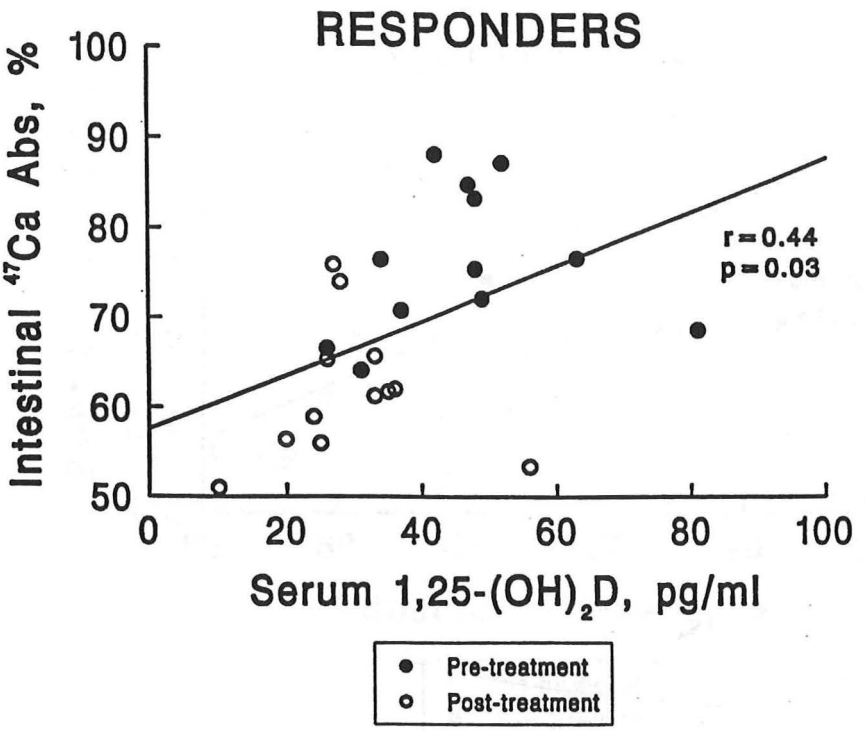
Test: Suppression of $1,25\text{-}(OH)_2D$ by Ketoconazole

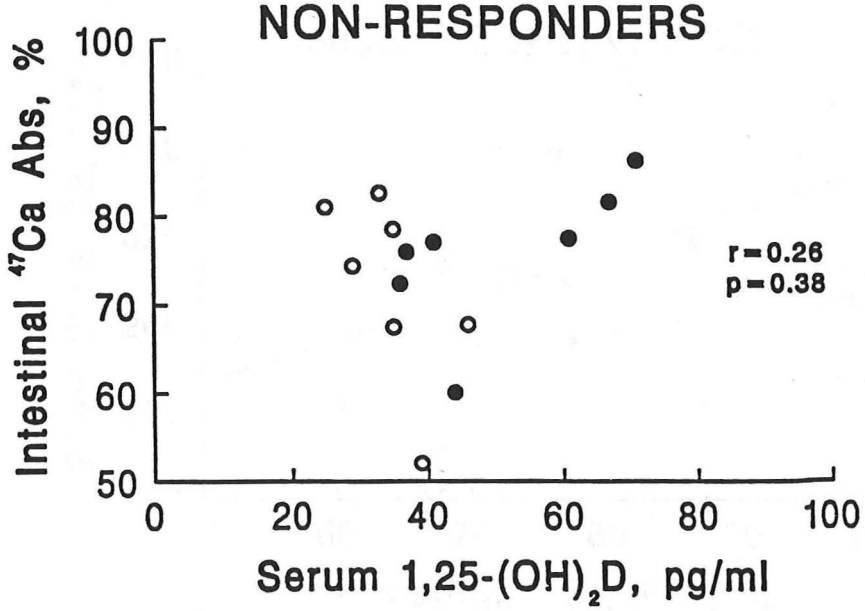
Results: 19 patients with absorptive hypercalciuria tested. There were 3 categories of response:

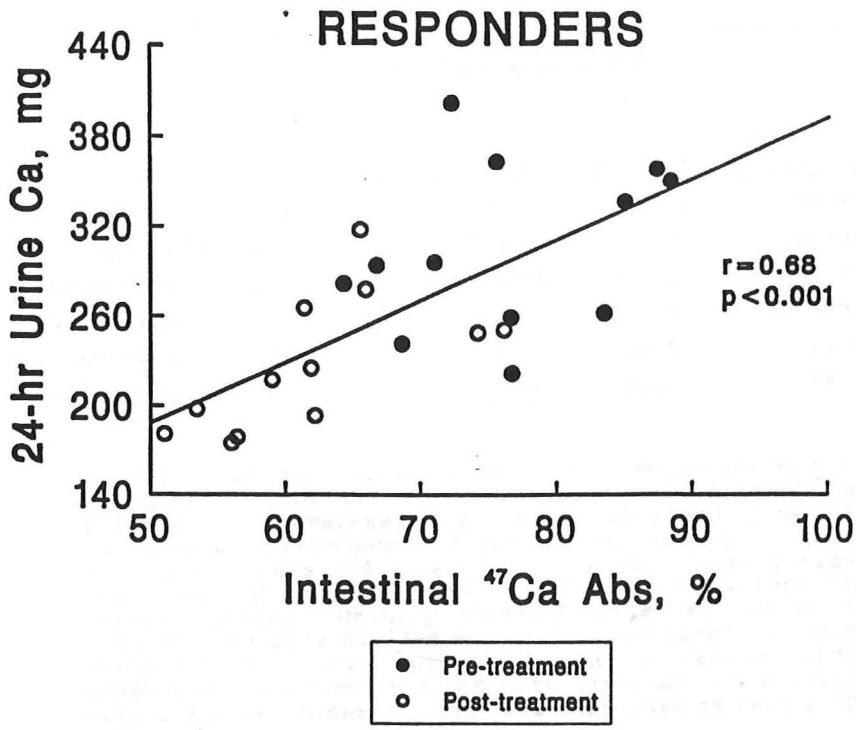
		$1,25\text{-}(OH)_2D$ pg/ml	Intes ^{47}Ca Abs percent	24-hr U_{Ca} mg
(1)	Responders with high $1,25\text{-}(OH)_2D$ (N=3)	65±15→39±15*	77±9→60±7*	287±63→236±70*
(2)	Responders with normal $1,25\text{-}(OH)_2D$ (N=9)	40±9→26±8*	76±8→62±8*	312±56→225±39*
(3)	Non-responders (N=7)	51±14→35±7*	76±8→72±11	292±54→288±41

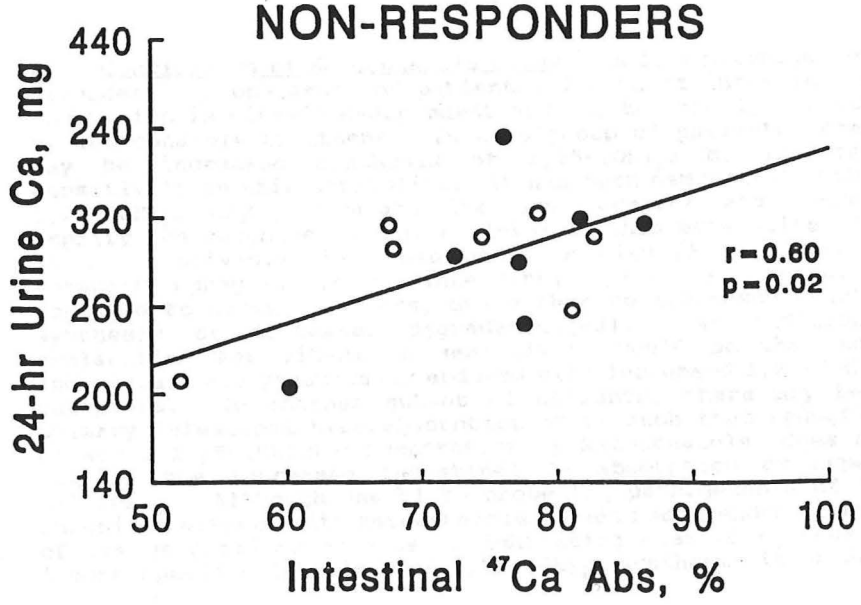
Data expressed as mean ± SD. * $P<.05$


RESPONDERS
NON-RESPONDERS
Serum 1,25-(OH)2D, pg/ml
100
80
60
40
20
0
Intestinal 47Ca Abs, %
100
90
80
70
60
50
24-hr Urine Ca, mg
450
400
350
300
250
200
150
CONTROL
KETOCONAZOLE
CONTROL
KETOCONAZOLE


RESPONDERS
Intestinal ^{47}Ca Abs, %
100
90
80
70
60
50
0
20
40
60
80
100
Serum 1,25-(OH)$_2$D, pg/ml
r=0.44
p=0.03
Pre-treatment
Post-treatment


NON-RESPONDERS
Intestinal ^{47}Ca Abs, %
100
90
80
70
60
50
0
20
40
60
80
100
Serum 1,25-(OH)$_2$D, pg/ml
r=0.26
p=0.38


RESPONDERS
24-hr Urine Ca, mg
440
380
320
260
200
140
50
60
70
80
90
100
Intestinal 47Ca Abs, %
r=0.68
p<0.001
Pre-treatment
Post-treatment


NON-RESPONDERS
24-hr Urine Ca, mg
440
240
320
260
200
140
50
60
70
80
90
100
Intestinal 47Ca Abs, %
r=0.60
p=0.02

Effect of Ketoconazole on Cholesterol, Triglycerides and Other Steroid Hormones†

	Normal Values	Control	Ketoconazole
Serum cholesterol, mg/dl	<200	225±41	177±33*
Serum triglycerides, mg/dl	10-190	177±69	184±90
Serum testosterone, ng/dl	200-1080	700±264	557±330*
Serum LH, mIu/ml	3-18	11.9±3.3	18.5±7.3*
24-hr urine cortisol, mcg	20-90	37±21	23±15*
Serum $ACTH_{IRMA}$, pg/ml	9-52	17±12	35±20*

† For the above parameters, responsiveness to ketoconazole did not differ between the two groups of patients. Therefore, the values obtained from all patients were combined. Serum cholesterol and triglycerides were measured in all 19 patients. Serum testosterol and LH were measured in all patients except the single female. Urinary cortisol was measured in 14 patients, equally divided between "responders" and "non-responders." Serum ACTH by immunoradiometric (IRMA) assay was performed in 8 patients (including 6 responders, 2 non-responders). Data are expressed as mean ± SD. * = P<.03.

F. Conclusions of Ketoconazole Study. AH is a heterogeneous disorder. In one group of patients, increased intestinal Ca absorption is vitamin D-dependent and may be largely reversed by ketoconazole treatment. In this group of patients, there may be increased synthesis of $1,25\text{-}(OH)_2D$ or increased sensitivity to this metabolite. It has been demonstrated that $1,25\text{-}(OH)_2D$ may upregulate its own receptor and thereby amplify the response to a given level of this metabolite(42). It is conceivable that some patients with AH may have an enhanced upregulation of intestinal $1,25\text{-}(OH)_2D$ receptors compared to normal subjects, due either to increased rate of synthesis or decreased degradation(43). An additional explanation for vitamin D sensitivity would be that some individuals are genetically endowed with increased $1,25\text{-}(OH)_2D$ receptors. In another subset of patients, there may be a primary intestinal hyperabsorption of Ca such that reduction of serum $1,25\text{-}(OH)_2D$ concentration by ketoconazole does not correct the increased intestinal Ca absorption or hypercalciuria. Although useful to probe the pathogenesis of AH, chronic treatment with ketoconazole is not recommended because of its generalized effects in inhibiting steroid synthesis. A more specific inhibitor of $1,25\text{-}(OH)_2D$ synthesis is needed.

G. Additional Insights from Genetically Hypercalciuric Rats

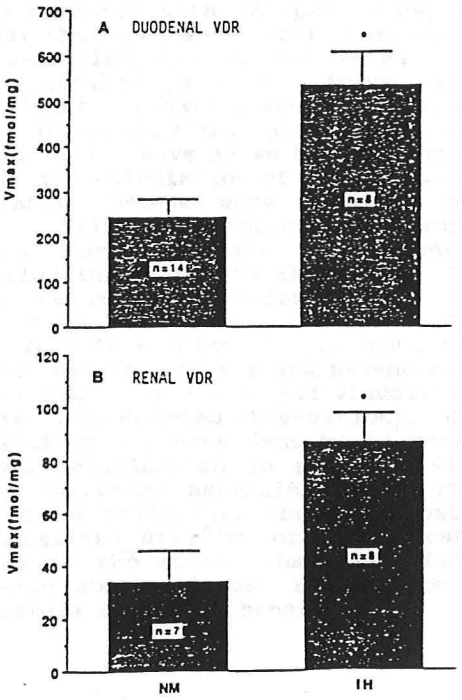
Selective breeding of spontaneously hypercalciuric male and female Sprague Dowley rats resulted in offspring with hypercalciuria, increased intestinal Ca absorption and normal serum $1,25\text{-}(OH)_2D$ levels(44).

Comparison of Hypercalciuric and Normocalciuric Rats

	Hypercalciuric	Normocalciuric
24-hr Urine Ca, mg	2.9±0.3	0.7±0.2*
Duodenal net Ca Abs, nmol/cm^2/hr	245±28	40±11*
Serum $1,25\text{-}(OH)_2D$, pg/ml	135±12	174±19

Data expressed as mean ± SE. * P<.01

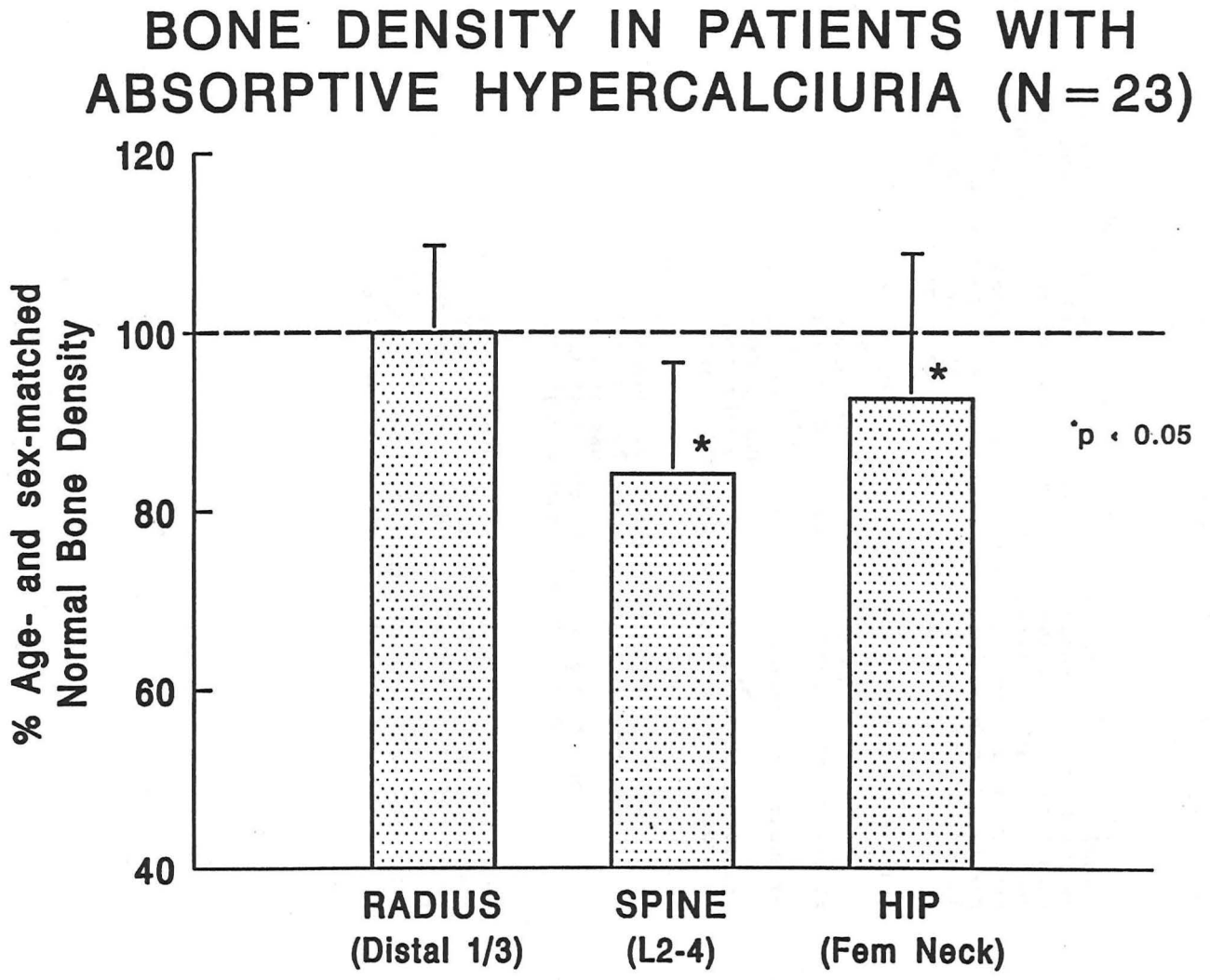
The role of the vitamin D receptor (VDR) in the regulation of intestinal Ca absorption was explored in 10th generation male genetic hypercalciuric rats and normocalciuric controls(44).



Hypercalciuric rat intestine contained twice the abundance of VDR compared to normocalciuric controls (536±73 vs. 243±42 fmol/mg protein, P<.001), with no difference in the affinity of the receptor for its ligand. Hypercalciuric rat intestine also contained greater concentrations of calbindin 9 Kd protein, an abundant enterocyte protein believed to play a role in Ca transport, whose synthesis is regulated by 1,25-$(OH)_2D$ at the genomic level. The accumulation of both intestinal and renal VDR in hypercalciuric rats suggests that the increased receptor number may be more generalized. The above studies strongly suggest that increased intestinal VDR number and normal levels of circulating 1,25-$(OH)_2D$ result in increased functional VDR-1,25-$(OH)_2D$ complexes, which exert biologic actions in enterocytes to increase intestinal Ca transport. Intestinal Ca hyperabsorption in the hypercalciuric rat may be the first example of a genetic disorder resulting from a pathologic increase in VDR.

H. Evidence for Low Spinal Bone Density in Patients with AH.

In the course of carefully evaluating patients with AH, including measurement of bone density at various sites, it became apparent that they had a significant reduction in spinal bone density (see accompanying figure). This trabecular bone loss affecting mainly the spine was subsequently confirmed in a larger study involving 62 patients with AH, but was not seen in 31 non-hypercalciuric stone-formers(45). At first, this observation might seem paradoxical. However, some balance studies in patients with idiopathic hypercalciuria have shown that while these patients exhibit enhanced rates of net intestinal Ca absorption, simultaneous rates of urinary Ca excretion are even greater so that calcium balances are slightly but significantly negative(46,47). Studies of the effects on Ca metabolism of experimentally raising serum 1,25-$(OH)_2D$ in healthy volunteers have shown that during dietary Ca restriction, despite the stimulation of intestinal Ca absorption, calcium balances became more negative because of hypercalciuria(48). Urinary hydroxyproline excretion increased, reflecting increased bone resorption(48). In addition to regulating intestinal Ca absorption, 1,25-$(OH)_2D$ is known to stimulate bone resorption(49) and to inhibit osteoblast proliferation and collagen synthesis(50). In the 23 patients with severe AH studied at the GCRC, mean lumbar bone density was only 84% that of age and sex-matched control subjects (P <.0001) and 6 of these individuals had spinal compression fractures (unpublished observation). Others also have noted diminished spinal bone density in patients with AH(51). Bone biopsy examination in patients with AH has revealed normal or increased osteoclastic resorption with impairment in bone formation(52,53). Therefore, patients with AH may have a generalized disorder of Ca homeostasis also involving bone, and to the extent that 1,25-$(OH)_2D$ may be contributing to these abnormalities, restoration of normal serum 1,25-$(OH)_2D$ levels should be beneficial.


BONE DENSITY IN PATIENTS WITH ABSORPTIVE HYPERCALCIURIA (N=23)
% Age- and sex-matched Normal Bone Density
120
100
80
60
40
*
*
*p < 0.05
RADIUS (Distal 1/3)
SPINE (L2-4)
HIP (Fem Neck)

I. Future Studies in AH

Protocol 456-9-91: A safe physiological and physicochemical correction of absorptive hypercalciuria with slow-release neutral potassium phosphate (Urophos-K).

Background: What is clearly needed is a drug which is directed at ameliorating or correcting the underlying disturbance in AH. Thus, in the vitamin D-dependent form of AH, an agent which inhibits $1,25\text{-}(OH)_2D$ synthesis may correct both increased Ca absorption and the spinal osteopenia. One potential candidate is orthophosphate. This treatment has been shown to reduce serum $1,25\text{-}(OH)_2D$ and urinary Ca(54) and to enhance inhibitor activity against the crystallization of Ca salts by increasing urinary pyrophosphate.

A. Problems with current orthophosphate preparations:
   1. Diarrhea
   2. Modest decline in urinary Ca
   3. Lack of demonstration of reduced intestinal Ca absorption
   4. Increased urinary saturation of brushite ($CaHPO_4.2H20$)
   5. Soft tissue calcification, renal functional deterioration and parathyroid stimulation.

B. Urophos-K
   1. Designed to obviate the above problems
   2. Consists of K-Phosphate in wax matrix for slow-release
   3. Each tablet contains 155 mg P, 8 meq K
   4. Anticipated dosage: 8 tablets daily

Aims:

1. Physiological Correction

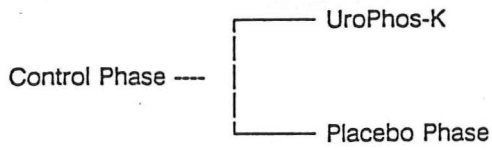
The hypothesis to be tested is that Urophos-K treatment would reduce $1,25\text{-}(OH)_2D$ synthesis or correct the upregulated state of the calcitriol receptor, lower intestinal Ca absorption and urinary Ca, and stimulate bone formation.

Methods:

Aims 1 and 2. Physiological and Physicochemical Correction

N=40 adult patients of either sex with AHI

Table 2. Schemes and Tests for Aims 1 and 2



Tests at Each Phase:

1. Physiology
   Serum Ca, P, Alkϕ, PTH, osteocalcin, procollagen, 25-OHD, calcitriol, ^{47}Ca absorption, fast & load, urinary Ca, hydroxyproline, deoxypyridinoline, bone density (radial shaft, L2-L4, femur).
2. Physicochemistry
   Urinary stone risk factors, saturation (AP and undissociated uric acid), inhibitor activity (FP and [Tm]), P_2O_7.
3. Gastrointestinal Tolerance

6. Idiopathic Osteoporosis

A. Comparison of Idiopathic Osteoporosis to AH

Idiopathic osteoporosis represents osteoporosis occurring in young men and women without secondary cause of bone loss, lack of sex steroids or elderly state. An impaired bone formation due to defective osteoblastic function was recently identified in such patients by our group(55,56). Hypercalciuria and increased intestinal Ca absorption, invariant features of AH, are frequently encountered in idiopathic osteoporosis. Increased intestinal ^{47}Ca absorption was found in about one third of patients with idiopathic osteoporosis(55,56). Approximately two-thirds of osteoporotic patients had an exaggerated calciuric response to a high calcium load (1g)(55,56). As in patients with AH, serum 1,25-$(OH)_2$D was increased in about one-third of patients with idiopathic osteoporosis.

The common denominators affecting both AH and idiopathic osteoporosis would therefore appear to be intestinal hyperabsorption of Ca and osteoblastic suppression. If one etiologic agent were responsible, the likely candidate would be 1,25-$(OH)_2$D. The finding of increased serum 1,25-$(OH)_2$D in

a minority of patients need not exclude calcitriol excess, since 1,25-$(OH)_2D$ is well-known to "upregulate" its receptor(42). As discussed earlier, 1,25-$(OH)_2D$ is not only a major stimulus of intestinal Ca absorption, but it has been shown to impair bone formation and collagen synthesis during long-term use(50,57).

Protocol 452-6-91: Are absorptive hypercalciuria and idiopathic osteoporosis "upregulated" states of the 1,25-$(OH)_2D$ receptor?

	Absorptive Hypercalciuria	Idiopathic Osteoporosis
Hypercalciuria	Yes	Frequent
Increased Intes Ca Abs	Yes	Frequent
Kidney Stones	Yes	Occasional
Decreased Spinal Bone Density	Frequent	Yes
Spinal Compression Fractures	Occasional	Yes
Decreased Bone Formation	Yes	Yes
Increased Serum 1,25-$(OH)_2D$	Frequent	Frequent

B. Future Studies in AH and Idiopathic Osteoporosis

Hypothesis: Certain patients with absorptive hypercalciuria or idiopathic osteoporosis may have an "up-regulation" of the 1,25-$(OH)_2D$ receptor.

Methods: Twenty-four (24) patients with absorptive hypercalciuria, 24 patients with idiopathic osteoporosis and 24 age and sex-matched normal subjects will be characterized with respect to calciotropic hormones, bone markers, bone density and intestinal calcium absorption. The fibroblasts grown from skin biopsies will be examined for:

(1) mRNA coding for 1,25-$(OH)_2D$ receptor

(2) Number and affinity characteristics of 1,25-$(OH)_2D$ receptors

(3) Stability of calcitriol receptor

(4) Ability of exogenous 1,25-$(OH)_2D$ to induce synthesis of 24,25-$(OH)_2D$

B. Future Studies in AH and Idiopathic Osteoporosis

7. Vitamin D Intoxication(1)

A.

Available Vitamin D Metabolites and Analogues

COMPOUND	NAME		RECOMMENDED DOSE	BIOLOGIC HALF-LIFE
	Generic	*Commercial*		
Vitamin D_2	Ergocalciferol	Calciferol	50,000–500,000 IU	15+ days
25-Hydroxyvitamin D_3	Calcifediol	Calderol	20–100 μg daily	15+ days
Dihydrotachysterol	—	Hytackerol	0.2–1.0 mg daily	1–2 days
1-alpha-Hydroxyvitamin D_3	Alpha-Calcidiol	Alpha-One	1–2 μg daily	1–2 days
1,25-Dihydroxyvitamin D_3	Calcitriol	Rocaltrol	0.25–1.0 μg daily	15 hours

B.

Laboratory Tests Helpful in the Differential Diagnosis of Hypercalcemia and Hypercalciuria

DISEASE	SERUM				URINE
	Ca	*iPTH*	*25*	*1,25*	*cAMP*
Primary hyperparathyroidism	↑	N-↑	N	N-↑	↑
Cancer					
Solid tumors	↑-↑↑	↓	N	↓	N-↑
Myeloma	↑-↑↑	↓	N	↓	N-↓
Lymphoma	↑	↓	N	↓-N-↑	N-↓
Granulomatous diseases	↑	↓	N	N-↑	N-↓
Intoxication with:					
D,25-D	↑	↓	↑	N	N-↓
1,25-D, 1-alpha-D	↑-↑↑	↓	N	↑	N-↓
DHT	↑-↑↑	↓	N	N	N-↓

C. Hypervitaminosis D Associated With Drinking Milk

Vitamin D has been added to milk in the United States since the 1930s to prevent rickets. Recently, an unusual occurrence of eight cases of vitamin D intoxication in Boston appears to have been caused by excessive vitamin D fortification of dairy milk(58). Two patients with unexplained vitamin D intoxication were described independently at a weekly interhospital endocrine conference in the fall of 1990. Six additional patients were then identified by physicians who had attended the conference. This report illustrates the importance of discussing cases that do not appear to have an explanation in a hospital or interhospital conference.

The patients, who ranged in age from 15 mos to 82 yr presented with symptoms typical for hypercalcemia: weakness, fatigue, altered mental status, anorexia, weight loss, constipation, irritability and vomiting. Some presented with bone pain and osteoporosis, and one had hypercalcemia detected on a routine chemical screen. Renal ultrasound showed nephrocalcinosis in one patient. Of the eight patients, seven had hypercalcemia (Sca 12.6±2.1 mg/dl), and one just had hypercalciuria. Serum 25-(OH)D levels were elevated at 293±174 ng/ml (normal 10-50 ng/ml), but 1,25-$(OH)_2$D levels were generally normal, and serum PTH_{IRMA} was usually suppressed. The

cases were clearly caused by vitamin D toxicity, but from what source?

The search for a common vehicle in these cases lead to a review of each patient's dietary history (with use of a questionnaire) that in no instance revealed the ingestion of an unusual type or amount of food. All eight patients however, drank milk produced by a local dairy in amounts ranging from 1/2 to 3 cups daily. According to Federal regulations, fortified milk should contain 400 IU of vitamin D per quart. Analysis of the dairy's vitamin D-fortified milk revealed concentrations of vitamin D_3 (cholecalciferol) that ranged from undetectable to as high as 232,565 IU per quart. In adults, it is estimated that continued ingestion of 60,000 units per day may cause intoxication.

Concentration of Vitamin D_3 in Milk from a Local Dairy.*

Type of Milk	Vitamin D_3	
	April 1991	June 1991
	IU/quart	
Raw	49	110
Nonfat	44,576	64,328
Low fat	29,250	795
Nonhomogenized	189	42
Homogenized whole	232,565	<40

*Vitamin D-fortified milk in Massachusetts should contain 400 to 500 IU per quart.[5] There are 946 ml in 1 quart.

The fortification of milk with vitamin D has substantial benefits in terms of preventing rickets and osteomalacia, but the potentially toxic side affects of excessive ingestion are equally well established and mandate careful monitoring of vitamin D-fortified foods.

The problem is not confined to Boston. In a screening survey, HPLC was used to measure vitamin D in samples of 13 brands of milk and five brands of infant formula purchased at random from local supermarkets in five Eastern States(59). Only 30% of the milk and none of the infant formula contained 80-120% of the amount of vitamin D stated on the label (60% of the milk contained less and 10% contained more vitamin D than labelled). Seventy percent of the infant formula contained more than 200% of the stated amount of vitamin D. Since both underfortification and overfortification are hazardous, better monitoring of the fortification process is needed(58-60).

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