

Pathophysiology of Spontaneous Hypercalciuria in Laboratory Rats

Role of Deranged Vitamin D Metabolism

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Abstract

Recent data suggest a causal role of deranged $1,25(\text{OH})_2\text{D}$ metabolism in the syndrome of idiopathic hypercalciuria. To test this hypothesis, we evaluated if vitamin D availability and/or increased serum $1,25(\text{OH})_2\text{D}$ were critical for the expression of hypercalciuria in laboratory rats. Ca balance, serum 25OHD_3 , and $1,25(\text{OH})_2\text{D}_3$ were studied in D-deprived (-D) and D-repleted (+D) male progeny (p) born to normocalciuric (NC) and spontaneously hypercalciuric (SH) rats. 7 of the 14 pSH and 2 of 21 pNC had SH, which was defined as urinary Ca greater than two standard deviations above the mean of values for control animals on days 5 and 6 of a low Ca +D diet (1.19 vs. 0.58 mg/d, $P < 0.001$). Fasting serum Ca and 25OHD_3 were similar to control. Serum $1,25(\text{OH})_2\text{D}_3$ was elevated in these nine SH rats (232 vs. 145 pg/ml, $P < 0.005$). However, during vitamin D deprivation, their Ca excretion was also increased (1.53 vs. 0.45 mg/d, $P < 0.001$), despite comparably reduced serum $1,25(\text{OH})_2\text{D}_3$ (102 vs. 106 pg/ml) and undetectable serum 25OHD_3 . Net intestinal Ca absorption on a low Ca diet was comparable during D repletion (-0.75 vs. -0.82 mg/d) or D deprivation (-0.80 vs. -2.15 mg/d), excluding primary hyperabsorption as the mediator of the hypercalciuria. Mild hypophosphatemia was present in SH on +D (5.8 vs. 6.9 mg/dl, $P < 0.005$) and -D diets (6.2 vs. 7.9 mg/dl, $P < 0.005$), and was associated with higher rates of cyclic adenosine monophosphate excretion (32.8 vs. 26.9 and 48.5 vs. 41.0 nmol/mg of creatinine, respectively).

Spontaneous hypercalciuria is therefore dissociable from increased Ca absorption, serum levels of 25OHD_3 , or $1,25(\text{OH})_2\text{D}_3$. The data are most compatible with the hypothesis of a renal Ca leak which stimulates parathyroid hormone activity and increases serum $1,25(\text{OH})_2\text{D}_3$, if provided adequate 25OHD_3 as substrate.

Introduction

Despite recent insights into the syndrome of idiopathic hypercalciuria (IH)¹ (1-28), it remains an unsettled issue as to

whether IH results from a renal tubular leak, a primary enhancement of intestinal absorption of Ca, or a defect in the regulation of $1,25(\text{OH})_2\text{D}$ production. Thus, the renal leak theory is not supported by the frequent normal (5, 12, 15) or suppressed (4, 8, 10, 14, 19) parathyroid hormone levels. Urinary cAMP and fasting urine Ca to creatinine ratio (2, 19, 25) were elevated in only a minority of the IH patients (5, 8, 19). Likewise, a primary intestinal hyperabsorption could not explain urine Ca excretion in excess of net Ca absorption on a low Ca diet (10, 11, 27) or the increased renal Ca clearance rate after an overnight fast (5).

Recently, it was hypothesized that the primary defect may reside in the disordered regulation of $1,25(\text{OH})_2\text{D}_3$ production (1, 10, 14), as suggested by the absolutely or relatively elevated serum $1,25(\text{OH})_2\text{D}_3$ levels in these patients (4, 10, 13, 14, 21, 23, 25, 28). When Ca intake is adequate, hyperabsorption predominates, and is expressed essentially as absorptive hypercalciuria. When Ca intake is low, increased bone resorption produces a picture similar to renal hypercalciuria (29, 30) which is associated with negative Ca balance (30). Patients with immobilization and displaying resorptive hypercalciuria (31) are characterized by low to normal PTH and urinary cAMP, fasting hypercalciuria, and normal serum Ca, which is similar to many IH patients (5, 10, 11).

We reasoned that if the hypercalciuria is due to an increased serum $1,25(\text{OH})_2\text{D}_3$, it should be abolished if $1,25(\text{OH})_2\text{D}_3$ can be normalized by restricting its substrate, 25OHD_3 . Vitamin D deprivation is only feasible in laboratory animals. Rats with spontaneous hypercalciuria (SH), recently described (32) and investigated by us (33), provide a useful model, since the hypercalciuria is inheritable (17, 24, 33) and it is associated with fasting normocalcemia (33), proximal tubular dysfunctions (33), parathyroid hormone (PTH)-independent defects in the reabsorption of Ca (33) and PO_4 (34), as well as normal response to thiazide (33) orthophosphate and PTH (35). The 7th generation normocalciuric (NC) and SH Wistar rats were therefore subject to the following studies to evaluate the role of deranged vitamin D metabolism.

Methods

Effects of vitamin D deprivation. Male progeny (p) born to the 7th generation NC ($n = 21$ from four crosses) and SH rats ($n = 14$ from three crosses) were weaned from their parents, placed in individual metabolic cages, and housed in special animal facilities devoid of sun or fluorescent light. They were fed a synthetic, vitamin D-deficient, Ca-deficient, and PO_4 -deficient metabolic diet (ICN, Cleveland, OH) supplemented to 1.8% Ca (with CaCO_3) and to 0.8% P (with sodium PO_4 , 4:1 dibasic to monobasic forms). In brief, the diet was normal with respect to all minerals, vitamins, and nutrients except for the absence of vitamin D (D-deprived diet). The generous Ca supplement during the D-deprived phase was designed to prevent the development of secondary hyperparathyroidism (36, 37). After 5 wk of D-deficient

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1. *Abbreviations used in this paper:* IH, idiopathic hypercalciuria; NC, normocalciuric; p, progeny; PTH, parathyroid hormone; SH, spontaneously hypercalciuric.

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diet, diet Ca and P were reduced to 0.5 and 0.6%, respectively, for 6 d, over the last 4 d of which urine was collected daily for Ca determination as previously described (38). Immediately thereafter, diet Ca was reduced to 0.007% (by analysis of six different batches of the same lot of food 0.0067 ± 0.0018 g%) by deleting the CaCO_3 supplement, leaving P (0.6%) and vitamin D (absent) unchanged. Over the ensuing 6 d, urine (in acidified vials), stool, and food aliquots were collected daily to determine Ca excretion, absorption, and retention as previously detailed (38). At 7 p.m. on day 7, the animals were fasted with H_2O ad lib. The next morning, they were bled by retro-orbital puncture, as previously described (33, 38), for measurements of Ca, 25OHD_3 , and $1,25(\text{OH})_2\text{D}_3$ under light ether anesthesia.

Effects of vitamin D repletion. Immediately after bleeding, they were fed for 4 d with a pellet form of regular rat chow (Ralston Purina Co., St. Louis, MO) that contained 5.6 IU of vitamin D_3 , 1.2% Ca, and 0.8% P, before resuming for 4 wk the ICN pulverized synthetic diet containing 0.87% Ca and 0.60% P, now supplemented with 2.2 IU of vitamin D_3 . The pelleted chow was necessary to prevent ingrown canine teeth that develops with the chronic exposure to the pulverized diet. During the 5th wk of the vitamin D repletion, urine was collected for two consecutive days, followed by full balance studies over an 8-d period, during which diet Ca was again deleted as P remained constant at 0.6%. For reasons that could only be attributed to lot differences, the Ca deficiency diet for this period was found by analysis of 10 separate batches to be 0.0170 ± 0.0025 g%.

After an overnight 14-h fast, the animals were bled (3 ml each) as described previously (33, 38) for measurements of serum Ca, 25OHD_3 , and $1,25(\text{OH})_2\text{D}_3$. Urine Ca was determined daily. Stool and food aliquots were digested for Ca analysis as previously described (38). Spontaneous hypercalciuria, defined as previously described, refers to Ca excretion 2 SD above the mean of the normal on days 5 and 6 of the low Ca, D-repleted diet in the absence of hypercalcemia (33). Urine cAMP was determined on days 5 and 6 of the low Ca diet during both the vitamin D-deprived and repleted phases.

Analyses and calculations. Ca in sera and urine were determined by previously published methods (33, 38). Urine cAMP was measured by radioimmunoassay using a New England Nuclear (Boston, MA) kit as previously described (33, 38). Serum 25OHD_3 and $1,25(\text{OH})_2\text{D}_3$

were determined by radioreceptor assay previously described (39). All data were subjected to statistical analyses using *t* tests, paired or unpaired, whichever was appropriate. A *P* value of ≤ 0.05 was considered significant. When urine Ca from more than 1 d of a given group was compared with that of another group, two-way analysis of variance was performed (40). Unless otherwise stated, all data are expressed and presented as mean \pm SEM.

Results

Effects of vitamin D deprivation on the expression of spontaneous hypercalciuria (Table I). As a group, on both a normal (0.5%) and low (0.007%) Ca diet, vitamin D-deprived progeny of SH rats excreted significantly more Ca than similarly treated progeny of NC (Table I). Body weight was not different between pSH and pNC rats either at the time of weaning (50.4 ± 1.7 vs. 53 ± 1.3 g), at the beginning (154 ± 5 vs. 156 ± 6 g), or at the end of the balance study (219 ± 11 vs. 220 ± 8 g). Similarly, after 5 wk of D repletion, urine Ca of progeny of SH rats exceeded that by progeny of NC rats, whether they were fed a normal (0.87%) or a low (0.017%) Ca diet (Table I). Body weight was also comparable, both at the beginning (331 ± 15 vs. 308 ± 7 g) and at the end of the balance studies (361 ± 15 vs. 355 ± 7 g) during vitamin D repletion. Two pups from the NC parents and seven from the SH parents were hypercalciuric by the prospective definition as previously described (33). Since none were hypercalcemic (Table II), these nine rats will hereafter be labeled as SH rats, in contrast to the remaining 24 NC rats (one rat died and another became anorexic and was excluded from further analysis). On a D-deficient diet, these 9 SH rats were also hypercalciuric, which indicated that the expression of this biochemical defect was not dependent on the adequacy of 25OHD_3 . Except for one rat with borderline high Ca excretion on the D-repleted diet (urine Ca = 0.82 mg/d), which was frankly hypercalciuric

Table I. Ca Excretion in Response to a Low Ca Diet in Progeny of NC and SH Rats

Diet Ca = 0.5%					Diet Ca = 0.007%					
Days	1	2	3	4	1	2	3	4	5	6
Vitamin D deprivation										
pNC (n = 21)	2.6 ± 0.1	2.1 ± 0.3	1.8 ± 0.3	1.8 ± 0.32	1.3 ± 0.2	0.94 ± 0.16	0.83 ± 0.13	0.75 ± 0.10	0.66 ± 0.13	0.49 ± 0.05
F ratio = 186, <i>P</i> < 0.00001*					F ratio = 102, <i>P</i> < 0.0001*					
pSH (n = 14)	5.8 ± 1.1	5.1 ± 1.3	4.2 ± 0.8	3.6 ± 0.6	2.5 ± 0.6	2.6 ± 0.8	2.0 ± 0.6	1.5 ± 0.4	1.2 ± 0.3	0.9 ± 0.2
Diet Ca = 0.87%										
Vitamin D repletion										
pNC (n = 21)	—	—	2.28 ± 0.27	2.89 ± 0.40	1.41 ± 0.21	1.02 ± 0.13	0.97 ± 0.13	0.73 ± 0.07	0.66 ± 0.08	0.62 ± 0.05
F ratio = 20.9, <i>P</i> < 0.0001*					F ratio = 7.17, <i>P</i> < 0.001*					
pSH (n = 12‡)	—	—	3.45 ± 0.40	4.20 ± 0.47	2.25 ± 0.37	1.35 ± 0.23	1.47 ± 0.21	1.13 ± 0.15	0.98 ± 0.12	0.76 ± 0.06

* *P* values denote statistical comparison between pNC and pSH for a given vitamin D status. ‡ One rat was anorexic, and one rat died from bleeding after the D-deprived phase; both are excluded in Table I.

Table II. Ca Metabolism in NC and SH Rats on a Low Ca Diet*

	Ingested Ca	Fecal Ca	Net Ca absorbed	Urine Ca	Retained Ca
	mg/d	mg/d	mg/d	mg/d	mg/d
Vitamin D deprivation					
NC	0.93	3.03	-2.15	0.54	-2.70
(n = 24)	±0.04	±0.45	±0.46	±0.07	±0.48
P value‡	NS	NS	NS	<0.001	NS
SH	1.12	1.93	-0.80	2.00	-2.83
(n = 9)	±0.05	±0.44	±0.41	±0.45	±0.80
Vitamin D repletion					
NC	3.20	4.03	-0.82	0.70	-1.47
(n = 24)	±0.08	±0.48	±0.47	±0.04	±0.26
P value‡	NS	NS	NS	<0.001	NS
SH	3.45	4.20	-0.75	1.38	-2.13
(n = 9)	±0.10	±0.85	±0.81	±0.11	±0.87

Mean±SE. * Balance data from days 3–6 of the diet. ‡ P values refer to statistical comparison between NC and SH rats for a given vitamin D status.

during D deprivation (urine Ca = 0.95 mg/d), all the 23 NC rats remained normocalciuric, regardless of whether they were vitamin D deprived or repleted.

Effects of vitamin D deprivation on Ca absorption and retention (Table II). Fecal Ca elimination on the first 2 d of the low Ca diet, analyzed separately, reflected residual unabsorbed Ca that was ingested previously as part of the normal Ca diet. They were therefore excluded from consideration of true Ca absorption during the more steady state of the subsequent days. From days 3–6 of the low Ca, D-repleted diet, net Ca absorption was not different between the NC and SH rats (Table II). Hence, the increased Ca excretion in SH was not a consequence of increased gastrointestinal absorption. Furthermore, urine Ca in each of the nine SH rats exceeded the corresponding net Ca absorption, which argued against the intestine as the source of the extra excreted Ca.

During vitamin D deprivation, fecal Ca exceeded ingested Ca even beyond the 2nd d of the low Ca diet, so that net Ca absorption became negative in most animals. The apparent Ca secretion was not different between the SH and NC rats (Table II). The tendency for the absolute value to be greater for NC rats was due to four animals which secreted an average of 5.5–9 mg Ca/d. Over the range of net Ca absorption, which spanned from -4 to 1 mg/d, where SH and NC rats overlapped (i.e., excluding these four data points), analysis indicated that the hypercalciuria in SH was not due to less severe Ca malabsorption. This impression is supported by the absolute value of Ca excretion in each of the nine SH rats, which was far in excess of the individual value for both the ingested Ca and net Ca absorbed. These data therefore exclude primary hyperabsorption as the pathogenetic mechanism for the SH.

Effects of D deprivation on serum 25OHD₃ and 1,25(OH)₂D₃ in SH (Table III). Serum Ca after an overnight fast was not significantly different between the NC and SH rats during either vitamin D deprivation or repletion. The absence of a difference is probably due to three factors. First, as indicated by the balance data (Table II), Ca retention between the two groups differed by only 0.13 mg/d (-2.83 vs. -2.70) in D deprivation and by only 0.66 mg/d (-2.13 vs. -1.47) in D repletion. Over the course of 6 d of balance studies, the

cumulative difference was therefore <1 and 4 mg, respectively. These were clearly too small a difference in retention to be reflected in the serum Ca between the NC and SH. Second, we did not have enough blood for ionized Ca (and necessarily simultaneous pH for proper interpretation) when the key variable of interest was the vitamin D metabolite. That would have been more sensitive and perhaps more revealing than the total serum Ca. Finally, in these intact animals, increased PTH secretion could have partially offset the tendency for the serum Ca to be reduced in the SH rats.

Serum PO₄ was lower in the SH rats for both D-deprived and repleted phases. Coupled with our preliminary data suggesting increased renal PO₄ excretion rates (34), and the increased cAMP excretion in the present male (Table III) and in previously published female SH rats (33), the hypophosphatemia is best interpreted as a reflection of renal PO₄ wastage secondary to increased PTH activity.

Serum 25OHD₃ measured in eight SH rats and eight NC rats was comparable after 5 wk of D-repleted diet. Serum 1,25(OH)₂D₃ was, however, significantly increased in the SH rats (Table III), resembling human IH (4, 10, 13, 14, 21, 23, 25, 28). The elevated 1,25(OH)₂D₃ could either be primary (and be responsible for the hypercalciuria) or secondary as a compensatory event to the hypercalciuria. Urinary cAMP excretion was increased (Table III), consistent with the notion of stimulated PTH activity and suggestive of a homeostatic linkage to the increased serum 1,25(OH)₂D₃. These findings are therefore incompatible with resorptive hypercalciuria mediated by a vitamin D-independent mechanism, such as immobilization, since, in this disorder, the PTH-endocrine system should be suppressed (31).

During vitamin D deprivation, as expected, serum 25OHD₃ was undetectable (≤2 ng/ml), and was equally so in both SH and NC rats, confirming the status of substrate depletion in our preparation. More importantly, serum 1,25(OH)₂D₃ measured in seven specimens pooled from SH rats and eight specimens pooled from NC rats was no longer different between SH and NC rats, since their values were comparably reduced (Table III). A similar range of 1,25(OH)₂D₃ (92±16 pg/ml) was recently reported in young rats after 6 wk of vitamin D

Table III. Effects of Vitamin D Deprivation on Ca Excretion and Other Metabolic Parameters of NC* and SH* Rats

Status of vitamin D	Deprived × 6 wk				Repleted × 5 wk			
	0.50		0.007		0.87		0.017	
	NC	SH	NC	SH	NC	SH	NC	SH
Serum‡ Ca (mg/dl)	—	—	10.8 ±0.1	11.0 ±0.1	—	—	10.7 ±0.1	10.9 ±0.3
			NS				NS	
Serum‡ P (mg/dl)	—	—	7.9 ±0.2	6.2 ±0.4	—	—	6.9 ±0.3	5.8 ±0.2
			P < 0.005				P < 0.005	
Serum‡ 25OHD ₃ (ng/ml)	—	—	Undetectable		—	—	16.7 ±0.7	17.9 ±0.8
							NS	
Serum‡ 1,25(OH) ₂ D ₃ (pg/ml)	—	—	102 ±6	106 ±6	—	—	145 ±10	232 ±17
			NS				P < 0.001	
Urine§ Ca (mg/d)	1.52 ±0.20	5.71 ±0.60	0.45 ^{¶¶} ±0.04	1.53 ^{¶¶} ±0.32	2.46 ±0.26	4.87 ±0.58	0.58 ^{¶¶} ±0.03	1.19 ^{¶¶} ±0.08
	P < 0.001		P < 0.001		P < 0.001		P < 0.001	
Urine§ cAMP/creatinine (nmol/mg)	—	—	41.0 ^{¶¶} ±1.3	48.5 ^{¶¶} ±1.9	—	—	26.9 ±1.0	32.8 ±1.9
			P < 0.03				P < 0.05	

NC rats ($n = 24$); SH rats ($n = 9$). P values given refer to statistical comparison between NC and SH for the parameter tabulated immediately above. * 2 of the 21 progeny from NC parents and 7 of the 12 progeny from SH parents were hypercalciuric as defined in text. ‡ Serum from overnight fast after 6 to 8 d of low Ca diet. § Urine from days 5 and 6 of the low Ca diet. ¶¶ $P < 0.001$ compared with the normal Ca diet within a given group and for a given vitamin D status. ¶¶ $P < 0.02$ compared with the D-repleted low Ca diet within a given group.

deprivation but supplemented throughout with a high Ca diet (36). Since the SH rats, identified from screening by a D-repleted diet, had always been hypercalciuric even in D deprivation (Table III), the dissociation between increased Ca excretion and increased serum 1,25(OH)₂D₃ strongly argued against a cause-and-effect relationship between these two phenomena. Thus, the hypercalciuria was independent of not only 25OHD₃ availability, but also of serum 1,25(OH)₂D₃ levels, contrary to the predictions by the hypothesis of a disordered regulation of vitamin D metabolism. Cyclic AMP excretion was again increased in SH rats (Table III), a finding incompatible with resorptive hypercalciuria (31).

Discussion

Mounting evidence suggests that if IH is a pathogenetically homogeneous syndrome, it cannot be adequately explained by either a primary hyperabsorption or an intrinsic tubular transport defect. One recent hypothesis suggests a basic abnormality in the regulation of 1,25(OH)₂D₃ synthesis (1, 14). Anatomically, a single lesion in the proximal tubule of the kidney could theoretically account for not only the disordered 1,25(OH)₂D₃ production, but also the exaggerated excretion rates of a host of solutes (15) normally handled by the proximal tubule, like Na (5), and PO₄ (5, 14, 20, 22).

We therefore used the spontaneously hypercalciuric rats to test this hypothesis. The low Ca diet protocol (containing 1–4 mg/d) was employed to facilitate the identification of hyper-

calciuria (33) by reducing the inherent variability in Ca excretion (Table I), to minimize contamination of urine by food and feces and to mimic the Ca restricted condition under which previous observations implying disordered 1,25(OH)₂D₃ synthesis were made (10, 14, 25). Additionally, a low Ca diet would facilitate the unmasking of resorptive hypercalciuria by minimizing the influx of intestinal Ca without obscuring hyperabsorption, since from 1 to 4 mg of Ca was still ingested daily (Table II).

Based on the previous definition of SH (33–35), a total of 9 SH male rats were found. Four potential mechanisms could mediate the hypercalciuria: (1) hyperabsorption from intrinsic intestinal abnormalities; (2) increased bone resorption; (3) increased 1,25(OH)₂D₃, producing a hybrid of increased absorption and enhanced resorption; and (4) intrinsic tubular Ca leak. Compared with the NC rat fed the same vitamin D-replete diet, net Ca absorption in SH rats was normal, and comparably negative with Ca restriction (Table II), mitigating against the role of primary hyperabsorption.

Although superficially there appears to be no change in net Ca absorption with a change in the vitamin D status (Table II), the comparison was not meaningful because of the differences in age (6 wk) and protocol (the insertion of the 0.5% Ca diet between the high [1.8%] and low [0.007%] Ca diets for only the D-deprived phase). When diet Ca was adequate (0.5–0.87%), net Ca absorption was significantly increased by D repletion (53.6±5.3 vs. 14.4±4.9 mg/d in SH and 55.9±6.1 vs. 15±3.5 mg/d in NC rats). This increase was

also evident in fractional terms (33.4 ± 3.2 vs. $16.4 \pm 6.4\%$ in SH and 34 ± 1.4 vs. $17.6 \pm 4.0\%$ in NC rats).

Despite the documented increase in serum $1,25(\text{OH})_2\text{D}_3$ in the D-repleted SH rats, Ca absorption was not significantly increased compared with the NC rats. These data, however, do not necessarily contradict each other, since the serum measurement was performed on day 7 of the low Ca diet after the balance studies. Based on the current concepts on resorptive hypercalciuria (31), PTH and cAMP should be suppressed by the increased serum $1,25(\text{OH})_2\text{D}_3$ in SH rats. The high serum $1,25(\text{OH})_2\text{D}_3$ and the increased urinary cAMP excretion (Table III) argue against the role of a primary increase in bone resorption. However, the increased serum $1,25(\text{OH})_2\text{D}_3$ was still compatible with a basic disturbance in $1,25(\text{OH})_2\text{D}_3$ production.

The hypercalciuria identified during vitamin D repletion was not abolished by vitamin D deprivation, at a time when serum 25OHD_3 was undetectable and serum $1,25(\text{OH})_2\text{D}_3$ levels were as low as the NC rats (Table III). Hence, SH is independent of vitamin D availability and serum 25OHD_3 . More importantly, the dissociation between the increased Ca excretion and increased serum $1,25(\text{OH})_2\text{D}_3$ documented here offers no support for a pathogenetic role of this metabolite in the generation of the hypercalciuria.

The cumulative data in the rat are therefore best interpreted by the alternate hypothesis of a renal Ca leak (33). In this view, the tubular defect produces negative Ca balance, stimulates PTH, and elicits a compensatory increase in $1,25(\text{OH})_2\text{D}_3$ production. When substrate was restricted, $1,25(\text{OH})_2\text{D}_3$ was no longer elevated in SH rats, despite a similar setting of increased PTH activity, as reflected by cAMP excretion (Table III).

If SH in rats were pathophysiologically akin to idiopathic hypercalciuria in man, our findings would suggest that the increased serum $1,25(\text{OH})_2\text{D}_3$ in these patients is a homeostatic response secondary to the hypercalciuria. The response to thiazide, of at least the renal hypercalciuric patients, with correction of their high urine Ca, high serum $1,25(\text{OH})_2\text{D}_3$, and intestinal hyperabsorption (25), is perfectly compatible with this notion. It is interesting to note that $25(\text{OH})\text{D}_3$ treatment of IH patients characterized by suppressible increases in serum PTH (22) did not aggravate their hypercalciuria. Similarly, high doses of cholecalciferol did not elicit a difference in the calciuric response between normal and hypercalciuric patients (27), as would be predicted if disordered control of $1,25(\text{OH})_2\text{D}_3$ production were the primary defect.

In summary, data from the SH rats suggest that their hypercalciuria is more likely the cause than the consequence of the increased serum $1,25(\text{OH})_2\text{D}_3$ levels. In vitro studies on the regulation of $1,25(\text{OH})_2\text{D}_3$ synthesis in these animals may potentially shed light on the genesis of the elevated serum concentration of this metabolite.

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