1,25-Dihydroxycholecalciferol Deficiency: The Probable Cause of Hypocalcemia and Metabolic Bone Disease in Pseudohypoparathyroidism

MARC K. DREZNER, FRANCIS A. NEELON, MARK HAUSSLER,* HARRY T. McPHERSON, AND HAROLD E. LEBOVITZ

The Departments of Medicine and Physiology, Duke University Medical Center, Durham, N.C.; and *The Department of Biochemistry, University of Arizona Medical College, Tucson, Arizona

ABSTRACT. Pseudohypoparathyroidism (PsH) is a genetic disease characterized by hypocalcemia, hyperphosphatemia, and metabolic unresponsiveness to parathyroid hormone (PTH). The administration of PTH elicits neither a significant rise in serum calcium (calcemic response) nor a decrease in the renal tubule reabsorption of phosphorus (phosphaturic response). The diminished phosphaturic response is due to an inability of PTH to generate cyclic AMP in renal tubule cells. We investigated the question of whether hypocalcemia and deficient calcemic response to PTH are due to a similar cyclic AMP defect in bone or to an acquired vitamin D deficiency.

Four patients were studied. The active form of vitamin D (1,25-dihydroxycholecalciferol) was measured in 3 and was low. Treatment with vitamin D restored the serum calcium and the calcemic response to PTH to normal without changing the impaired renal response. Bone biopsy was performed in 2 patients and showed morphologic evidence of increased osteoclastic activity and osteomalacia. The data indicate that the hypocalcemia and bone disease in PsH are due to active vitamin D deficiency, possibly resulting from the genetic renal lesion. (J Clin Endocrinol Metab 42: 621, 1976)

PSEUDOHYPOPARATHYROIDISM (PsH) is a disorder characterized by hypocalcemia, hyperphosphatemia, and deficient calcemic and phosphaturic responses to exogenously administered parathyroid hormone (PTH). These data suggest that the disorder is due to end-organ unresponsiveness to PTH, and this has been confirmed by measurements indicating that serum PTH is markedly elevated. Chase et al. (1) have shown that PTH action on the kidney is mediated through an increase in renal cyclic adenosine 3',5'-monophosphate (cyclic AMP). In PsH the urine cyclic AMP response to exogenous PTH is markedly diminished, consistent with renal refractoriness to PTH. Evidence that bone cells are also innately unresponsive to PTH has been largely from inference, based on the systemic hypocalcemia and a subnormal rise in serum calcium when PTH is given to patients with PsH. We have investigated the question of whether calcemic unresponsiveness in PsH might be an acquired lesion or due to disordered vitamin D metabolism in PsH.

Materials and Methods

Four patients (32, 29, 45, and 32 years old) with PsH were hospitalized in the Duke University Medical Center Clinical Research Unit. Informed consent was obtained for all studies. The patients received a diet containing 1200 mg of phosphorus and 900 mg of calcium/day. Studies requiring hourly urine determinations were preceded by a water load (20 mg/kg), and urine was collected by spontaneous voiding at designated intervals. Bovine parathyroid extract for parenteral use (Eli Lilly Co.) was of proven activity.

Serum calcium, phosphorus, alkaline phosphatase, and creatinine were determined on the multichannel Technicon AutoAnalyzer. Serum magnesium was measured by the method of Thiers (2). Serum parathyroid hormone assays were performed in the laboratories of Dr. Claude Arnaud (3) (Cases 1 and 2) and Dr. John Potts, Jr. (4) (Cases 3 and 4). Serum 25-hydroxycholecalciferol was measured by the methods of Belsey et al. (5) (Case 1) and Haddad and Chyu (6) (Cases 2, 3, and 4). The plasma concentration of 1,25-dihydroxycholecalciferol was measured by the method of Brumbaugh et al. (7) (Cases 1, 2, and
4), and the data are presented as the mean of triplicate determinations of a single blood specimen.

Urine specimens were stored at -20 °C before determination of cyclic AMP (8), phosphorus (9), and creatinine (10). The tubular maximum for reabsorption of phosphate, normalized to glomerular filtration rate (T_m/GFR), was calculated by the method of Bijvoet (11).

Fat was measured in 72 hour fecal collections by the method of Van de Kamer (12). Urinary d-xylose was measured (13) in 5 hour urine specimens after the ingestion of 25 gm of d-xylose. Calcium absorption was calculated by measurement (14) of dietary calcium intake minus fecal calcium excretion, over a 5 day period.

Special studies were undertaken in two patients (Cases 1 and 2). Under general anesthesia, the right anterior iliac crest was biopsied and specimens were preserved in 70% alcohol. Dr. Jennifer Jowsey assessed morphology and performed quantitative analysis on these undecalcified specimens according to methods previously described (15,16).

### Results

**The Diagnosis of pseudohyopoparathyroidism**

All four patients had hypocalcemia and hyperphosphatemia (Table 1), and Cases 2 and 3 showed the typical skeletal abnormalities of PsH described by Albright et al. (17). In addition, all showed the markedly blunted increase in urine cyclic AMP excretion in response to parathyroid extract that is the single best criterion for establishing the diagnosis of PsH (1). These data imply renal unresponsiveness to PTH action. This was confirmed by finding an elevated (>4.8 in all cases) theoretical tubular maximum for the reabsorption of phosphate per liter of glomerular filtrate (T_m/GFR) and, where tested (Cases 1, 2, and 3), an insignificant fall in T_m/GFR (Δ T_m/GFR in Table 1) in response to bovine parathyroid extract.

| Table 1. Metabolic data in 4 patients with pseudohyoparathyroidism |
|-------------------------------------------|----------------|-----------------|-----------------|
|                                          | Calcium (mg/dl) | Phosphorus (mg/dl) | PTH** (%) | 25-HCC** (ng/ml) | 1,25-DHCC** (ng/ml) | ΔT_m/GFR (μmoles/g creatinine) | Δ Ca (mg/dl) |
| Normal values Before treatment           | 8.5-10.5       | 2.4-4.5          | 100       | 19.9-39.9         | 2.6-5.8           | 0.78-1.50                      | 20-500     | >1.0 |
| Case 1                                  | 7.1-8.3        | 4.0-5.0          | 1230      | 18.0*            | 1.1              | 0.72                          | 12.5       | 0.9 |
| Case 2                                  | 6.4-7.1        | 4.5-5.7          | 123       | 25.3             | 0.8              | 0.68                          | 19.1       | 0.9 |
| Case 3                                  | 6.0-6.8        | 5.6-5.9          | 240       | 26.2             | —                | 0.49                          | 9.5        | —   |
| Case 4                                  | 6.0-6.6        | 4.6-4.9          | 403       | 41.7†            | 2.1†             | —                             | 11.2       | 0.8 |
| After treatment with vitamin D          | 8.5-8.7        | 3.6-4.4          | 128†      | —                | —                | 0.50                          | 10.0       | 2.6† |
| Case 1                                  | 8.8-10.2       | 4.2-4.6          | —         | —                | —                | 0.95                          | 2.5        | 1.9† |
| Case 2                                  | 8.9-10.0       | 4.2-4.4          | 87        | —                | —                | 0.82                          | 11.7       | 2.3† |
| Case 4                                  | 9.9-8.6        | 3.2-4.2          | 70        | —                | —                | 0.06                          | 12.2       | 2.5† |

* Maximal observed decrement in T_m/GFR (ΔT_m/GFR) and maximum cyclic AMP excretion after 200 U of intravenous parathyroid extract. ΔCa represents maximal increase in serum calcium in response to 200 U of intramuscular parathyroid extract every 6 hours for 3 days.

**PTH = serum parathyroid hormone measured by different assays with different normal ranges. Results are expressed as per cent increase above uppermost normal concentration. 25-HCC = 25-hydroxycholecalciferol. 1,25-DHCC = 1,25-dihydroxycholecalciferol.

+ Normal range for the assay in this patient is 15-100 ng/ml.
† Ergocalciferol 50,000 U/day had been prescribed for this patient. She took this sporadically and presented hypocalcemic; this compound may interfere with the assay and spuriously elevate this value.
‡ Serum PTH measured with intravenous calcium load (serum calcium 10.8 mg/dl).
§ Response necessitated that the test be ended after a single intramuscular injection of parathyroid extract (200 U).
Calcium dynamics and bone metabolism in pseudohypoparathyroidism

All our patients had hypocalcemia; all had normal serum magnesium concentrations (2.0, 1.8, 2.1, 2.0 meq/l [normal range: 1.7–2.2 meq/l]) and all could acidify urine maximally. The serum PTH concentrations were increased (Table 1). These observations suggest that our patients lacked bone responsiveness to PTH. The administration of bovine parathyroid extract appeared to confirm this inference. In response to 200 U of parathyroid extract given intramuscularly every 6 hours for 72 hours, the patients showed a diminished calcemic response (Table 1; Fig. 1.) Both the magnitude of the response and the late occurrence of the maximal increment in serum calcium are typical of PsH (MacGregor (18) used a rise

![Graph showing calcemic response to parathyroid extract in baseline state and after treatment with ergocalciferol. The graph compares before and after treatment, showing a normal calcemic response to parathyroid extract after treatment.](image-url)
in serum calcium of <1.0 mg/dl to diagnose PsH).

However, two of our patients (Cases 2 and 3) had radiographic evidence of bone demineralization and one (Case 1) had radiographic changes of osteitis fibrosa cystica. We therefore performed iliac crest biopsies on two patients (Cases 1 and 2). In Case 1, sections from the biopsy showed areas of active bone resorption and osteitis fibrosa cystica, as well as widened osteoid seams (Fig. 2). The bony cortices and trabeculae were thin, and the calcification fronts abnormal, being either absent or broad and diffuse. Quantitative analysis (Table 2) confirmed these observations. The bone biopsy from Case 2 likewise showed increased bone resorption and widened osteoid seams (Table 2). However, the assessment of calcification fronts and the surface area of the bone covered by unmineralized osteoid tissue was precluded by a period of bed rest (10 days) before biopsy which resulted in decreased bone formation (19). These results are indicative of increased PTH action on bone. In addition, the widened osteoid seams, as well as the abnormal calcification fronts in Case 1, are compatible with osteomalacia. Serum alkaline phosphatase activity (bone isoenzyme) was increased in Cases 1, 2, and 4, lending further evidence of increased bone turnover.

Vitamin D metabolism in pseudohypoparathyroidism

There was no evidence in the patients’ histories to suggest dietary insufficiency of vitamin D. Fecal fat excretion and d-xylose absorption ruled out malabsorption in the three patients studied (Cases 1, 2, and 4). Since, in man, vitamin D (cholecalciferol) must be activated by hydroxylation to 25-
1,25-DHCC DEFICIENCY IN PsH

TABLE 2. Quantitative microradiography of bone biopsy

<table>
<thead>
<tr>
<th></th>
<th>Formation* (%)</th>
<th>Resorption* (%)</th>
<th>Unmineralized osteoid § (%)</th>
<th>Osteoid width (µm)</th>
<th>Cortical thickness (mm)</th>
<th>Trabecular thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals† (age 25–37 yrs)</td>
<td>2.5 ± 0.56†</td>
<td>3.5 ± 0.70†</td>
<td>0</td>
<td>13.8 ± 1.40†</td>
<td>1.11 ± 0.47†</td>
<td>123.3 ± 15.0†</td>
</tr>
<tr>
<td>Case 1</td>
<td>24.1</td>
<td>7.7</td>
<td>8</td>
<td>20.8 ± 4.60</td>
<td>0.6</td>
<td>105</td>
</tr>
<tr>
<td>Case 2</td>
<td>0.6</td>
<td>5.8</td>
<td>0</td>
<td>16.9 ± 3.80</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

* Percentage of bone surface undergoing formation (or resorption).
§ Percentage of bone surface (in association with bone formation) covered by unmineralized osteoid tissue.
† Unpublished observations of J. Jowsey.
‡ Mean ± SD.

hydroxy-cholecalciferol (25-HCC) and subsequently further hydroxylated to 1,25-dihydroxy-cholecalciferol (1,25-DHCC) (20), we measured the concentrations of these products in several patients. The 25-HCC concentration was normal in three subjects (Cases 1, 2, and 3) and marginally elevated in Case 4, a patient for whom vitamin D had previously been prescribed (see Table 1). Plasma 1,25-DHCC concentration was, however, low in the three subjects studied (Cases 1, 2, and 4) (Table 1). Thus, these patients with PsH are deficient in 1,25-DHCC. We found evidence of functional 1,25-DHCC deficiency in a study of calcium absorption in Case 1. In the untreated state, 231 mg of a 1100 mg daily calcium intake was absorbed (normal values: 325 ± 25 mg (21). After treatment with oral vitamin D₂, 466 mg was absorbed daily.

Treatment with ergocalciferol restores calcemic response

It has long been known that dietary vitamin D deficiency (rickets) creates a state of calcemic unresponsiveness to PTH (22). If the calcemic unresponsiveness in PsH is secondary to 1,25-DHCC deficiency, then treatment with large amounts of ergocalciferol or dihydrotachysterol might be sufficient to repair the deficit in active vitamin D. Accordingly, we treated our patients with sufficient ergocalciferol (50–100,000 U/day) to raise serum calcium into the normal range. The time elapsed from the initiation of therapy to the attainment of a normal serum calcium, and subsequent retesting, ranged from 2 weeks (Cases 1, 2, and 4) to one year (Case 3). Table 1 shows that ergocalciferol therapy raised plasma calcium and lowered plasma phosphorus in all four patients. There was no change in renal phosphaturic or cyclic AMP response to intravenous parathyroid extract (Table 1). However, the calcium response to intramuscular parathyroid extract was markedly enhanced (Table 1 and Figure 1). In every case the increment in serum calcium in response to a single dose of parathyroid extract (200 U im) was 1.9 mg/dl or greater and the response occurred by 8 hours after administration. In each individual the actual serum calcium concentration rose to 10.8 mg/dl or greater, and, therefore, only a single dose of parathyroid extract could safely be administered. To demonstrate that the restoration of the calcemic response was not simply a result of the raised serum calcium concentration found in treated patients, we infused calcium (prior to treatment with ergocalciferol) in two subjects (Cases 1 and 2). When serum calcium had reached steady concentrations, greater than 10 mg/dl in both patients and serum immuno-reactive PTH, measured by amino terminal assay, was suppressed (data not shown), we gave a single dose of 200 U of parathyroid extract by intramuscular injection. In both cases the impaired rise in serum calcium (<0.8 mg/dl) persisted.

Discussion

Since Albright et al. (17) first described PsH it has been recognized that renal unresponsiveness to PTH is the hallmark of this disease. A vigorous and continuing
effort has characterized the biochemical defect at this organ (1,23), and our patients satisfy the criteria of renal unresponsiveness. It is, furthermore, undoubted that patients with PsH have a diminished calcemic response to intramuscular PTH (18). Again, our patients satisfy this criterion. Nevertheless, 20% of the patients with PsH reported by Bronsky et al. (24) had radiographic evidence of osteopenia. Bone biopsies of 7 previously reported patients (25-27) with PsH have shown evidence of increased bone turnover, with or without widened osteoid seams, and 10 patients have been reported to have radiographic evidence of osteitis fibrosa cystica (28,34). It has been difficult to reconcile these observations, as well as the occasional finding of normocalcemia in PsH (35), with the supposition that the bone cells in this disorder are intrinsically incapable of responding to PTH.

The possibility that the calcemic unresponsiveness is secondary to some renal lesion has been entertained previously. Rasmussen (36) outlined a mechanism which postulated osteitis fibrosa cystica and subsequent calcemic unresponsiveness due to hyperphosphatemia, and Frame (37) theorized that disordered Vitamin D metabolism might be involved. Our data demonstrate bone changes (and calcemic unresponsiveness) consistent with impaired vitamin D metabolism and studies in Case 1 showed impaired absorption of calcium, corrected by vitamin D treatment. Although it is not known precisely how the kidney controls the enzymatic hydroxylation of precursor 25-HCC to the active product 1,25-DHCC, PTH (perhaps mediated by cyclic AMP, or a decrease in renal cell phosphate concentration, or both) appears to enhance 1-hydroxylation of 25-HCC (38). Individuals with PsH have two factors possibly impairing activation of vitamin D: they cannot generate renal cyclic AMP in response to PTH, and they have elevated plasma phosphorus. In any case, they have subnormal serum concentration of 1,25-DHCC consistent with an ineffective synthesis of 1,25-DHCC.

All our patients with PsH have acquired calcemic unresponsiveness to PTH, shown by the restoration of this responsiveness following treatment with therapeutic doses of ergocalciferol (Table 1). Studies of patients with nutritional vitamin D deficiency rickets (22) and of animals with induced vitamin D deficiency (39), have demonstrated that active vitamin D metabolites are required for PTH to elicit a calcemic response. The cause of the absent calcemic response to PTH in vitamin D deficiency states has been extensively studied, but the mechanism remains unclear. The major conclusion from both animal and human studies is that in vitamin D deficiency, in spite of clear evidence of hyperparathyroidism, there is failure to mobilize sufficient calcium from bone to maintain calcium homeostasis. The major identifiable defect in the bone appears to be a failure of osteoclastic maturation or differentiation (39). In PsH there is both increased PTH and decreased 1,25-DHCC concentrations, and the lack of a calcemic response to PTH is therefore expected. The absence of a similar defect in patients with idiopathic or surgical hypoparathyroidism, despite similar depressed concentrations of 1,25-DHCC (unpublished observations) has been noted. However, the loss of the calcemic response to PTH has thus far been associated only with conditions in which endogenous PTH concentration is increased and 1,25-DHCC concentration decreased (e.g., vitamin D deficiency rickets; renal failure). Thus, loss of calcemic response appears secondary to the chronic effects of increased endogenous PTH on bone turnover and bone cell activity, coupled with active vitamin D deficiency. The absence of an elevated concentration of endogenous PTH in idiopathic and surgical hypoparathyroidism may therefore preserve calcemic responsiveness. It is reasonable to suggest, however, that the absent osseous response to PTH may be secondary to sustained hypocalcemia. Indeed long-term treatment with calcium sufficient to maintain normocalcemia might suppress PTH and its effects on bone turnover, resulting in the laying down of new calcification fronts in bone and the restoration
of calcemic responsiveness (40). It was not possible to perform such studies in this investigation.

We envisage the sequence of metabolic events in PsH as follows: a genetic inability of the kidney cells to respond to PTH with a rise in cyclic AMP results in an elevated Tm/GFR and a raised concentration of phosphorus in plasma. Furthermore, the kidney is unable to convert 25-HCC to 1,25-DHCC. The lack of active vitamin D3 results, eventually, in calcemic unresponsiveness. In addition, there may be roentgenographic or morphologic evidence of osteomalacia, osteopenia, or osteitis fibrosa cystica depending, perhaps, on the rate of development of the 1,25-DHCC deficiency and the concentration of plasma PTH. (For instance, Case 1 had markedly raised serum 25-OH vitamin D3; to the nurses and staff of the Clinical Research Unit for assistance in the care of these patients, and to Ms. Holly B. Curtis and Ms. Kristine Bursac for expert technical assistance. This study was supported by grants (AM 01324, 5 T1 AM 5074, 1 P 22 AM 02296-01, and AM 15781) from the National Institute of Arthritis, Metabolism, and Digestive Diseases; by grant (MO 1 FR 30) from the Clinical Research Center Branch Division of Research Facilities and Resources, U.S. Public Health Service; and by an American Diabetes Association Research and Development Award.

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Addendum

After submission of our manuscript, Stögman and Fischer (Am J Med 59: 140, 1975) reported a case of PsH in which treatment with vitamin D2 likewise restored calcemic responsiveness. This report is in line with previous reports demonstrating that vitamin D2 therapy improves calcemic responsiveness in PsH (42, 43). The case reports noted present data consistent with our observations in patients with PsH.

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