PLASMA 25-HYDROXYVITAMIN D AND NOT 1,25-DIHYDROXYVITAMIN D IS ASSOCIATED WITH PARATHYROID ADENOMA SECRETION IN PRIMARY HYPERPARATHYROIDISM: A CROSS-SECTIONAL STUDY

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Abstract

Background: Primary hyperparathyroidism (PHPT) is associated with reduced plasma 25-hydroxyvitamin D (P-25OHD) and usually increased plasma 1α,25-dihydroxyvitamin D (P-1,25(OH)2D). Parathyroid tissue expresses the vitamin D receptor and it is thought that circulating 1,25(OH)2D participate in the regulation of parathyroid cell proliferation, differentiation and secretion.

Aim: To investigate the relations between circulating levels of 1,25(OH)2D and 25OHD respectively and parathyroid adenoma weight (AW), plasma-parathyroid hormone (P-PTH) and PTH secretion expressed as P-PTH/AW.

Design: Cross-sectional study.

Material: One hundred and seventy-one consecutive hypercalcaemic caucasian patients aged 19–87 years (median 63, 84% females) with surgically proven parathyroid adenoma.

Results: A weak positive correlation was found between P-25OHD and P-1,25(OH)2D (r=0.24, P<0.005). AW depended on sex and body mass index. Following adjustment, it was correlated positively to P-PTH, calcium (Ca) and alkaline phosphatase (AP) and inversely to plasma phosphate in a multiple regression model. AW was not associated with vitamin D metabolites. Preoperative P-PTH correlated positively to plasma levels of Ca and AP, but inversely to phosphate and 25OHD (P<0.001) levels. P-PTH was not associated with P-1,25(OH)2D (P=0.65). The P-PTH:AW ratio correlated inversely to P-25OHD (P<0.05), but showed no relations to plasma levels of Ca, phosphate or 1,25(OH)2D (P=0.22).

Conclusion: In this material, low levels of 25OHD were related to higher levels of P-PTH and higher PTH:AW ratios in patients with PHPT suggesting that vitamin D deficiency increase PTH secretion activity. Neither PTH secretion nor AW was associated with circulating levels of 1,25(OH)2D.

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Introduction

Parathyroid hormone (PTH) stimulates the renal 25-hydroxyvitamin D-1α-hydroxylase (1), whereas the enzyme activity is reduced by hypercalcaemia, hyperphosphataemia, decreased renal function and excess 1α,25-dihydroxyvitamin D (1,25(OH)2D) (2). In consequence, plasma 1α,25-dihydroxyvitamin D (P-1,25(OH)2D) is usually high normal or elevated in primary hyperparathyroidism (PHPT) (3–5), although low levels have also been reported depending on available plasma 25-hydroxyvitamin D (P-25OHD), renal function and the degree of hypercalcaemia (3, 4, 6–8).

In general, 1,25(OH)2D regulates gene transcription through a nuclear high-affinity receptor (vitamin D receptor, VDR)) (9) and the initiation of rapid cellular responses through a putative plasma-membrane-associated receptor (10). Parathyroid tissue expresses VDR and 1,25(OH)2D regulates parathyroid cell proliferation, differentiation and secretion (11) and also increases the expression of the Ca sensing receptor (CaSR) (2, 12). However, both VDR and CaSR expression are reduced in parathyroid adenoma (PA) cells (13–15) suggesting a partial resistance to 1,25(OH)2D in primary, but not in secondary hyperparathyroidism (11).

Many tissues, including parathyroid cells, express the low-density lipoprotein receptor-related protein (LRP)-2 or megalin, which in the kidney very likely mediates tubular reabsorption of the 25OHD3-vitamin D binding protein complex (16), in combination with a 25-hydroxyvitamin D-1α-hydroxylase (17, 18). Theoretically, the parathyroids thereby possess the capability to produce 1,25(OH)2D locally from circulating 25OHD and...
P-25OHD may participate in the regulation of parathyroid cell proliferation, differentiation and secretion. This is further corroborated by the finding of increased 25-hydroxyvitamin D₃ 1α-hydroxylase and reduced 25-hydroxyvitamin D₃ 24-hydroxylase expression in PAs (19). It has recently been documented that PHPT patients have reduced plasma levels of 25OHD independent of age, sex and season (20). Furthermore, based on sporadic P-25OHD measurements, it has been suggested that chronic vitamin D deficiency may accelerate PA growth and PTH secretion and thereby aggravate bone turnover and bone loss (14, 21, 22).

In a cross-sectional design, we investigated possible relations between the two above-mentioned vitamin D metabolites (P-1,25(OH)₂D and P-25OHD) and parathyroid adenoma weight (AW), plasma-parathyroid hormone (P-PTH) and PTH secretory activity respectively in 172 patients with surgically proven PA in order to disclose the pathophysiological importance of the two circulating metabolites for adenoma size and PTH secretion. PTH secretory activity was assessed as the ratio of P-PTH to AW taking potential confounders into account.

**Materials and methods**

**Materials**

Figure 1 depicts the study profile and Table 1 characterizes the included patients. From January 1994 to January 2003, we investigated a total of 357 consecutive caucasian patients with the suspicion of PHPT, because of hyperparathyroid hypercalcaemia. Hypercalcaemia was defined by elevated albumin adjusted plasma calcium (Ca) (> 2.52 mmol/l) and hyperparathyroidism or high normal (upper one-third of reference range) of plasma intact parathyroid hormone (P-PTH > 5 pmol/l). The upper one-third of the normal range was included, since the normal range for P-PTH depends on vitamin D status in the reference population (23, 24). During the screening phase, we excluded seven patients because of suspicion of familial hypocalciuric hypercalcaemia due to a Ca/creatinine clearance rate < 0.01 or a clinical significant mutation in the CaSR gene. Ten were excluded because of MEN1 (n = 7), MEN2a (n = 1) or isolated familial PHPT (n = 2). Among the remaining 337 PHPT patients, 249 underwent neck exploration and 197 (79%) had a PA removed. Parathyroid AW was available in 171 of the patients, all of whom were normocalcaemic postoperatively. These patients constitute the final material for the present investigation. The histological diagnosis was made from frozen section of the removed pathological parathyroid tissue. All removed parathyroid tissue was re-evaluated on paraffin-embedded sections by the same pathologist (F Melsen). The histological adenoma diagnosis was based on an enlarged gland with a confluence of dense parathyroid cells, no or sparse stromal and cytoplasmatic fat, and often a rim of normal parathyroid tissue outside the adenoma (25). Among the remainder, 26 (10%) had hyperplasia and the histological classification was uncertain in 26 (10%) of the patients. Double adenomas were removed in four patients. The weights of the double adenomas were added in the analysis.

We collected preoperative data as part of a standardized protocol aimed at confirming the diagnosis and assessing possible complications. The database was accepted by the Danish Data Protection Agency.

**Methods**

We measured PA wet weight to the nearest milligram shortly after removal using an electronic scale.

We measured plasma and urinary Ca, albumin and creatinine by standard laboratory methods. We corrected total plasma Ca for individual variations in albumin by the equation,

\[
\text{adjusted plasma Ca (mmol/l)} = \text{ plasma Ca total (mmol/l)} - 0.00086 \\
\times (6.50 - \text{plasma albumin (µmol/l)})
\]

We measured P-PTH by an IMMULITE automated analyser (Diagnostic Products Corporation, Los Angeles, CA, USA). The coefficient of variation (CV) in our lab. was less than 7%. We measured P-25OHD by an equilibrium RIA procedure (DiaSorin, Inc., Stillwater, MN, USA) with inter- and intraassay CV values of 13 and 10% respectively. P-1,25(OH)₂D was measured by RIA (Nichols Institute, San Clemente, CA, USA) with inter- and intraassay CV values of 11 and 11% respectively. Cross-reaction with 25OHD is 0.001%. We measured alkaline phosphatase (AP) spectrophotometrically using an automated instrument (Hitachi 917, Roche). The total CV was less than 8%.
Patient (Pt) creatinine clearance rate was calculated from the plasma creatinine and the 24-h renal excretion of creatinine. All measurements were accredited by DANAK (Skovlunde, Denmark).

The activity of the PA was estimated as the ratio of P-PTH to parathyroid AW, i.e. the P-PTH level obtained by a certain volume of PA tissue.

**Statistical analysis**

We expressed basic variables by their medians and ranges and assessed between group differences by the Mann–Whitney test. We used parametric statistics and multiple linear regressions following logarithmic transformation of data when necessary to assess relations between variables. The analysis was performed with backward stepwise estimation with a significance level for the removal of 0.05. We also used a hierarchic multiple linear regression model with preselected inclusion of either 25OHD or 1,25(OH)2D in order to compensate for collinearity between the two vitamin D metabolites. Statistical analyses were performed with STATA version 8.2 software (StataCorp., College Station, Texas, USA).

**Results**

**Baseline characteristics**

The clinical characteristics of the 171 patients with PHPT and PA are given in Table 1 together with locally established laboratory reference values. All patients were hypercalcaemic and 85% had elevated P-PTH compared with a normal reference population including vitamin D-insufficient, as well as vitamin D-sufficient individuals. All had P-PTH above 5 pmol/l. The PA patients had reduced P-25OHD compared with controls during both summer (72%, P < 0.001) and winter (11%, P < 0.05). The percentage of patients with 25OHD below 50 and 75 nmol/l was 79 and 96% respectively compared with 35% (P < 0.001) and 60% (P < 0.001) of the controls. P-1,25(OH)2D was elevated in 29% (P < 0.001) and reduced in 3% (P > 0.74). Well, over half (56%) of the patients had elevated plasma AP (P-AP). The renal Ca excretion was increased in 56% and the endogenous creatinine clearance rate was reduced in 35%.

**Relation between vitamin D metabolites**

P-1,25(OH)2D correlated positively to plasma ln(25OHD) (r = 0.24, P < 0.005; Fig. 2). Overall, only 6% of the variation in P-1,25(OH)2D could be explained by variations in ln(P-25OHD).

**Parathyroid adenoma weight and vitamin D metabolites**

Table 2 explores the influence of age, sex, body mass index (BMI) and renal function on AW. Age did not influence AW. There was a significant effect of sex with a larger number of females with small PA. High BMI was associated with higher AW. AW was not related to renal function.

Table 3 gives unadjusted and multiple adjusted relationships between AW and biochemical variables related to Ca and vitamin D metabolism. Larger adenomas were positively associated with higher plasma levels of P-25OHD and P-1,25(OH)2D.
P-PTH and vitamin D metabolites

Table 4 gives unadjusted and multiple adjusted relationships between P-PTH and biochemical variables related to Ca and vitamin D metabolism. In the unadjusted analyses, P-PTH correlated positively to P-Ca and P-AP and inversely to plasma phosphate and P-25OHD. There was no significant correlation to P-1,25(OH)₂D. Adjustment for age, sex, BMI and creatinine did not change these results. In a multiple backward regression analysis including age, sex, BMI, plasma Ca, plasma phosphate, plasma creatinine, P-25OHD and P-1,25(OH)₂D, P-PTH depended (P<0.05) positively on plasma Ca and inversely on P-25OHD. The equation was

\[ \ln(P-PTH) = 5.71 \times \ln(Ca) - 0.36 \times \ln(25OHD) \]

\[ -2.05, \ R^2 = 0.51. \]

P-1,25(OH)₂D neither correlates to P-PTH levels in this model nor in any hierarchic multiple regression analysis with a primary inclusion of P-1,25(OH)₂D with or without exclusion of P-25OHD.

Parathyroid activity and vitamin D metabolites

Parathyroid activity was estimated as the ratio of P-PTH to AW. Table 5 gives simple and multiple adjusted relationships between parathyroid activity and various variables related to Ca and vitamin D metabolism. In the unadjusted analysis, plasma phosphate correlated positively with the P-PTH:AW ratio. No relationship was found to plasma levels of Ca or vitamin D metabolites. In the adjusted analysis also including plasma creatinine, the association with plasma phosphate became insignificant. However, in this analysis, P-25OHD correlated inversely to the PTH:AW ratio (P<0.05). No relationship was found between P-1,25(OH)₂D and PTH:AW in any of the analyses. In a multiple backward regression analysis including age, sex, BMI, plasma Ca, plasma phosphate, plasma creatinine, P-25OHD and P-1,25(OH)₂D, parathyroid activity depended (P<0.05) positively on the female sex, inversely on BMI and P-25OHD. The equation was

\[ \ln(PTH/adeno	ext{ra} \text{t} \text{e} \text{ weight}) = 0.607 \times (\text{sex (females)}) \]

\[ -0.036 \times \text{BMI} - 0.317 \]

\[ \times \ln(P-25OHD) + 4.718, \]

\[ R^2 = 0.13 \]

P-1,25(OH)₂D neither correlates to PA activity in this model nor in any hierarchic multiple regression analysis with a primary inclusion of P-1,25(OH)₂D and in or exclusion of P-25OHD. Exclusion of three large PAs weighing 12.9, 25.5 and 26.5 g, or exclusion of three small adenoma (<0.01 g), or exclusion of four double adenomas did not change these associations in the multiple regression analysis (data not shown).

Discussion

The present cross-sectional study has shown that declining levels of 25OHD are related to increasing

Table 2 Age, sex, BMI and renal function among patients with primary hyperparathyroidism (PHPT) stratified according to the median parathyroid adenoma weight (AW).

<table>
<thead>
<tr>
<th></th>
<th>AW &lt; 0.745 g</th>
<th>AW &gt; 0.745 g</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>171</td>
<td>85</td>
<td>86</td>
</tr>
<tr>
<td>% Females</td>
<td>171</td>
<td>63.0 (18.6–85.5)</td>
<td>62.6 (29.4–87.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>171</td>
<td>90.5</td>
<td>77.3</td>
</tr>
<tr>
<td>Pt-creatinine clearance rate (ml/min)</td>
<td>123†</td>
<td>25.5 (16.7–41.4)</td>
<td>28.1 (17.5–51.8)</td>
</tr>
<tr>
<td></td>
<td>146‡</td>
<td>80.2 (25.2–199.8)</td>
<td>82.2 (15.9–170.2)</td>
</tr>
</tbody>
</table>

*The distribution of females were analysed with χ² for trend. †The number of available data is 66 and 57 respectively. ‡The number of available data is 74 and 72 respectively.
levels of P-PTH and higher PTH secretion activity (PTH:AW ratio) in patients with PHPT caused by PA. There was no relation between P-25OHD and AW after adjustment for age, sex, BMI and renal function. This suggests that vitamin D deficiency mainly increases PTH secretion activity in existing adenomas. Our findings could not reproduce an effect of vitamin D insufficiency on adenoma growth previously reported based on point estimates of P-25OHD (14). Neither PTH secretion nor AW was associated with circulating levels of 1,25(OH)2D.

The parathyroid glands express VDR and 1,25(OH)2D is thought to inhibit parathyroid tissue growth and secretion (11, 18) in part by increasing the expression of the CaSR (2, 12). The inhibitory effect of 1,25(OH)2D on PTH secretion is well established in patients with end-stage renal failure and secondary hyperparathyroidism (26). However, the present study has failed to show any relationship between P-1,25(OH)2D and adenoma size or secretion in PHPT patients. One explanation for the lack of effect of 1,25(OH)2D on PTH secretion in our study could be that the expression of VDR and CaSR is reduced in PA cells (13–15) in combination with the sparse increase in P-1,25(OH)2D observed in PHPT. The possible partial resistance of PA cells to 1,25(OH)2D is supported by in vitro studies showing that 1,25(OH)2D inhibits cell proliferation and apoptosis in secondary but not in PHPT, suggesting that 1,25(OH)2D may reduce gland mass in the former but not in the latter condition (11). However, in a recent case report, Kinoshita et al. (27) showed that ectopic 1,25(OH)2D production in sarcoidosis may reduce P-PTH in a patient with PHPT.

Another explanation could be that a balanced negative feedback system exists between P-PTH and 1,25(OH)2D levels. In this system, any increase in P-PTH will enhance renal 1,25(OH)2D production, which again will decrease PTH secretion until a new steady-state situation is reached. However, in such a system, a residual increase in P-PTH and 1,25(OH)2D would be necessary in order to maintain the new balance. Hence, in a cross-sectional study, a positive relationship should be observed between P-PTH and P-1,25(OH)2D. However, any other factor decreasing the response of the renal 1α-hydroxylase to excess PTH, i.e. hypercalcaemia, reduced renal function and excess 1,25(OH)2D, as found in PHPT, would tend to mitigate the effect of PTH on P-1,25(OH)2D and thereby create an inverse relationship between PTH and 1,25(OH)2D. The outlined homeostatic mechanisms may in combination explain the lack of association between plasma levels of 1,25(OH)2D and PTH.

The most likely explanation for the observed inverse relationship between P-25OHD and P-PTH and P-PTH/AW ratio is that the parathyroid cells – like many other normal cells participating in Ca homeostasis (keratinocytes, placenta and bone cells) and several malignant cells (colon, prostate, breast and lung) – express a 1α-hydroxylase that intracellularly converts 25OHD to 1,25(OH)2D (17, 18, 28). Parathyroid cells, like many other tissues, also express the low density LRP-2 or megalin (17). In the proximal tubulus of the kidney, this receptor apparently mediates the reabsorption of the.

Table 3 Simple linear regression and adjusted multiple regression analysis (partial correlation coefficients) of relations between parathyroid adenoma weight (AW) and various clinical indices of disease severity in patients with primary hyperparathyroidism (PHPT).

<table>
<thead>
<tr>
<th>AW (g) (ln)</th>
<th>Simple linear regression</th>
<th>Adjusted for age, sex, BMI and P-creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>P-PTH (pmol/l)</td>
<td>171</td>
<td>0.49</td>
</tr>
<tr>
<td>P-Ca (mmol/l)</td>
<td>171</td>
<td>0.48</td>
</tr>
<tr>
<td>P-phosphate (mmol/l)</td>
<td>161</td>
<td>0.35</td>
</tr>
<tr>
<td>P-25OHD (mmol/l)</td>
<td>161</td>
<td>0.17</td>
</tr>
<tr>
<td>P-1,25(OH)2D (pmol/l)</td>
<td>150</td>
<td>0.01</td>
</tr>
<tr>
<td>P-AP (U/l)</td>
<td>161</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 4 Simple linear regression and adjusted multiple regression analysis (partial correlation coefficients) of relations between plasma-parathyroid hormone (P-PTH) and various indices of calcium (Ca) and vitamin D metabolism in primary hyperparathyroidism (PHPT).

<table>
<thead>
<tr>
<th>P-PTH (pmol/l) (ln)</th>
<th>Simple linear regression</th>
<th>Adjusted for age, sex, BMI and P-creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>P-Ca (mmol/l)</td>
<td>171</td>
<td>0.62</td>
</tr>
<tr>
<td>P-phosphate (mmol/l)</td>
<td>161</td>
<td>−0.24</td>
</tr>
<tr>
<td>P-25OHD (mmol/l)</td>
<td>161</td>
<td>−0.31</td>
</tr>
<tr>
<td>P-1,25(OH)2D (pmol/l)</td>
<td>150</td>
<td>−0.14</td>
</tr>
<tr>
<td>P-AP (U/l)</td>
<td>160</td>
<td>0.53</td>
</tr>
</tbody>
</table>

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filtered 25OHD\textsubscript{3}-vitamin D binding protein complex (16). In the parathyroids, a similar effect would enhance the cellular uptake of circulating 25OHD. The increased 25-hydroxyvitamin D\textsubscript{3} 24-hydroxylase expressions observed in PAs may further enhance the intracellular production of 1,25(OH)\textsubscript{2}D from 25OHD (19). Hence, in PHPT even decreased circulating levels of 25OHD (20) may provide an adequate amount of substrate for a local production of 1,25(OH)\textsubscript{2}D that can overcome the partial resistance to 1,25(OH)\textsubscript{2}D described above (13–15). The large difference in molar concentrations between 25OHD and 1,25(OH)\textsubscript{2}D (1000/1) further supports such a mechanism. A local tissue production of 1,25(OH)\textsubscript{2}D from 25OHD also explains why the development of osteomalacia (29), proximal myopathy (30, 31) and secondary hyperparathyroidism among the elderly (32, 33) depends on circulating P-25OHD levels.

The observed inverse relationship between P-25OHD and adenoma in the univariate linear regression is in accordance with the results reported by Rao et al. (14). However, after adjustment for age, sex and BMI, there was no significant relation between 25OHD and AW. This may be explained in several ways. P-25OHD is a point estimate and may not reflect individual average vitamin D status during adenoma growth. Furthermore, the observed positive effects of BMI on adenoma size, which is in accordance with a recent metaanalysis (34) showing that body weight and BMI are increased by 0.3 s.d. (95% CI 0.19–0.40) in PHPT compared with normocalcaemic controls, may interact. At present, it is unknown whether PHPT leads to increased body weight through stimulation of lipogenesis (35) or obesity facilitates the development of hyperparathyroidism through deposition and catabolism of vitamin D metabolites in the adipose tissue (36–38). The finding that the increase in body weight appears to antedate the development of hypercalcaemia favours the last mechanism (39). In both situations, adiposity and adenoma development may build up over time inducing a positive relationship between the variables in a cross-sectional study.

Our study supports the concept that treatment with vitamin D may have some benefits in the preoperative management of PHPT patients and, perhaps in combination with an antiresorptive agent, may be valuable as long-term treatment in unoperated patients. However, large-scale randomized trials are warranted to assess possible benefits and risks of such a treatment.

The higher occurrence of small adenomas among females may be explained by a higher diagnostic awareness of osteoporosis and disturbances in Ca metabolism in this sex compared with males leading to an earlier detection of disease. However, the exclusion of small adenomas did not change results.

The usually accepted suppression of the remaining normal parathyroid glands in PHPT caused by PAs support the concept that the P-PTH:AW ratio reflects the PTH secretion activity of the adenoma. However, this ratio have not been reported or evaluated before.

Strengths and limitations to study

The main strengths of our study are its large number of consecutively admitted patients, which support the external validity of the study and the uniform protocol for data collection.

In this study, we have used PTH:AW ratio as an estimate of parathyroid activity. To our knowledge, this ratio has not been used before in similar analyses. However, the ratio has been used and reported in another context (40).

The main weakness of the study is the cross-sectional design, which impede causal interpretations. Hence, the study cannot reveal whether parathyroid function has influenced measured variables or the variables have influenced PA function and size. In particular, it cannot be concluded that plasma levels of 25OHD influence P-PTH or parathyroid activity (PTH:AW). It may be that a high secretion of PTH and hypercalcaemia in some way reduces P-25OHD (20). However, our interpretation of the study is in accordance with two studies showing that treatment with vitamin D reduces P-25OHD in patients with PHPT without raising plasma Ca levels (41, 42).

In conclusion, this study showed that in PHPT plasma levels of 1,25(OH)\textsubscript{2}D correlates positively to P-25OHD. Furthermore, decreased levels of P-25OHD are related to increased levels of P-PTH and higher PTH:AW ratios suggesting that vitamin D deficiency increase PTH secretion activity without demonstrable effect on adenoma growth. Neither PTH secretion nor AW was associated with circulating levels of 1,25(OH)\textsubscript{2}D.
References


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