Elevated 1,25-Dihydroxyvitamin D Levels in Patients with Chronic Obstructive Pulmonary Disease Treated with Prednisone

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ABSTRACT

Glucocorticoid administration is a well established cause of osteopenia. Mechanisms underlying the deleterious effect of glucocorticoids on bone may include direct inhibition of bone formation as well as indirect effects through changes in intestinal calcium absorption, renal calcium excretion, and/or levels of the calcitropic hormones. To further examine the potential role of the calcitropic hormones we measured serum levels of PTH and 1,25 dihydroxyvitamin D [1,25(OH)2D], as well as serum and urine calcium levels of calcium and vertebral bone density in patients with chronic obstructive pulmonary disease being managed with or without prednisone. Patients treated with prednisone had lower spinal bone density (53 vs. 106 mg/cm²) and higher serum calcium (2.40 vs. 2.33 mmol/L), urine calcium (6.9 vs. 2.7 mmol/24h), and 1,25(OH)2D levels (147 vs. 95 pmol/L). Compared to the patients not treated with glucocorticoids, PTH levels also tended to be higher (33 vs. 26 µg/ml), but the difference was not significant. Serum and urine calcium levels correlated positively with 1,25(OH)2D levels, but none of these measurements correlated with PTH levels. Our results suggest that prednisone treatment alters the regulation of 1,25(OH)2D production, and this may contribute to the loss of bone mineral induced by prednisone. (J Clin Endocrinol Metab 76: 456-461, 1993)

THE DEVASTATING effect of long-term, high dose glucocorticoid administration on bone is well known (1-4). Glucocorticoids both stimulate bone resorption as assessed by osteoclast counts (5, 6), urinary hydroxyproline levels (7), and calcitropic studies (8), and inhibit bone formation as assessed by decreased mineral apposition rates (5, 9, 10) and osteocalcin levels (11-13). These skeletal effects occur concomitant with the ability of glucocorticoids to inhibit intestinal calcium absorption (14, 15) and renal calcium reabsorption (16, 17).

The roles of the calcitropic hormones, PTH and 1,25 dihydroxyvitamin D [1,25(OH)2D], in mediating the actions of glucocorticoids on bone mineral homeostasis are not well established. Increased bone resorption is thought to be the result of secondary hyperparathyroidism (18), although elevated PTH levels are not universally found (6, 7, 10, 12, 19-22). In vitro studies indicate that glucocorticoids can poten¬tiate the action of PTH on bone (23, 24), suggesting a way that glucocorticoids could stimulate bone resorption via PTH without raising PTH levels. The fall in serum osteocalcin levels and the inhibition of intestinal calcium transport would be expected to follow a glucocorticoid-induced reduction in 1,25(OH)2D production, yet 1,25(OH)2D production has been reported to be normal (21) in subjects with endogenous or exogenous Cushings syndrome. In some (25, 26) but not all (27) studies the administration of 1,25(OH)2D fails to com¬pletely reverse the glucocorticoid-induced decrease in intestinal calcium transport, suggesting resistance at the target tissue level. However, when 1,25(OH)2D administration does improve intestinal calcium absorption it may aggravate the glucocorticoid-induced hypercalciuria (27) and further suppress bone formation (27). Thus, too little 1,25(OH)2D is not a common factor in glucocorticoid-induced bone disease, but too much 1,25(OH)2D may be detrimental.

The concept that elevated 1,25(OH)2D levels as well as elevated PTH levels could contribute to the disordered bone mineral metabolism resulting from glucocorticoid administration has received little attention. Acute administration of glucocorticoids to normal subjects increased 1,25(OH)2D levels in several studies (20, 22, 28); the increase in 1,25(OH)2D levels was accompanied by an increase in urine calcium levels (22). Surgical correction of endogenous Cushings syndrome has been reported to lower 1,25(OH)2D levels (19). However, several studies of patients on long-term glucocorticoid therapy have found normal levels of 1,25(OH)2D (12, 21, 29-31). These long-term studies either did not measure serum PTH and urine calcium levels or found them to be normal (21, 31).

Because vitamin D (or one of its metabolites) and calcium therapy are often recommended for the prevention or treatment of glucocorticoid-induced bone disease, we sought to clarify whether 1,25(OH)2D production was altered by glucocorticoid administration in a way that would increase the risk of toxicity (hypercalcemia and/or hypercalciuria) from such therapy. We measured the levels of 1,25(OH)2D and PTH in conjunction with serum and urine calcium levels in patients with chronic obstructive pulmonary disease who were treated with or without prednisone. The results showed...
elevated levels of 1,25(OH)\textsubscript{2}D in many of the subjects treated with prednisone that correlated with elevated serum and urine calcium levels. Our data suggest that abnormal regulation of the calcitropic hormones in patients treated with glucocorticoids contributes to their disordered bone mineral homeostasis and could predispose them to toxicity after overzealous treatment with vitamin D and calcium supplementation.

Materials and Methods

Subjects

Thirty-six subjects were recruited from the Pulmonary Clinic at the Veterans Administration Medical Center (VAMC), San Francisco, where they were being treated for chronic obstructive pulmonary disease. Subjects taking medications or evidencing medical problems predisposing to abnormalities in bone mineral homeostasis (except for glucocorticoid administration and a history of smoking) were excluded from the study. In particular, none of the subjects were given calcium or vitamin D supplementation, were taking diuretics, had a history of alcohol or drug abuse, or had a history of hepatic, renal, intestinal, or pancreatic disease. Twenty-two of the subjects were being treated with oral prednisone for at least 1 yr at the time of study; 14 subjects serving as controls had never been treated with oral or inhaled glucocorticoids. Informed consent was obtained from each subject at the time the study was initiated. The following measurements were made on each subject.

Biochemical measurements

Routine biochemical measurements were performed by the clinical laboratory of the VAMC and were compared to their normal ranges. Vitamin D metabolites were measured after chromatographic separation using serum binding and calf thymus cytosol binding assays as previously reported (32). The results were compared to the normal range established in our laboratory (33). PTH was measured by the method of Arnaud et al. (34) using an antiserum (GP-1M) directed to the midregion of the molecule, and the results were compared to the normal range established for that assay in the Endocrine Research Laboratory at the VAMC.

Bone densitometry

The density of cancellous bone in the lumbar vertebrae was determined by quantitative computed tomography using a modified GE 9800 computer tomography (CT) scanner (General Electric, Schenectady, NY) and a mineral calibration phantom according to the method of Genant et al. (35): the results were compared to values of normal men of comparable age (35). The results are expressed in absolute units.

Statistical analyses

All analyses were performed with the appropriate programs in Statstix (Analytic Software, St. Paul, MN). Such programs included summary statistics for individual variables, Pearson correlation matrix for all variables, linear correlations between two variables, and unpaired t tests. Data in the text and figures are expressed as mean ± SD unless otherwise indicated.

Results

The subjects treated with prednisone were comparable in age to those whose chronic obstructive pulmonary disease (COPD) was managed without glucocorticoids (62 ± 9 vs. 65 ± 10 years) (Table 1). Prednisone dose ranged from 7.5-60 mg/day, although most of the subjects were treated with 10-20 mg/day. The duration of glucocorticoid treatment ranged from 1-16 yr. Incomplete records made it difficult to precisely quantitate total prednisone administered for a number of the subjects taking glucocorticoids over an extended period of time. The severity of the COPD tended to be worse in the subjects treated with prednisone as indicated by a lower forced expiratory volume in 1 second (FEV\textsubscript{1}) (1.2 ± 0.9 vs. 1.9 ± 1.0 L/sec in the prednisone treated vs. nonprednisone-treated subjects, respectively). The forced vital capacity (FVC) was not significantly different (Table 1).

Bone densitometry measurements of the lumbar spine showed a highly significant decrease in bone density of the subjects taking prednisone compared to those not treated with glucocorticoids (53 ± 30 vs. 106 ± 38 mg/cm\textsuperscript{2}, respectively) (Fig. 1, Table 2). When expressed as a percent of the mean normal age-adjusted value, the bone density of subjects treated with prednisone was 42 ± 23% of normal, whereas the bone density of subjects not treated with glucocorticoids was 88 ± 31% of normal. However, the estimated total dose of prednisone consumed (dose × years of administration) was not significantly correlated with bone density (r = 0.346).

The degree of pulmonary impairment also appeared to affect bone density independent of glucocorticoid use. As seen in Fig. 2 when data from both groups were combined, the bone density of the spine was highly correlated to the FEV\textsubscript{1} (r = 0.768, P < 0.001). The correlation remained significant when the prednisone-treated and control groups were analyzed separately (r = 0.717, P < 0.001; r = 0.708, P < 0.01, respectively). However, as seen by the distribution of data points from each group in Fig. 2, the bone density measurements in the control group were higher than those in the prednisone-treated group at comparable levels of FEV\textsubscript{1}.

Serum and urine calcium levels were significantly higher in the subjects treated with prednisone (2.40 ± 0.11 vs. 2.33 ± 0.10 mmol/L and 6.9 ± 3.4 vs. 2.7 ± 1.5 mmol/24 h, respectively) (Fig. 3, Table 2). Although only 1 prednisone-treated subject had a serum calcium level above the normal range, 11 such subjects excreted an abnormally large amount of calcium. The serum phosphorus levels were comparable and normal in the two groups, but the alkaline phosphatase levels were slightly higher in the subjects treated with prednisone compared to those not treated with glucocorticoids (1.57 ± 0.61 vs. 1.25 ± 0.18 µKat/L) (Table 2).
FIG. 1. Spinal bone density measurements performed by quantitated computer tomography (QCT) in patients with COPD treated with or without glucocorticoids (GC). The error bars enclose mean ± SD for each group. The subjects treated with glucocorticoids have significantly lower bone density.

Serum levels of 1,25(OH)2D and PTH were higher in the subjects treated with prednisone compared to those not treated with glucocorticoids (147 ± 30 pmol/L, 33 ± 8 vs. 26 ± 13 μ eq/mL for 1,25(OH)2D and PTH, respectively) (Fig. 4, Table 2). The 1,25(OH)2D data are especially striking in that over 50% of the subjects treated with prednisone had 1,25(OH)2D levels that exceeded 2 SD of the normal range. The subject with the highest 1,25(OH)2D level also had the highest serum calcium level and sixth highest urine calcium level. When data from both groups are combined, the urine and serum levels of calcium were found to correlate to the levels of 1,25(OH)2D (r = 0.610, P < 0.001 and r = 0.482, P < 0.01, respectively) (Fig. 5), but not to the levels of PTH. PTH and 1,25(OH)2D levels did not correlate significantly (r = 0.214), nor did 1,25(OH)2D levels correlate to bone density. As shown in Fig. 5, subjects treated with prednisone had higher urine calcium (but not serum calcium) levels than control subjects with equivalent 1,25(OH)2D levels.

Discussion

Although the deleterious effect of glucocorticoid therapy on bone is well established, the diseases for which the glucocorticoids are being used often contribute to the changes observed in the skeleton and in the hormones regulating bone mineral metabolism. Thus, it is not always easy to separate out the effects of the disease from the effects of the drug used to treat the disease when one is attempting to determine the mechanism by which glucocorticoids lead to osteoporosis. Acute studies in which relatively short courses of glucocorticoids were administered to normal volunteers have demonstrated increases in both PTH (7, 36) and 1,25(OH)2D (20, 22, 28). These changes in the calcitropic hormones have been ascribed to direct effects on PTH secretion (36) or secondary to decreased intestinal calcium absorption (7, 28) and increased renal calcium excretion (16). Although mild hyperparathyroidism (6, 37) has been demonstrated in some but not all (10, 38) studies of patients on long-term glucocorticoid therapy, 1,25(OH)2D levels have been normal (21, 29, 39), as have the urine calcium levels when measured (31). Thus, the concept has arisen that glucocorticoid administration leads to secondary hyperparathyroidism which can be effectively and safely treated with calcium and vitamin D or one of its metabolites (6). Our data suggest that this concept may be incorrect.

The choice of patients with COPD as subjects for this study was fortuitous in that the control subjects not taking glucocorticoids were nearly normal in their bone density and

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<th>TABLE 2. Biochemical and radiological evaluation</th>
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<tr>
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<th>No GC Rx</th>
<th>P</th>
<th>Normal range (or mean ± SD)</th>
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<tr>
<td>SCr (mmol/L)</td>
<td>40 ± 0.11</td>
<td>23.3 ± 0.10</td>
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<td>SP (mmol/L)</td>
<td>0.99 ± 0.15</td>
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<td>NS</td>
<td>0.81-1.45</td>
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<td>AlkPase (μKat/L)</td>
<td>0.57 ± 0.61</td>
<td>1.25 ± 0.18</td>
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<tr>
<td>Scr (mmol/L)</td>
<td>102 ± 19</td>
<td>100 ± 14</td>
<td>NS</td>
<td>53-124</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>80 ± 21</td>
<td>89 ± 28</td>
<td>NS</td>
<td>85-125</td>
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<td>UCa (mmol/24 h)</td>
<td>6.9 ± 3.4</td>
<td>2.7 ± 1.5</td>
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<td>PTH (μ eq/mL)</td>
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<td>26 ± 13</td>
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<td>45 ± 20</td>
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<td>25OHD (mmol/L)</td>
<td>58 ± 26</td>
<td>51 ± 28</td>
<td>NS</td>
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<td>1,25-(OH)2D (pmol/L)</td>
<td>147 ± 50</td>
<td>95 ± 30</td>
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<td>45 ± 20</td>
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<tr>
<td>Bone density (mg/cm²)</td>
<td>53 ± 30</td>
<td>106 ± 38</td>
<td>0.0002</td>
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Biochemical and radiological assessment at the time of study. Bone density was evaluated by quantitated computed tomography of the lumbar spine and is expressed both in absolute terms and as a percent of the age adjusted mean normal value. The significance of the difference between the two groups is shown by the P value. The normal range is shown for comparison to the patient values.
PREDNISONE INCREASES 1,25(OH)₂D

Fig. 2. Correlation of spinal bone density measurements (QCT) with the FEV₁. Measurements from all subjects were combined for this analysis. The correlation was positive and significant (P < 0.001). Data from subjects treated with prednisone are shown in solid circles; data from subjects not treated with prednisone are shown in open circles.

Fig. 3. Serum and urine calcium measurements in subjects treated with or without glucocorticoids (GC). The error bars enclose mean ± SD for each group. Serum and urine calcium values were significantly higher in the group treated with glucocorticoids (P = 0.044 and P < 0.0001, respectively).

calcitropic hormone levels. The two exceptionally low bone density measurements in the nonglucocorticoid-treated group were obtained in patients over 70 yr old who had the lowest FEV₁ values in the control group. Both the pulmonary disease and the limited activity it caused appear to impact on the development of osteoporosis independent of treatment. As seen in Fig. 2, the severity of the pulmonary disease as reflected by FEV₁ correlates with the degree of osteopenia regardless of glucocorticoid use, although for comparable degrees of pulmonary impairment the subjects treated with prednisone had more osteopenia. The two elevated PTH measurements obtained in the control group using the midregion assay were repeated using the two-site immunoradiometric assay (40) to measure intact PTH. Both PTH measurements were normal using this assay. None of the calcitropic hormone levels in the control subjects were outside the normal range.

The major new observation in this study is that subjects chronically treated with prednisone had elevated 1,25(OH)₂D or calcium measurements in the control subjects; open circles are data from nonglucocorticoid-treated subjects.

Fig. 4. Serum 1,25(OH)₂D and PTH levels in subjects treated with or without glucocorticoids (GC). The error bars enclose mean ± SD for each group. 1,25(OH)₂D values are significantly higher in the group treated with glucocorticoids (P < 0.001); PTH values tend to be higher in the group treated with glucocorticoids (P = 0.063).

Fig. 5. Correlation of urine (UCa) and serum (SCa) calcium levels with 1,25(OH)₂D levels in all subjects. These correlations are significant and positive (P < 0.001 and P < 0.01, respectively). Closed circles are data from glucocorticoid-treated subjects; open circles are data from nonglucocorticoid-treated subjects.
1,25(OH)₂D levels. The 1,25(OH)₂D concentrations correlated with both serum and urine calcium levels, which also were increased compared to the group not receiving glucocorticoids. As indicated in Fig. 5, the effect of 1,25(OH)₂D on hypercalcuria appears to be additive to that of glucocorticoid administration in that for a given 1,25(OH)₂D level subjects treated with prednisone had more hypercalcuria. The serum 1,25(OH)₂D levels were not correlated with serum phosphorus or PTH levels. Thus, the data support the hypothesis that prednisone therapy alters 1,25(OH)₂D production through a mechanism other than secondary hyperparathyroidism, and that the rise in 1,25(OH)₂D at least contributes to the rise in serum calcium and urinary calcium excretion.

Other studies have not shown increased 1,25(OH)₂D levels in patients treated for months or years with glucocorticoids (12, 21, 29-31), and 1,25(OH)₂D production and clearance were found to be normal in one of these studies (21). Such results are in contrast not only to our own study but to studies in which short courses of glucocorticoids administered to normal subjects raised the 1,25(OH)₂D levels (20, 22, 28). However, in those long-term studies in which PTH and calcium measurements were made (12, 21, 31) glucocorticoid administration also failed to alter PTH and urinary calcium excretion again in contrast to our own study and the results achieved with short-term glucocorticoid administration (22). A number of differences among these studies could contribute to the disparate results. That severity of illness, type of disease, or duration of exposure might play a role is suggested by the observation that normal subjects given short courses of glucocorticoids show an increase in serum 1,25(OH)₂D and urine calcium levels, but this does not readily explain the differences between our study and the long-term studies of others. The studies of Seeman et al. (21) and LoCascio et al. (31) evaluated a heterogenous group of patients treated primarily for connective tissue disorders. However, the studies by Keid et al. (30) and Grecu et al. (29) evaluated patients treated for chronic asthma, patients likely to be similar to our own. Doses of glucocorticoids used in treatment appeared to be comparable among the studies. None of the studies except our own compared patients treated with glucocorticoids to patients with the same disorder who were not treated with glucocorticoids or reported renal function which if impaired could alter the ability of the kidney to respond with increased 1,25(OH)₂D production.

The tendency toward increased PTH levels in our patients may reflect the effect of prednisone on PTH secretion but could also reflect the normal effect of age on PTH levels. The subjects in this study were generally older than the population used to develop the normal range for PTH, and PTH levels have been shown to increase with age (41-43). However, in the combined data PTH levels correlated only with age (r = 0.299, P < 0.1). These elevated PTH values were found despite increased serum calcium and 1,25(OH)₂D levels, although no correlation was observed between PTH and either calcium or 1,25(OH)₂D. Thus, the data support the hypothesis that PTH secretion in prednisone-treated patients is less responsive than normal to the inhibitory effects of calcium and 1,25(OH)₂D.

The concomitant increase in serum calcium, PTH, and 1,25(OH)₂D levels in patients treated with prednisone is reminiscent of the situation in patients with primary, not secondary, hyperparathyroidism. However, the lack of correlation of PTH levels with either serum calcium or 1,25(OH)₂D levels suggests that PTH oversecretion is not the major cause of increased calcium and 1,25(OH)₂D levels. Furthermore, mild increments in PTH, either secondary or primary, might be expected to mitigate, not aggravate, urinary calcium losses. The data might be better explained by postulating that glucocorticoids alter calcium transport across a number of biological membranes leading to reduced intestinal calcium absorption, reduced renal calcium reabsorption, and reduced calcium inhibition of PTH secretion and 1,25(OH)₂D production. Further study is required to verify or reject this hypothesis. However, the therapeutic implications of this hypothesis, if valid, are substantial. Pharmacological doses of vitamin D and calcium could lead to an unacceptably high risk of hypercalcemia and hypercalcuria in patients lacking the normal feedback inhibition by calcium of PTH secretion and 1,25(OH)₂D production.

References