Vitamin D status: effects on parathyroid hormone and 1,25-dihydroxyvitamin D in postmenopausal women¹,²

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ABSTRACT
Background: Low serum 25-hydroxyvitamin D [25(OH)D] concentrations are commonly found in the elderly and are associated with hip fracture. Treatment with vitamin D and calcium can reduce the risk of fracture. The relation between the rise in parathyroid hormone (PTH) with age and the decrease in 25(OH)D is not clear. Neither is there any consensus on the serum concentration of 25(OH)D required for bone health.

Objective: Our objective was to study the relations between serum PTH, serum vitamin D metabolites, and other calcium-related variables in postmenopausal women.

Design: This was a cross-sectional study of 496 postmenopausal women without vertebral fractures attending our menopausal osteoporosis clinics.

Results: PTH was significantly positively related to age and serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] and inversely related to 25(OH)D and plasma ionized calcium. There was a step-like increase in PTH as serum 25(OH)D fell below 40 nmol/L. In women with 25(OH)D concentrations > 40 nmol/L, 1,25(OH)₂D was positively related to 25(OH)D; in women with 25(OH)D concentrations ≤40 nmol/L, the relation was the inverse. In women with 25(OH)D concentrations ≤40 nmol/L, 1,25(OH)₂D was most closely related to PTH; in women with 25(OH)D concentrations > 40 nmol/L, 1,25(OH)₂D was most closely (inversely) related to plasma creatinine. Therefore, with serum 25(OH)D concentrations increasingly ≤40 nmol/L, serum 1,25(OH)₂D becomes critically dependent on rising concentrations of PTH.

Conclusion: The data suggest that aging women should maintain 25(OH)D concentrations > 40 nmol/L (which is the lower limit of our normal range for healthy young subjects) for optimal bone health.

Subjects and Methods
This study comprised 496 postmenopausal women attending our menopause and osteoporosis clinics for assessment of osteoporosis. Patients with spinal fractures, primary hyperparathyroidism, or Paget disease or receiving treatments known to affect bone metabolism (such as calcium, vitamin D, estrogens, selective estrogen receptor modulators, bisphosphonates, calcitonins, corticosteroids, or diuretics) were excluded. Postmenopausal status was confirmed by a serum follicle-stimulating hormone concentration > 20 IU/L. Written, informed consent was not obtained because the subjects were being investigated for evidence of osteoporosis. Regulations of the Royal Adelaide Hospital Research Ethics Committee regarding the use of clinical material in an investigative format were followed.

Physical examination included measurement of height, of weight, and of skinfold thickness with Harpenden calipers (British Indicators Ltd, St Albans, United Kingdom) as the mean of 3 sites on the back of each hand (9). Investigations then osteomalacia is not usually found in bone biopsies in these cases, even in persons with extremely low serum 25(OH)D concentrations (4), the bone density in these patients tends to be low, probably because of high serum PTH concentrations (5, 6). Vitamin D therapy (with calcium) has been shown to lower PTH and reduce fracture rates in nursing home residents (7).

Although serum PTH is known to rise with age (8), the exact relation between this rise and the concomitant age-related decrease in 25(OH)D concentrations is not clear. More importantly, the serum concentration of 25(OH)D required for optimal bone health is unknown. To shed more light on these issues, we studied the interrelations between age and serum concentrations of PTH, 25(OH)D, and 1,25-dihydroxyvitamin D [1,25(OH)₂D] in 496 healthy postmenopausal women.

INTRODUCTION
The low serum 25-hydroxyvitamin D [25(OH)D] concentrations often found in the elderly (1), particularly in those with hip fracture (2), are frequently associated with high serum parathyroid hormone (PTH) concentrations (3) and are suspected of accelerating bone loss and raising the risk of fracture. Although

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followed a standard protocol. All patients fasted overnight, voided on waking, and attended the laboratory between 0900 and 1000 for venipuncture and to provide a urine sample. They then drank 5 μCi of 45Ca in 250 mL water with 20 mg Ca as a carrier. A single blood sample was collected exactly 1 h later. We measured plasma calcium, albumin, globulins, bicarbonate, and anion gap by standard methods; serum 25(OH)D by competitive protein binding (10); 1,25(OH)2 D by HPLC and radioimmunoassay (11); and intact PTH by immunometric assay (DPC, Los Angeles, CA). Plasma ionized calcium (Ca2+) was calculated by an iterative computer program (12). The hourly fractional rate of calcium absorption was calculated from the radioactivity in the blood collected 1 h after the dose of 45Ca (13).

The relations between PTH and other variables were examined by one-way analysis of variance and simple and multiple linear regression by using MINITAB (release 9.2; Minitab Inc, State College, PA). Means were compared by Student’s t test for unpaired samples.

### RESULTS

Relevant physical and biochemical variables measured in the women are given in Table 1. The women’s median age was 62 y (range: 35–88 y), their median weight was 66 kg (range: 40–120 kg), and their median height was 158 cm (range: 130–183 cm).

Univariate correlation coefficients between the variables are shown in Table 2. Serum PTH was significantly positively related to age, body mass index (BMI), radiocalcium absorption, and serum 1,25(OH)2 D and inversely related to plasma ionized calcium and 25(OH)D. With multiple linear regression, PTH remained inversely related to ionized calcium and serum 25(OH)D and positively related to age, serum 1,25(OH)2 D, and BMI (Table 3).

When the patients were divided into groups by using 10-nmol/L steps of serum 25(OH)D, it became clear that the inverse relation between PTH and 25(OH)D was not linear but was mainly due to significantly higher PTH concentrations in women with 25(OH)D concentrations £ 40 nmol/L than in those with 25(OH)D concentrations > 40 nmol/L (Figure 1). Serum PTH was 4.1 ± 0.08 pmol/L (x ± SE) in the women with 25(OH)D concentrations > 40 nmol/L (n = 386) and 5.6 ± 0.04 pmol/L in the women with 25(OH)D concentrations ≤ 40 nmol/L (n = 111) (P < 0.001). Similarly, when the women were grouped by 0.05-nmol/L steps of ionized calcium, the negative correlation with PTH was essentially due to higher PTH concentrations in women with ionized calcium concentrations < 1.20 mmol/L (Figure 2).
As shown in Table 2, there was a significant positive correlation between serum 1,25(OH)$_2$D and 25(OH)D. However, inspection of the data showed that the relation between these 2 variables was not a simple linear one either. On the contrary, the stepwise analysis of serum 25(OH)D used above showed that the relation between these 2 variables was positive in women with 25(OH)D concentrations > 40 nmol/L but inverse in women with serum 25(OH)D concentrations ≤ 40 nmol/L (Figure 3). When mean 1,25(OH)$_2$D was plotted at each 10-nmol/L step of 25(OH)D, there was a clear change in the relation between the 2 at a 25(OH)D concentration of 40 nmol/L. Above a serum 25(OH)D concentration of 40 nmol/L, 1,25(OH)$_2$D = 104 + 0.22 × 25(OH)D (in pmol/L) ($r = 0.11$, $P = 0.029$); less than or equal to a 25(OH)D concentration of 40 nmol/L, 1,25(OH)$_2$D = 141 – 1.00 × 25(OH)D ($r = -0.21$, $P = 0.027$).

Radiocalcium absorption was positively related to PTH, BMI, and 1,25(OH)$_2$D and negatively related to age in the whole set and in each subset [ie, those with 25(OH)D concentrations ≤ and > 40 nmol/L], but there was no significant correlation between radiocalcium absorption and 25(OH)D. However, when 1,25(OH)$_2$D was regressed on PTH in univariate fashion in the 2 subsets, both the intercepts and the regression slopes differed significantly (Figure 4).

Serum 1,25(OH)$_2$D was positively related to serum PTH and 25(OH)D and negatively to plasma creatinine by univariate regression in the whole set (Table 2). These relations remained with multiple linear regression (Table 4).

In the patients with 25(OH)D concentrations > 40 nmol/L, age, serum 25(OH)D, PTH, and plasma creatinine all remained significant determinants of 1,25(OH)$_2$D with multiple linear regression, but plasma creatinine was the most significant (Table 5). In contrast, in those with 25(OH)D concentrations ≤ 40 nmol/L, 1,25(OH)$_2$D was significantly related only to PTH (Table 6).

On univariate linear regression, serum 25(OH)D was significantly positively related to skinfold thickness and average daily hours of sunlight 2 mo before sampling and significantly inversely related to age and BMI (Table 2). These relations remained significant with multiple linear regression (Table 7).

When 25(OH)D was regressed on body weight and height simultaneously, it was found to be more closely positively related to height than inversely related to weight (Table 8).
serum 1,25(OH)2 D tends to rise as 25(OH)D falls, suggesting 25(OH)D concentrations > 40 nmol/L. Below this concentration, PTH of 10% at 25(OH)D concentrations between 30 and 40 nmol/L and of 30% at 25(OH)D concentrations < 30 nmol/L. Any impairment of 1,25(OH)2 D production would presumably accentuate the effect of vitamin D deficiency on serum PTH. Our data suggest that PTH becomes increasingly important in maintaining plasma 1,25(OH)2 D concentrations as serum 25(OH)D falls below 40 nmol/L. Below this concentration, PTH appears to be a more significant determinant of 1,25(OH)2 D than it is above this concentration, when other factors, such as serum 25(OH)D, become more important (Table 5).

We also confirmed, in larger numbers than in our earlier study (9), that 25(OH)D concentrations are related to average daily hours of sunlight, skinfold thickness, BMI, and age, with age being the least significant determinant. The most significant determinant of serum 25(OH)D was the average daily hours of sunlight available 2 mo before the blood sample was drawn. Serum 25(OH)D was also inversely related to BMI. Because a high BMI is in general associated with obesity, we assumed previously that subjects with high fat mass also have a larger pool into which 25(OH)D is distributed and therefore a lower serum 25(OH)D concentration (9). However, in the current data set the inverse relation between 25(OH)D and BMI was due to a positive correlation between 25(OH)D and height (r = 0.14, P = 0.002) rather than an inverse correlation between 25(OH)D and body weight (r = 0.04, P = 0.365). At present, there is no simple explanation for this.

There is currently no consensus on what represents an optimal serum 25(OH)D concentration. Serum concentrations are known to be low in persons with hip fracture (2) and seasonal variation in serum 25(OH)D is associated with seasonal variation in hip fracture rates (18). Vitamin D taken with calcium reduces PTH and markers of bone resorption and also reduces fracture rates in nursing home residents (7).

A 25(OH)D concentration < 30 nmol/L has been suggested by some authors to represent vitamin D deficiency (3, 19), but others have suggested concentrations < 37.5 nmol/L (20), 62 nmol/L (21), 77 nmol/L (22), or even 120 nmol/L (23) as being deleterious to bone, all on the basis of changes in PTH. Krall el al (24) showed seasonal changes in serum PTH when serum 25(OH)D

### DISCUSSION

We showed that serum 25(OH)D concentrations < 40 nmol/L are associated with significantly higher serum PTH. Our data suggest that the positive relation that is known to exist between serum 1,25(OH)2 D and 25(OH)D (14) (presumably because the latter is the substrate for the former) is only operative at 25(OH)D concentrations > 40 nmol/L. Below this concentration, 1,25(OH)2 D production is reported to be impaired in the elderly (17) but our data do not show this. If impaired production does occur, it may be due either to the decrease in 25(OH)D with age or to diminishing renal function; in our data, serum 1,25(OH)2 D was inversely related to plasma creatinine (Table 2).

A serum 25(OH)D concentration of 40 nmol/L (which is the lower limit of our reference range) appears to represent a critical point in vitamin D metabolism at which vitamin D deficiency begins to unduly stress normal calcium homeostasis. The higher 1,25(OH)2 D concentrations with 25(OH)D concentrations > 40 nmol/L and of 30% at 25(OH)D concentrations < 30 nmol/L. Any impairment of 1,25(OH)2 D production would presumably accentuate the effect of vitamin D deficiency on serum PTH. We also confirmed, in larger numbers than in our earlier study (9), that 25(OH)D concentrations are related to average daily hours of sunlight, skinfold thickness, BMI, and age, with age being the least significant determinant. The most significant determinant of serum 25(OH)D was the average daily hours of sunlight available 2 mo before the blood sample was drawn. Serum 25(OH)D was also inversely related to BMI. Because a high BMI is in general associated with obesity, we assumed previously that subjects with high fat mass also have a larger pool into which 25(OH)D is distributed and therefore a lower serum 25(OH)D concentration (9). However, in the current data set the inverse relation between 25(OH)D and BMI was due to a positive correlation between 25(OH)D and height (r = 0.14, P = 0.002) rather than an inverse correlation between 25(OH)D and body weight (r = 0.04, P = 0.365). At present, there is no simple explanation for this.

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### TABLE 4

<table>
<thead>
<tr>
<th>1,25(OH)2 D</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.31 × 25(OH)D</td>
<td>0.072</td>
</tr>
<tr>
<td>+ 3.2 × PTH</td>
<td>0.81</td>
</tr>
<tr>
<td>− 377 × creatinine</td>
<td>117</td>
</tr>
<tr>
<td>+ 110 pmol/L</td>
<td>—</td>
</tr>
</tbody>
</table>

\[ r = 0.26, P < 0.001, n = 496. \]

### TABLE 5

<table>
<thead>
<tr>
<th>1,25(OH)2 D</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>0.29 × 25(OH)D</td>
<td>0.10</td>
</tr>
<tr>
<td>+ 2.2 × PTH</td>
<td>0.95</td>
</tr>
<tr>
<td>− 481 × creatinine</td>
<td>150</td>
</tr>
<tr>
<td>+ 118 pmol/L</td>
<td>—</td>
</tr>
</tbody>
</table>

\[ r = 0.22, P < 0.001, n = 386. \]

### TABLE 6

<table>
<thead>
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<th>1,25(OH)2 D</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0 × PTH</td>
<td>1.1</td>
<td>0.001</td>
</tr>
<tr>
<td>− 0.82 × 25(OH)D</td>
<td>0.43</td>
<td>0.061</td>
</tr>
<tr>
<td>− 1.07 × creatinine</td>
<td>225</td>
<td>0.655</td>
</tr>
<tr>
<td>+ 120 pmol/L</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\[ r = 0.40, P < 0.001, n = 110. \]

### TABLE 7

<table>
<thead>
<tr>
<th>25(OH)D</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3 × skinfold thickness</td>
<td>0.50</td>
<td>0.011</td>
</tr>
<tr>
<td>− 0.26 × age</td>
<td>0.12</td>
<td>0.032</td>
</tr>
<tr>
<td>+ 2.4 × sunlight</td>
<td>0.44</td>
<td>0.000</td>
</tr>
<tr>
<td>− 0.63 × BMI</td>
<td>0.21</td>
<td>0.002</td>
</tr>
<tr>
<td>+ 60.3 nmol/L</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\[ r = 0.32, P < 0.001, n = 496. \]
was ≤63 nmol/L, Dawson-Hughes et al (25) showed that bone loss was less in women with serum 25(OH)D concentrations of 100 nmol/L than in women with concentrations of 66 nmol/L, and Malabanan et al (26) showed that raising 25(OH)D from 43 to 88 nmol/L caused a 22% decrease in PTH. Our data showed a clear change in the relation between 1,25(OH)2 D and 25(OH)D when 25(OH)D concentrations > 40 nmol/L with women with concentrations > 60 nmol/L. PTH was also significantly higher in those subjects with 25(OH)D concentrations > 40 nmol/L than in those with 25(OH)D concentrations above this. The cutoff of 40 nmol/L also happens to be the lower end of the reference range for 25(OH)D in Adelaide.

Ours is the first study to show the inverse relation between 1,25(OH)2 D and 25(OH)D when 25(OH)D concentrations > 40 nmol/L. The data suggest that the skin thinning that occurs with age lowers serum 25(OH)D, which presumably could be positive to negative) when we compared women with 25(OH)D concentrations above this. The cutoff of 40 nmol/L also happens to be the lower end of the reference range for 25(OH)D in Adelaide.

REFERENCES