Effects of Age and Renal Function on Vitamin D Status in Men

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Background: In general, no study has examined the relationship between vitamin D status and key parameters of bone mineral homeostasis in healthy men in Iran. The effects of age and renal function on vitamin D status were determined in a cross-sectional study on a healthy population of Iranian men.

Methods: From January through February 2001, 520 men, aged 20 – 74 years were selected through a cluster randomized sampling from Shiraz. Serum 25-hydroxyvitamin D, intact parathyroid hormone, calcium, phosphorus, alkaline phosphatase, creatinine, and albumin were measured. Pearson and partial correlation coefficients were used to determine the association between variables.

Results: Five hundred twenty men with a mean±SD age of 45±15 years and a mean±SD 25-hydroxyvitamin D level of 35±17 nmol/L were enrolled into the study. Over 33.9% of men in Shiraz had a low level of 25-hydroxyvitamin D (≤25 nmol/L). Serum 25-hydroxyvitamin D did not decline with age in men. Serum parathyroid hormone increased significantly by age in men, but it did not correlate with the decline in creatinine clearance. Men had a decline in serum phosphorus (r=0.23, P<0.0001) and calcium (r=0.14, P=0.001) concentrations by age. Creatinine clearance declined with age.

Conclusion: In healthy men, the serum level of 25-hydroxyvitamin D did not decline with age. Nonetheless, the prevalence of vitamin D deficiency is high which warrants consideration of dietary vitamin D supplement in men.

Keywords: Calcium • men • phosphorus • PTH • 25-hydroxyvitamin D

Introduction

Vitamin D (VitD) metabolites and parathyroid hormone (PTH) participate in the regulation of calcium homeostasis and bone metabolism. It is well known that VitD deficiency causes rickets in children and osteomalacia in adults. Low levels of VitD metabolites are associated with malabsorption of calcium, which results in bone loss. The serum level of 25-hydroxyvitamin D (25-OH-VitD) has been accepted as an index for VitD status. The reported studies concerning with aging have given conflicting results. In few studies conducted in the European countries the data have been equivocal, with some showing the occurrence and others the absence of a decline in VitD status with age. However, these studies were limited to specific age and race groups, and in general, no study has examined the relationship between VitD status and the key parameters of bone mineral homeostasis in our healthy men population. In addition, there is no consensus concerning the relationship of age to serum calcium and phosphorus levels. On the other hand, in our area where VitD intake is usually low and dairy products are not fortified with VitD, the VitD status mainly depends on sunlight exposure. Because of the absence of any data on VitD status of men, we undertook this study to determine the prevalence of hypovitaminosis D, its relation to serum PTH concentration, and renal function as a
key factor in VitD activation in an urban population of normal adult men.

Patients and Methods

Participants

The cross-sectional study was conducted from January through March 2001. Men aged 20 – 74 years were selected through a cluster randomized sampling. The Shiraz city map was divided into 40 areas. In each area, houses the postal code of which ended in 0 were selected. These addresses were visited and one man from each house was invited to participate in the study. The exclusion criteria included any history of fracture or metabolic bone diseases; any malignancy at any age; history of thyroid, parathyroid, or adrenal disease; history of drug abuse or alcoholism; any hepatic or renal disease; use of drugs which may affect bone and calcium metabolism, including consumption of calcium, VitD supplements, and anticonvulsant medication; and gastrointestinal resection. The participants were then asked to come in a fasting state to the Endocrine and Metabolism Research Center, Nemazee Hospital, affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. We invited 600 men to participate. After taking the informed consent, history was taken, blood samples were collected, and a physical examination with particular attention to thyroid, heart, lung, and abdomen plus height and weight was carried out.

Biochemical tests

Fasting blood samples were obtained from participants between 8:00 and 10:00 am. The sera were stored at -20°C until analysis was done in the laboratory of the Endocrine and Metabolism Research Center. Serum calcium (normal range: 8.6 – 10.3 mg/dL), phosphorus (normal range: 2.6 – 4.5 mg/dL), alkaline phosphatase (normal range: <211 IU/L), and creatinine (normal range: 0.6 – 1.1 mg/dL) were measured with enzyme kits (Pars Azmun, Tehran) and a Hitachi 902 autoanalyzer (Boehringer Mannheim, Mannheim, Germany). Serum albumin (normal range: 3.4 – 4.7 g/dL) was measured by turbidimetric assay using a specific antibody. Serum 25-OH-VitD level (normal range: 23 – 113 nmol/L) was measured using an immunoradiometric assay with the IDS gamma-B 25-OH-VitD kit (IDS, Fountain Hills, Arizona). Serum intact PTH (normal range: 13 – 54 pg/mL) was quantified using an immunoradiometric assay (DiaSorin, Stillwater, Minnesota). Inter- and Intra-assay for 25-OH-VitD were 8%, 6.8% and for PTH were 8.9% and 6.1%, respectively. We classified serum levels of 25-OH-VitD into four groups for deficiency status—levels ≤12.5 nmol/L as severe, 12.5 – 25 as moderate, 25 – 35 as mild, and >35 nmol/L as normal. Creatinine clearance was estimated by the Cockcroft and Gault formula, which takes serum creatinine (mg/dL), weight (kg), age (years), and sex into account:

\[
\text{Creatinine clearance} (\text{mL/min}) = \frac{(140 - \text{age}) \times \text{weight}}{72 \times \text{serum creatinine}}
\]

Height and weight were measured with a stadiometer and balance scale after the participants had removed their shoes. All measurements were done on the same equipment. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m²).

Statistical analysis

Data are presented as mean±SD. Pearson and partial correlation coefficients were used to determine the association between variables. Statistical analyses were performed by SPSS for Windows®, release 9.0 (SPSS Inc.). P values <0.05 (two-sided test) were considered statistically significant.

Results

Of 600 invited men, 560 came for the assessment; 40 refused to participate which translates to a response rate of 93.3%. Of the 560 men, 40 were excluded for the above-mentioned reasons leaving a final sample of 520 men.

The mean±SD age of these 520 participants was 45±15 years. The anthropometric and biochemical variables of the participants are shown in Table 1. The mean±SD 25-OH-VitD and PTH levels was 35±17 nmol/L and 30±15 pg/mL, respectively. The mean±SD serum calcium and phosphorus levels and alkaline phosphatase activity was 9.8±0.7 mg/dL, 3.4±0.7 mg/dL, and 196±60 IU/L, respectively.

There was no correlation between age or BMI and 25-OH-VitD levels (Table 2). The minimum, 25 percentile, median, 75 percentile, and maximum of VitD in various age groups are shown in Figure 1. Almost 34% of the participants had a low level of 25-OH-VitD (<25 nmol/L).
As shown in Table 2, PTH rose significantly with age ($r=0.17$, $P<0.0001$) but not with BMI ($r=0.05$, $P=0.3$). After adjustment for age there was a significant, though weak, inverse correlation between PTH and 25-OH-VitD ($r=0.12$, $P=0.005$) (Figure 2).

The participants had a decline (with age) in total serum calcium ($r=0.14$, $P=0.001$) (Table 2). This correlation was not significant after the effect of serum albumin was controlled. There was however a significant decline in the serum phosphorus level with age ($r=0.23$, $P<0.0001$) (Table 2).

The serum alkaline phosphatase activity was significantly correlated with age ($r=0.10$, $P=0.017$) (Table 2). The serum alkaline phosphatase was correlated with both serum phosphorus and PTH levels ($r=0.13$, $P=0.002$; and $r=0.18$, $P=0.0001$, respectively).

There was a significant decline in creatinine clearance with age in our participants ($r=-0.13$, $P=0.001$) (Table 2). There was no significant correlation between creatinine clearance and PTH ($r=0.031$, $P=0.47$).

### Table 1. Anthropometric and biochemical parameters of the participants.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age groups (yr)</th>
<th></th>
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<th></th>
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</thead>
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<tr>
<td></td>
<td>20 – 29 (n=110)</td>
<td>30 – 39</td>
<td>40 – 49</td>
<td>50 – 59</td>
<td>&gt;60</td>
<td>Total</td>
<td></td>
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<tr>
<td>Age (yr)</td>
<td>24±3</td>
<td>35±2</td>
<td>44±3</td>
<td>54±3</td>
<td>65±4</td>
<td>45±15</td>
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<tr>
<td>Height (cm)</td>
<td>172±6</td>
<td>170±7</td>
<td>169±7</td>
<td>168±7</td>
<td>164±6</td>
<td>168±7</td>
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<tr>
<td>Weight (kg)</td>
<td>70±12</td>
<td>74±14</td>
<td>75±11</td>
<td>71±11</td>
<td>72±17</td>
<td>72±14</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9±1.8</td>
<td>26.2±1.7</td>
<td>26.3±1.4</td>
<td>26.2±1.2</td>
<td>26.5±1.7</td>
<td>26.2±1.6</td>
<td></td>
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<tr>
<td>25-OH-VitD (nmol/L)</td>
<td>36±21</td>
<td>33±17</td>
<td>35±17</td>
<td>32±15</td>
<td>35±16</td>
<td>35±17</td>
<td></td>
</tr>
<tr>
<td>25-OH-VitD≤ 12.5nmol/L</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.3%</td>
<td>0</td>
<td>0.2%</td>
<td></td>
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<tr>
<td>12.5&lt;25-OH-VitD≤ 25nmol/L</td>
<td>33.3%</td>
<td>34.8%</td>
<td>33.8%</td>
<td>34.7%</td>
<td>31.9%</td>
<td>33.7%</td>
<td></td>
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<tr>
<td>25&lt;25-OH-VitD≤ 35nmol/L</td>
<td>29.8%</td>
<td>33.7%</td>
<td>30%</td>
<td>29.3%</td>
<td>26.6%</td>
<td>29.9%</td>
<td></td>
</tr>
<tr>
<td>25-OH-VitD&gt; 35nmol/L</td>
<td>36.9%</td>
<td>31.5%</td>
<td>36.3%</td>
<td>34.7%</td>
<td>41.5%</td>
<td>36.3%</td>
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<tr>
<td>PTH (pg/mL)</td>
<td>26±12</td>
<td>28±13</td>
<td>29±13</td>
<td>35±20</td>
<td>33±13</td>
<td>30±15</td>
<td></td>
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<tr>
<td>Calcium (mg/dL)</td>
<td>10±0.6</td>
<td>9.9±0.6</td>
<td>9.7±0.7</td>
<td>9.7±0.5</td>
<td>9.7±0.9</td>
<td>9.8±0.7</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.6±0.9</td>
<td>3.4±0.6</td>
<td>3.3±0.5</td>
<td>3.2±0.5</td>
<td>3.1±0.5</td>
<td>3.4±0.7</td>
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</tr>
<tr>
<td>ALP (IU/L)</td>
<td>214±63</td>
<td>192±54</td>
<td>191±52</td>
<td>193±63</td>
<td>192±60</td>
<td>196±60</td>
<td></td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>1.3±0.4</td>
<td>1.2±0.3</td>
<td>1.2±0.4</td>
<td>1.2±0.5</td>
<td>1.3±0.3</td>
<td>1.2±0.4</td>
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</tr>
<tr>
<td>Clcr (mL/min)</td>
<td>84±8</td>
<td>86.2±11</td>
<td>84.9±9</td>
<td>82.2±12</td>
<td>81.1±11</td>
<td>83.8±10</td>
<td></td>
</tr>
</tbody>
</table>

Note: The serum values were obtained and the creatinine clearance was estimated; BMI= body mass index; 25-OH-VitD= 25-hydroxy-vitamin D; PTH= parathyroid hormone; ALP= alkaline phosphatase; Alb= alkaline phosphatase; Cr= creatinine; Cl= creatinine clearance.

### Table 2. Correlation coefficients between some studied parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age</th>
<th>BMI$^1$</th>
<th>25-OH-VitD$^1$</th>
<th>PTH$^1$</th>
<th>Ca$^1$</th>
<th>P$^1$</th>
<th>ALP$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-OH-VitD (nmol/L)</td>
<td>-0.023</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>0.17**</td>
<td>0.058</td>
<td>-0.17**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>-0.14**</td>
<td>0.03</td>
<td>-0.05</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>-0.23**</td>
<td>-0.028</td>
<td>0.04</td>
<td>-0.063</td>
<td>0.22**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>-1.05*</td>
<td>-0.062</td>
<td>-0.03</td>
<td>0.18**</td>
<td>0.15**</td>
<td>0.13**</td>
<td></td>
</tr>
<tr>
<td>Clcr (mL/min)</td>
<td>-0.13**</td>
<td>0.012</td>
<td>-0.016</td>
<td>0.06</td>
<td>-0.04</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>

$^{*} P<0.05; **P<0.001; ^1$Age-adjusted; Note: The serum values were obtained and the creatinine clearance was estimated; BMI= body mass index; 25-OH-VitD= 25-hydroxy-vitamin D; PTH= parathyroid hormone; Ca=calcium; P=phosphorus; ALP= alkaline phosphatase; Cl= creatinine clearance.

**Discussion**

In the sample of healthy men we studied, although serum 25-OH-VitD did not decline, the PTH level increased with age which was associated with a decline in both serum calcium and phosphorus levels. We did not find any significant correlation between creatinine clearance and parameters of the VitD endocrine system. The absence of a decline in 25-OH-VitD levels with age, however, does not support the belief that a compromise in either VitD status or the hormonal form of the vitamin is a normal concomitant of the aging process.$^8,9$

The age-related increase in PTH observed in this study, similar to that of other reports,$^1,10,11$ can not attribute to an age-related decline in VitD status, because 25-OH-VitD levels were stable with age. However, the inverse correlation of PTH with 25-OH-VitD, which was independent of age, suggests that VitD status may be a potentially important determinant of PTH levels. It has been hypothesized that elevated 25-OH-VitD levels may decrease PTH secretion via direct interaction with...
1,25-(OH)₂-VitD receptors in the intestine to enhance calcium absorption. 12 On the other hand, 1,25-(OH)₂-VitD dramatically suppresses PTH gene transcription and decreases PTH level. 13

The absence of correlation between PTH and creatinine clearance in men (r=0.03, P=0.6) does not corroborate the view that declining renal function is the primary mechanism responsible for the age-related increase in intact PTH. It is likely that presence of deficits in glomerular filtration rate (GFR), substantially greater than those observed in our healthy subjects, may be necessary to produce the strong correlation between creatinine clearance and PTH observed by others. 14

We found a significant decline in total serum calcium and phosphorus levels in our participants. A decline in total serum calcium was not significant after the effect of serum albumin was controlled (r=0.07, P=0.13). Some studies showed no decline in total serum calcium, 9 while others reported a fall in total serum calcium. A decline in serum phosphorus level has been reported in men, as in our study. The decline in serum phosphorus in men is compatible with the age-related increase in PTH. So, the increase in PTH and decrease in serum phosphorus levels most probably cause an increase in 1,25-(OH)₂-VitD to maintain the serum calcium level by advancing age.

One of the most important findings in our study is a relatively high percentage of VitD deficiency in men of Shiraz. Sedrani et al. 17,18 reported similar results about VitD deficiency in general Saudi Arabian (35%) population. Reiszadeh et al. reported a prevalence rate of 35% VitD deficiency in men residing in Tehran. 19 Nevertheless, in North America, hypovitaminosis D was rarely, if ever, reported in healthy young adults. 20–22

In Scandinavian countries, VitD deficiency was reported in about 4 – 9% of young adults during winter and in up to 5% during summer. 23,24

Diet and sunlight are two sources of VitD in human. Individuals in Iran have a limited intake of milk. Furthermore, milk in Iran is generally not fortified with VitD. On the other hand, Shiraz is located in a subtropical region (latitude 29° N) and has an acceptable annual bright sunshine. So, a lower percentage of hypovitaminosis D was expected in our general population. However, the extent of solar-ultraviolet (UV) exposure is determined primarily by lifestyle rather than outdoor UV irradiation.

Low level of 25-OH-VitD, with normal calcium concentration and absence of osteomalacia, necessitate the measurement of the active form of VitD, 1,25-(OH)₂-VitD for reliable interpretation of the results. 25

This study had some limitations. Those excluded from the study were only based on personal interview and their statement about their disease, not based on established documents. In

Figure 1. The minimum, 25 percentile, median, 75 percentile, and maximum of VitD in various age groups.

Figure 2. Scatter plot of PTH and Vit D in men.
addition, the data of this study were limited to Shiraz City and just to winter; so, our data would be assumed just as an estimation of the condition in southern Iran.

We concluded that in our healthy men, the serum level of 25-OH-VitD does not decline with age and that the PTH level increases with age which is associated with a decline in serum calcium and phosphorus levels. Also, it seems that the prevalence of VitD deficiency is high in men residing in our region, which warrants consideration of dietary VitD supplement.

Acknowledgment

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References