Original Article

Serum 25-Hydroxyvitamin D in relation to Parathyroid Hormone, and Calcium Intake In Saudi Healthy Premenopausal Women. Short Title: Vitamin D, Parathyroid Hormone, and Calcium Intake

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1. Introduction

Adequate supply of cutaneous and/or dietary vitamin D is considered essential for bone health even though, it remains controversial how much of the vitamin is required for the maintenance of bone strength and prevention of osteofragility and/or fractures [1-3]. Vitamin D status can be determined by means of serum 25-hydroxyvitamin D [25(OH)D] measurements, however, a universal serum value constituting deficiency, insufficiency and/or sufficiency is still debatable [4,5]. A further difficulty in assigning a universal reference value arises from the multitude of contributory factors to calcium homeostasis. However, no consensus exists as to define the recommended vitamin D intake; this is partly due to considerable variation in serum 25(OH)D levels associated with any given serum PTH values and also the variations in the threshold levels [3,6]. Such variations could be related to: 1) the use of different methods for the measurements of both serum 25(OH)D and parathyroid hormone (PTH) as well as, defining baseline values [7]; 2) different dietary calcium intakes [8]; and 3) the characteristics of the population under investigation [9]. The interrelationship between calcium intake and vitamin D requirements has been recently documented but it was shown that calcium intake was not related to the maintenance of normal PTH values among individuals with serum 25(OH)D > 25 nmol/L [10,11]. The interrelationship between calcium intake and vitamin D requirements among Saudi women has not been studied previously. Thus, the main objective of the present study was: 1) to evaluate the relationship between dietary calcium intake, serum 25(OH)D and serum intact-PTH in relation to calcium homeostasis; and 2) to define the level of serum 25(OH)D associated with raised serum intact-PTH in the subjects studied.

SUBJECTS AND METHODS

Study design:

This is a cross-sectional study. A total of 138 healthy Saudi premenopausal women living in the Jeddah area were recruited from the pool of women attending the clinics at the Center of Excellence for Osteoporosis Research (CEOR) who were randomly selected during a health survey from 40 primary health care center scattered around the city of Jeddah. Women studied were medically examined and interviewed using a locally developed and validated standardized questionnaire to collect information on anthropometric parameters, life style habits, and level of physical activity. The study was approved by the Bioethics and Research Committee at King Abdulaziz University Hospital (KAUH). Prevalence of vitamin D insufficiency and deficiency was calculated using the threshold levels of 25(OH)D (50 nmol/L) and 25 nmol/L as suggested by the Endocrine Society [12]. Participants were divided into tertiles of daily dietary calcium intake and serum 25(OH)D. The interrelation between dietary calcium intake and vitamin D requirements among Saudi premenopausal women in relation to vitamin D status. Thus, the objective of the present study is to assess the inter-relations between dietary calcium intake, 25-hydroxyvitamin-D [25(OH)D] and PTH among Saudi healthy premenopausal women. Subjects and Methods: A total of 138 Saudi healthy premenopausal women living in the Jeddah area were studied. Each woman completed a questionnaire on life-style factors, anthropometric data and dietary calcium intake. Fasting blood samples were collected for the measurement of serum 25(OH)D, parathyroid hormone (PTH), calcium, phosphate and magnesium. Bone mineral density (BMD) was measured by a dual-energy X-ray absorptiometry. Results: All women showed vitamin-D deficiency (serum 25(OH)D < 50 nmol/L). Stratifying women by tertiles of daily dietary calcium intake showed that women with the lowest tertile exhibited significantly lower serum 25(OH)D (P < 0.026), as compared with the group with the highest tertile. Stepwise multiple linear regression analysis showed that changes in serum 25(OH)D contributed to the variation in daily dietary calcium intake. Conclusions: Vitamin-D deficiency is common among Saudi healthy premenopausal women and such was associated with low daily dietary calcium intake.

Keywords:
activity in leisure time, and the use of vitamins and medications. Dietary calcium intake was calculated based on a locally validated food frequency questionnaire (FFQ) and included pictures of portion sizes with approximately 40 food items containing calcium included in the FFQ. Women with cancer or chronic diseases including osteoarthritis or established osteoporosis or with evident endocrine disorders (diabetes mellitus, hyperthyroidism, hyperparathyroidism, etc.) or on any form of drug treatment with possible effect on bone metabolism (e.g., calcitonin, glucocorticoids, anticonvulsants and/or thyroid hormones) were excluded from the final analysis. Women who reported a recent clinical fracture (within 2 years) were also excluded. In addition, all healthy women included in the present study exhibited: 1) normal blood counts; 2) normal values for renal creatinine; and 3) normal values for liver function tests. Accordingly, a total of 138 healthy premenopausal Saudi women (age range 20–39 years) living in Jeddah area, participated in the present study.

The study protocol was in agreement with CEOR ethical standards and the Helsinki Declaration (2008). The study was approved by the Ethics Committee, and written informed consent was obtained from all women participated in the present study. Determination of Bone Mineral Density

Bone mineral density (BMD) (g/cm2) was determined for the anteroposterior lumbar spine (L1-L4) and mean of proximal right and left femur by dual-energy X-ray absorptiometry (DEXA) using LUNAR Prodigy Model, Lunar Corp., Madison, WI), according to standard protocol as described by the manufacturer.

Determination of Serum 25(OH)D, PTH and other analytes

Overnight fasting blood samples were collected in the morning between 8:00 – 11:00 hrs. under standardized conditions. Serum samples were stored at -85°C within 30 min after centrifugation at 2500 g for 10 min. The samples were stored until analyzed for the determinations of serum 25(OH)D, intact-PTH, minerals [serum calcium, phosphate and magnesium] and other biochemical analytes according to the manufacturer's instructions. Serum 25(OH)D was determined using LIAISON system (DiaSorin Inc., Stillwater, MN), according to the manufacturer's instructions.

Statistical Analysis

Results are presented as means (± SD) and categorical variables are expressed as frequencies. Data were analyzed using SPSS-Statistical Package (version 15.0 for Windows Smart Viewer) supplied by SPSS Inc. 2000, Mapinfo Corp. Tokyo, NY, USA. Results that were not normally distributed were log-transformed before analysis. Associations between continuous variables were examined using Pearson’s correlation coefficient. Associations between continuous variables were tested using multiple regression and partial correlation analysis. Women were stratified by tertiles of dietary calcium intake (mg/day) and various variables were compared according to the tertiles for tendency.

RESULTS

The main anthropometric and clinical characteristics of studied women are presented in Table 1. The mean age was 29.52 ± 5.79 years with a range of 20-39. About 26.8% of participants were obese (BMI > 30 kg/m2), whereas 42.1% were overweight (BMI ≥ 25.0 – <30 kg/m2). Of participants studied, 14.2% performed high physical activities compared to 58.0% with sedentary lifestyle and 13.5% had vitamin D deficiency [serum 25(OH)D < 12.5 nmol/L]). The overall mean (± SD) calculated sun exposure index was 3.58 ± 2.57. The overall mean (± SD) serum 25(OH)D level was 30.34 ± 6.18 nmol/L. All women studied exhibited serum 25(OH)D levels < 50.0 nmol/L. However, none of the women had severe vitamin D deficiency [serum 25(OH)D < 12.5 nmol/L]). In addition, 25.6% of participants had moderate vitamin D deficiency (≥25.0 – <30.0) and 73.9% were considered to be with mild vitamin D deficiency [serum 25(OH)D < 12.5 nmol/L].

When stratifying participants by tertiles of daily dietary calcium intake, the group with the lowest intake (< 380 mg/day) showed significantly lower serum 25(OH)D level (P<0.026), compared with the group with the highest dietary calcium intake (>1000 mg/day), respectively (Table 2).

Table 1: Demographic and anthropometric characteristics of the studied healthy premenopausal women

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number =138</th>
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</thead>
<tbody>
<tr>
<td>Age( yrs)</td>
<td>29.52 ± 5.80</td>
</tr>
<tr>
<td>Parity</td>
<td>3.06 – 2.59</td>
</tr>
<tr>
<td>Body mass index (Kg) / m²</td>
<td>27.37 – 5.17</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>431.9 – 110.1</td>
</tr>
<tr>
<td>Sun exposure time (h/day)</td>
<td>0.77 – 0.53</td>
</tr>
<tr>
<td>Sun exposure index</td>
<td>3.58 – 2.57</td>
</tr>
<tr>
<td>Serum FSH (UL)</td>
<td>3.40 – 1.26</td>
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<tr>
<td>Serum LH (UL)</td>
<td>7.31 – 1.78</td>
</tr>
<tr>
<td>Serum E₂ (pmol/L)</td>
<td>394.9 – 77.6</td>
</tr>
<tr>
<td>Serum 25(OH)D(nmol/L)</td>
<td>30.34 – 6.18</td>
</tr>
<tr>
<td>Serum intact-PTH(pmol/L)</td>
<td>2.66 – 0.69</td>
</tr>
</tbody>
</table>
In Saudi healthy premenopausal women, vitamin D deficiency and/or insufficiency together with dietary calcium intake and serum 25(OH)D levels in relation to changes in serum intact-PTH among Saudi healthy premenopausal women. It is known that PTH is a major regulator of calcium and phosphate levels and is considered to be the most reliable marker of inadequate calcium availability, and itself is modulated by calcitriol and serum calcium [8,9]. Thus, vitamin-D deficiency or insufficiency or calcium deficiency are generally associated with an increase in PTH levels, however, the relative contribution of each nutrient to these processes is still under investigation and has not been studied previously in Saudi healthy premenopausal women.

In conclusion, vitamin D deficiency [serum 25(OH)D < 50 nmol/L] is very common among Saudi healthy premenopausal women. Further, the present study, together with previous reports [10-11], indicate that the optimum levels of serum 25(OH)D are influenced by age and dietary calcium intake. With the emerging evidence that vitamin D sufficiency may be linked to the prevention of several health conditions including cardiovascular diseases, autoimmune diseases, common cancers and pregnancy and its outcomes; it is essential that further studies are required to review recommendations for vitamin D nutrition and to identify the appropriate preventive practical measures to remedy vitamin D deficiency among Saudi healthy women. 9

### DISCUSSION

The present study examined the extent of vitamin deficiency and/or insufficiency in relation to dietary calcium intake and serum 25(OH)D levels in relation to changes in serum intact-PTH among Saudi healthy premenopausal women. It is known that PTH is a major regulator of calcium and phosphate levels and is considered to be the most reliable marker of inadequate calcium availability, and itself is modulated by calcitriol and serum calcium [8,9]. Thus, vitamin-D deficiency or insufficiency or calcium deficiency are generally associated with an increase in PTH levels, however, the relative contribution of each nutrient to these processes are still under investigation and has not been studied previously in Saudi healthy premenopausal women.

In Saudi healthy premenopausal women, vitamin D deficiency and insufficiency were common; all participants exhibited serum 25(OH)D levels that are commonly considered to represent deficiency (< 50 nmol/L), and with no women studied sh o w i n g _ l e v e l s _ o f _ s u f f i c i e n t _ c a l c i u m _ a v a i l a b i l i t y _ (≥ 75.0 nmol/L). These results have several important implications and demonstrate the deleterious effects of vitamin D deficiency on bone health among the studied premenopausal women. In addition, the present results are concurrent with previous studies in Caucasians [12-15], Asians [16,17] and others [18] confirming the observation that vitamin D deficiency and/or insufficiency is endemic among populations of the world, and countries with sunny climates throughout the year are no exception.

In the present study, daily dietary calcium intake is low, reflecting the common dietary pattern among Saudi healthy premenopausal women: this is consistent with the few studies published previously in Arabian populations showing inadequate intake of this nutrient [19,20] which is much lower than that observed in Caucasian [21] or Asian, (e.g. [16,22]) populations. Further, the dietary calcium intake of the present study failed to meet the recommended daily intake for calcium in the majority of subjects [21].

The results of the present study are similar to those described by Adami et al [11], but contrast from that described by Steิงrimsdóttir et al [10] with some relevant differences: someβ interaction was evident with age and the effect of calcium intake was more obvious at all serum 25(OH)D levels. Such differences are possibly explained by: 1) the low dietary calcium intake obtained for Saudi women which was much lower than that described for Icelanders [10], and other European [3] populations; and 2) the high prevalence of vitamin-D deficiency and/or insufficiency among Saudi women.

The strengths of the present study include its very strict detailed inclusion criteria including BMD measurements. Furthermore, both serum 25(OH)D and intact-PTH levels were determined independently by laboratory services at CEOR in a blinded manner. The main limitations of the present study were, its cross-sectional design, and hence not sufficient to demonstrate causality and was based on a single assay of 25(OH)D and intact-PTH levels. The possibility of selection bias also arises. Finally, the accuracy of self-reported data related to lifestyle factors may have been subject to report bias, such that these may be unrecognized confounding.

In conclusion, vitamin D deficiency [serum 25(OH)D < 50 nmol/L] is very common among Saudi healthy premenopausal women. Further, the present study, together with previous reports [10-11], indicate that the optimum levels of serum 25(OH)D are influenced by age and dietary calcium intake. With the emerging evidence that vitamin D sufficiency may be linked to the prevention of several health conditions including cardiovascular diseases, autoimmune diseases, common cancers and pregnancy and its outcomes; it is essential that further studies are required to review recommendations for vitamin D nutrition and to identify the appropriate preventive practical measures to remedy vitamin D deficiency among Saudi healthy women. 9

### References


