Vitamin D Status and Its Seasonal Variations and Association With Parathyroid Hormone Concentration in Healthy Women in Riga

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Key Words: *vitamin D; vitamin D deficiency; parathyroid hormone.*

Summary. *The aim of the study was to describe the vitamin D status and its seasonal variations in women living in Riga, Latvia, to examine an association between the concentrations of plasma 25-hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH), and to determine the threshold for plasma 25(OH)D above which there is no further suppression of PTH.*

Material and Methods. The data of 189 healthy Caucasian women were analyzed. The serum levels of 25(OH)D, PTH, and phosphorus were measured twice a year. All the participants were divided into 3 groups according to vitamin D supplementation and the reproductive status.

Results. The overall mean level of 25(OH)D was 32.8 ng/mL with significantly lower levels being in winter when compared with those in summer (28.2 ng/mL vs. 37.5 ng/mL, respectively; P<0.05). PTH was negatively associated with 25(OH)D. A threshold level of plasma 25(OH)D above which no further suppression of PTH occurred was found to be 38 ng/mL. Postmenopausal women not taking vitamin D supplements and without exposure to sunlight had 25(OH)D deficiency in winter and summer (92% and 88%, respectively). The most significant seasonal fluctuations were seen in the women of the reproductive age not taking vitamin D supplements and without exposure to sunlight, of which 47% had 25(OH)D deficiency in summer and 69% in winter.

Conclusions. An optimal concentration of 25(OH)D was found to be 38 ng/mL. According to this definition, 70.4% of all the healthy women were classified as vitamin D deficient in winter and 59.8% in summer. The highest proportion of vitamin D deficient individuals was found in the group representing the postmenopausal women not taking vitamin D supplements.

Introduction

A low vitamin D level not only contributes to the development of osteoporosis but is also associated with cancer (1), multiple sclerosis (2), infections (3), cardiovascular diseases (4), diabetes (5), and rickets. A normal level of vitamin D is ensured by both sufficient intake of exogenous vitamin D and adequate production of endogenous vitamin D. As a dietary source of vitamin D is limited almost exclusively to fish and its products, the synthesis of vitamin D in the epidermis of the skin under exposure to ultraviolet rays is considered as a primary source. Occasionally, this is the cause of seasonal variations observed in the populations living at the northern latitudes where exposure to natural sunlight is limited during an extended period in winter. It has been reported that no or only marginal production of calcitriol occurs during mid-October to mid-April at 52° N (6). In line with these biochemical data, the incidence of hip fractures is increased in the wintertime placing seasonal variations as an important phenomenon (7).

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A low vitamin D level is associated with a decreased absorption of calcium in the intestine, which results in an increased production of parathyroid hormone (PTH) known as secondary hyperparathyroidism. Subsequently, this process will lead to a release of calcium from bones, and if sustained, to osteoporosis and fractures. Many studies suggest that secondary hyperparathyroidism will develop long before vitamin D will decline below the level defined as vitamin D hypovitaminosis according to population-based studies (8, 9). Recently, it has been suggested that vitamin D deficiency should be defined as the lowest level of vitamin D that prevents secondary hyperparathyroidism. This level may differ by countries according to the latitude as the sun is an important source of vitamin D. In previous studies, the serum concentration of 25-hydroxyvitamin D [25(OH)D] below which PTH begins to rise has been estimated to be between 10 and 48.8 ng/mL (10). The wide range of these estimates may be related to various factors including but not limited to the latitude and lifestyle. Therefore, the studies defining hypovitaminosis D according to secondary hyperparathyroidism should be performed in each country. To our knowledge, no relevant data are available for the population living in Riga, Latvia.

The main problem associated with vitamin D deficiency is the development of osteoporosis. According to the data of the World Health Organization, osteoporosis is the second most common disorder following cardiovascular diseases (11). Although this disease is observed among women and men, postmenopausal osteoporosis is the most common type accounting for 90% of cases (12). Therefore, women, especially in the postmenopausal stage of their life, are at a particular risk of vitamin D deficiency.

The aim of the study was to describe the vitamin D status and its seasonal variations in women living in Riga, Latvia, to examine the association between the concentrations of plasma 25(OH)D and PTH, and to determine the threshold for plasma 25(OH)D above which there is no further suppression of PTH.

Material and Methods

The study population included all women aged 28–69 years who attended Paula Stradins Clinical University Hospital for annual check-ups during the period of February 2007 to March 2007. In total, 196 healthy Caucasian women were included into this cross-sectional study. Of them, 7 subjects failed to attend the second visit with relocation reported as the most common reason. As a result, both visits were attended, and complete data were obtained from 189 subjects (response rate, 96.4%).

All the participants provided written informed consent, and the study was conducted in accordance to the principles of Helsinki declaration of 1975 (revised in 1983). The study was approved by the Ethics Commission of Riga Stradins University.

The serum concentrations of 25(OH)D, PTH, and phosphorus were measured in 189 healthy volunteers twice, i.e., during February-March 2007 representing the winter season and July-August 2007 representing the summer season. The serum levels of 25(OH)D were measured by an electrochemiluminescence immunoassay in one laboratory with the measuring range from 4.0 to 100 ng/mL defined by the lower detection limit and the maximum of the master curve. The method was standardized against liquid chromatography-tandem mass spectrometry (13), and calibration was performed once per reagent lot using a fresh reagent, i.e., no more than 24 hours since the reagent kit was registered on the analyzer. Serum intact PTH was measured with an Immulite intact PTH immunoassay kit. The samples showing visible signs of hemolysis were not analyzed to avoid possible interference with the results.

Additionally, waist circumference was recorded, and height and weight were measured to calculate body mass index.

The data regarding dietary and over-the-counter vitamin D intake were collected as well as the information on the exposure to natural or artificial sunlight. The participants taking vitamin D supplements reported a daily dose ranging between 400 and 1000 IU. The degree of sunlight exposure was assessed by asking the subjects a simple question, which enabled them to be categorized into 2 groups. The subjects were classified as individuals exposed to artificial sunlight or natural sunlight if they fulfilled at least 1 of the following criteria: exposure to artificial sunlight at least once per week, exposure to natural sunlight at least 5 hours per week with or without sun-blocking agents, and exposure to natural sunlight for 2 to 5 hours per week without sun-blocking agents. Otherwise, they were classified as the subjects without exposure to artificial or natural sunlight.

The medical histories were reviewed, and the individuals with serious conditions were excluded from the study (Crohn's disease, cystic fibrosis, celiac disease, renal diseases, hyperparathyroidism, Cushing's syndrome, thyrotoxicosis, active rheumatoid arthritis, organ transplantation, diabetes, oncological diseases, etc.). Then, the volunteers were divided into 3 groups: group 1, postmenopausal women not taking vitamin D supplements and without exposure to natural or artificial sunlight; group 2, women of the reproductive age not taking vitamin D supplements and without exposure to natural or artificial sunlight; and group 3, women of any age taking vitamin D supplements and/or with exposure to natural or artificial sunlight.

The characteristics of all the groups are presented in Table 1.

For further analysis, group 1 was divided into 3 subgroups according to BMI ($\leqslant$25, 25.1–29.9, and $\geqslant$30 kg/m^2, respectively). The characteristics of these 3 subgroups are shown in Table 2.

The descriptive statistics including means, standard deviations, numbers, and percentages were used to summarize the demographic characteristics of the sample. The Loess method (14), which is the technique for determining the shape of the function that best summarizes the scatter plot between 2 continuous variables, was used to describe the relationship between 25(OH)D and PTH. The Student *t* test for 2 means was used to determine significant differences. A *P* value of less than 0.05 was considered statistically significant.

Results

Characteristics of Study Population. The data from 189 of the 194 healthy volunteers was used in this study. For the analysis, 7 participants were excluded from the study because they failed to attend the second visit. At the beginning of the study, the subjects' age ranged between 28 and 69 years with

Table 1. Characteristics of all Participants by Groups

Season	Variable	Group 1	Group 2	Group 3
Overall	Reproductive status	Postmenopausal	Premenopausal	Any
	Vitamin D supplementation and/or exposure to sunlight	No	No	Yes
Winter	Number	112	42	35
	Age, years	61.8 (9.9)	36.4 (12.7)	48.6 (17.5)
	Body mass index, kg/m^2	26.5 (2.3)	22.7 (1.8)	23.8 (1.9)
	25(OH)D, ng/mL	18.7 (4.9)	28.3 (7.7)	58.7 (8.5)
	PTH, pg/mL	62.1 (12.1)	41.0 (5.8)	43.8 (4.3)
	Phosphorus, mmol/L	1.4 (0.3)	1.26 (0.2)	1.29 (0.3)
Summer	Number	107	38	44
	Age, years	62.4 (8.1)	35.6 (12.7)	49.6 (18.8)
	Body mass index, kg/m^2	27.8 (2.8)	22.7 (1.8)	23.9 (2.1)
	25(OH)D, ng/mL	26.5 (5.1)	40.2 (7.8)	62.1 (8.0)
	PTH, pg/mL	61.3 (9.4)	39.8 (4.8)	43.4 (3.9)
	Phosphorus, mmol/L	1.38 (0.2)	1.37 (0.3)	1.27 (0.2)

Values are mean (standard deviation) unless otherwise stated. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

Table 2. Characteristics of all Participants by Body Mass Index

Season	Variable	Body Mass Index, kg/m^2		
		⩽25.0	25.1–29.9	⩾30.0
Winter	Number	47	37	28
	Age, years	60.8 (6.3)	61.9 (5.9)	63.3 (7.1)
	Body mass index, kg/m^2	22.3 (1.4)	27.8 (1.1)	31.8 (1.2)
	25(OH)D, ng/mL	19.5 (2.9)	18.2 (2.7)	18.0 (3.5)
	PTH, pg/mL	62.3 (11.1)	62.0 (6.1)	62.0 (4.8)
	Phosphorus, mmol/L	1.41 (0.2)	1.40 (0.1)	1.40 (0.3)
Summer	Number	44	36	27
	Age, years	61.5 (5.5)	62.6 (5.8)	63.6 (6.9)
	Body mass index, kg/m^2	23.6 (1.3)	28.4 (1.6)	33.8 (2.1)
	25(OH)D, ng/mL	34.2 (6.6)	22.1 (6.5)	19.8 (6.1)
	PTH, pg/mL	61.3 (9.4)	39.8 (4.8)	43.4 (3.9)
	Phosphorus, mmol/L	1.38 (0.3)	1.37 (0.1)	1.39 (0.2)

Values are mean (standard deviation) unless otherwise stated. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

a mean age of 53.7 years (SD, 17). As expected, the mean age in the group 1, which represented the postmenopausal women, was significantly higher compared with both the group 2 and the group 3 (61.8 [SD, 9.9] vs. 36.4 [SD, 12.7] and 48.6 [SD, 17.5], respectively; $P<0.05$). Furthermore, body mass index was significantly higher in the group 1 compared with the group 2 (26.5 [SD, 2.3] vs. 22.7 [SD, 1.8], respectively; $P<0.05$).

The characteristics of the groups slightly changed for the second study visit as 5 participants from the group 1 and 4 participants from the group 2 qualified for the group 3 due to vitamin D supplementation, which was initiated after the first study visit. However, the previously described significant differences in particular characteristics between the groups remained the same.

The characteristics of the groups are described in Table 1.

Vitamin D Status, its Seasonal Variations, and Associations With Parathyroid Hormone. No values were found below or above the measuring range. The overall mean level of 25(OH) D was 32.8 ng/mL, and it was significantly lower in the winter season when compared with summer (28.2 vs. 37.5 ng/mL, respectively; $P<0.05$). In the winter season, there was a significant difference in the 25(OH)D level comparing the groups with the lowest mean level of 18.7 ng/mL being in the group 1, which represented the postmenopausal women not taking vitamin D supplements, and with the highest of 58.7 ng/mL in the group 3, which represented the women of any age with regular vitamin D supplementation ($P<0.05$). The difference in the level of 25(OH)D between these groups remained significant in summer, too (26.5 vs. 62.1 ng/mL, respectively; $P<0.05$).

Serum 25(OH)D showed a significant seasonal relationship with the lowest values being in winter. There was a significant difference in the vitamin D level measured in summer and winter in the groups 1 and 2 (differences of 7.8 and 11.9, respectively; $P<0.05$). The group 3, which included the women regularly taking vitamin D supplements, did not show a significant rise in the 25(OH)D level in summer (a difference of 3.4, $P>0.05$).

The mean levels of serum 25(OH)D, serum PTH, and phosphorus in the 3 groups in both seasons are presented in Table 1.

Serum PTH was negatively associated with serum 25(OH)D both in winter and summer. We estimated that 38 ng/mL was a threshold concentration of plasma 25(OH)D above which no further suppression of PTH occurred. Below and above this concentration, the slopes of the regression lines were −0.18 (different from 0; P=0.003) and −0.01 (P=0.76). The relation between the vitamin D status and PTH did not differ between the groups and was not affected by vitamin D supplementation or exposure to sunlight. The estimated level of 38 ng/mL was defined as vitamin D deficiency in this study for further description of the vitamin D status.

According to this definition, 70.4% of all the healthy volunteers involved in our study were classified as vitamin D deficient in winter with the highest and lowest proportions being in the group 1 and the group 3, respectively (91.9% and 2.8%, respectively). During summer, the proportion of vitamin D-deficient individuals decreased to 59.8% on average. Like in winter, in summer, the highest proportion of vitamin D-deficient individuals was observed in the group 1 and lowest in the group 3 (87.8% and 2.3%, respectively). The distribution of vitamin D deficient individuals by groups is shown in Fig.

No significant difference was found in the level of phosphorus between the seasons or different groups. Moreover, there was no association between the level of phosphorus and 25(OH)D or PTH.

Analysis of Subgroup. As the group of the postmenopausal women was largest in our study, we used it for the further analysis. This group was divided into 3 subgroups according to body mass index (⩽25, 25.1–29.9, and ⩾30 kg/m^2 respectively). There were no significant differences in age comparing the 3 subgroups. A significant difference in the vitamin D level in the samples collected during summer across different subgroups with the highest level to be found in the persons with a lower BMI (34.2 ng/mL, 22.1 ng/mL, and 19.8 ng/mL, respectively; P<0.05). There was no significant difference between the subgroups in the samples collected during winter. A significant difference between the seasons was found only in the subgroup with BMI of ⩽25 kg/m^2 (a difference of 14.7, P<0.05).

No significant associations with the levels of PTH or phosphorus were found.

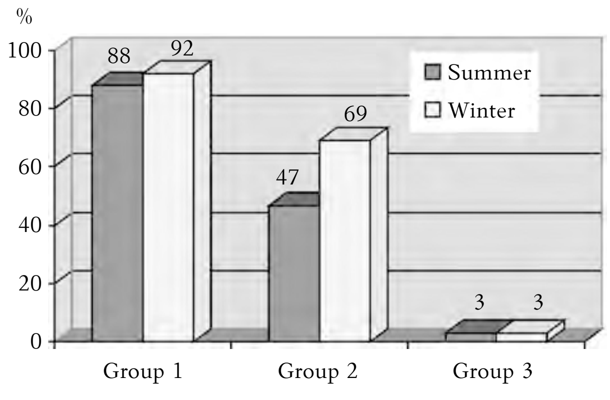

Fig. The distribution of vitamin D deficient individuals with a threshold 25-hydroxyvitamin D level lower than 38 ng/mL by groups (expressed in % of all study participants)

Discussion

Vitamin D has attracted attention in a view of the worldwide epidemic of osteoporosis and the overwhelming evidence of a high prevalence of deficiency in specific populations, particularly in elderly people (15) and postmenopausal women. The present study was conducted to assess the prevalence of vitamin D insufficiency in the population of healthy women, to estimate the relative influence of sun exposure on the concentration of 25(OH)D, and to examine relationships between PTH and 25(OH)D. To describe the vitamin D status in our study, the 25(OH)D concentration was measured. The biologically most active vitamin D metabolite, 1 alpha 25OH2D, is inapplicable for this purpose for several reasons described in literature (16). The circulating concentration of 25(OH)D is considered a good marker of the vitamin D status as it represents the cumulative effects of dietary intake of vitamin D and exposure to sunlight. However, the use of plasma 25(OH)D to assess the vitamin D status has been recently debated. The use of a lower reference value from an abnormal population has several consequences because the level of plasma 25(OH)D depends on unchangeable ecological factors (season, local weather condition, and latitude) and individual lifestyle factors (skin pigmentation, thickness, sunbathing habits, etc.). Vitamin D deficiency was defined previously by the occurrence of frank osteomalacia with obvious clinical symptoms. However, there is an increasing body of evidence that some alteration in calcium and bone metabolism can occur at the 25(OH)D level that was earlier thought to be adequate. These unfavorable changes include impaired calcium absorption and a slight elevation in the serum PTH level leading to an increased bone turnover and accelerated bone loss (17, 18). The cutoff level between this state of insufficiency and the repletion remain to be defined by each country. Riga is located at the latitude of 56°58 N. It is reported that no cutaneous vitamin D production occurs at the latitude 54°58 N from October to April (19) during that period; the maintenance of vitamin D level depends on oral vitamin D intake and the stores of vitamin D built up during the previous summer. In respect of this study, it should be noted that there is no food fortification with vitamin D in Latvia.

Definition of Vitamin D Deficiency. Vitamin D deficiency leads to an increased level of PTH, which results in a high bone turnover state known as secondary hyperparathyroidism. Traditionally, the threshold for vitamin D deficiency has been set as the mean result obtained in population-based studies. However, defining the optimal level of 25(OH)D that would ensure the development and maintenance of a healthy skeleton has proven not to be an easy task. The threshold for vitamin D deficiency is generally considered to be at a serum 25(OH)D level below 20 ng/mL (20). Recently, it has been shown that abnormalities of the parathyroid hormone axis already develop at the level of vitamin D that was earlier thought to be adequate (21). A higher level of PTH combined with an insufficient level of vitamin D facilitates Ca removal from bones and thereby increases the risk of osteoporosis and bone fractures. It has been suggested that vitamin D sufficiency may be best defined as the lowest threshold level of 25(OH)D that prevents increases in PTH. Several studies have been performed to establish this threshold in different populations; however, no data from Latvia are available.

Studies performed in countries at different latitudes have demonstrated an increase in PTH at the threshold level of vitamin D between 10 and 48.8 ng/mL (22, 23). There is a trend observed in these studies showing a lower vitamin D threshold in the countries at lower latitudes. Our study was conducted in Riga that is located at the latitude of 56°58 N. We found the threshold vitamin D level of 38 ng/mL to be sufficient to prevent a rise in the PTH concentration. Our results are consistent with the results obtained in the studies conducted at the similar latitude (24). This level was defined as indicating vitamin D deficiency in our study.

The results of our study revealed a high prevalence of vitamin D deficiency among women living in Riga by a previously identified threshold level. The similar results of the inadequate vitamin D level were also reported in the European study conducted by Bruyère et al. (25). However, it should be noted that this European study included only osteoporotic women while our study focused on the generally healthy population.

Vitamin D Status and its Seasonal Variations. The sun is an important source of vitamin D; therefore, seasonal fluctuations are expected in the countries at high latitudes. A peak of the plasma 25(OH)D concentration is obvious in late summer around 1 to 2 months after the maximum solar radiation, with a nadir in late winter. Plasma PTH mirrors these changes with greater values in late winter due to secondary hyperparathyroidism and low values in late summer. The time of sample collection in our study was based on previous statements. The results demonstrated a significant difference in the vitamin D level between the times of sampling with the lowest level being during winter months and highest in summer while the PTH level mirrored these changes. These findings are consistent with previously reported studies, particularly those performed among postmenopausal women (26). However, in our study, it should be stressed that the prevalence of vitamin D insufficiency remains high (59.8% at a threshold of 38 ng/mL) even during the summer months. This leads to a recommendation that vitamin D supplementations should be advised regardless of the season.

Association with Body Mass Index. An association between vitamin D and body mass index has been described previously as a negative correlation between both parameters (27). The results of our study also demonstrated a significant difference in the level of 25(OH)D in summer across the different subgroups with the lowest level to be found in the persons with higher BMI. Although the explanation for a higher prevalence of vitamin D deficiency in obese individuals is unknown, it has been postulated that the production of 1.25-dihydroxyvitamin, an active vitamin D metabolite, is enhanced, and thus, its higher concentration exerts negative feedback control on the hepatic synthesis of 25(OH)D (28).

However, in our study, no associations in the samples collected during summer were found. Furthermore, a remarkably small difference in the obese individuals between the seasons was documented, while the nonobese participants maintained a significant increase in 25(OH)D in summer. These findings are in accordance with the results of the study by Wortsman et al., demonstrating an increase in 25(OH)D lower by 57% in obese individuals following exposure to UVB irradiation (29). A lack of seasonal fluctuations in the obese individuals observed in our study may be explained also by enhanced vitamin D uptake by the adipose tissue (30). As a result, obese individuals possibly have greater storage of vitamin D that can be used in winter to maintain a stable level of 25(OH)D.

Conclusions

Based on the maximal suppression of PTH, 38 ng/mL was defined as an optimal concentration of 25(OH)D. According to this definition, 70.4% of all the healthy women were classified as vitamin D deficient in winter and 59.8% in summer. The highest proportion of vitamin D deficient individuals both in winter and summer were found in the group representing the postmenopausal women not taking vitamin D supplements. Therefore, it can be concluded that both postmenopausal and premenopausal women with limited exposure to sunlight may benefit from additional vitamin D supplementation.

Statement of Conflict of Interest

The authors state no conflict of interest.

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