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Importance of Vitamin D and Vitamin D levels Status in Puerto Ricans

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Abstract

There is growing and compelling evidence demonstrating the extra-skeletal role of vitamin D and the importance of maintaining adequate levels of this nutrient. Currently, there is very limited information available on the vitamin D status in children and adults in underserved groups, including Puerto Ricans. We assessed the vitamin D status of 4,090 Puerto Ricans living in six geographical regions in the island. Only 31.5% of the studied population had sufficient vitamin D levels (>30 ng/ml). The 18–39 year age group and the females showed inadequate (<30 ng/ml) levels of vitamin D (76.9% and 69.8%, respectively). Participants aged 60 or older showed the highest mean values of serum 25(OH)D (28.8 ng/ ml) and the highest percentage (37.1%) of suffi cient levels (>30 ng/ml). Future studies are certainly warranted to understand the prevalence of vitamin D deficiency and influencing factors (including obesity) in Puerto Ricans.

Keywords

Vitamin D; Puerto Ricans; obesity; Puerto Rico

Vitamin D is a fat-soluble steroid hormone formed as vitamin D3, or cholecalciferol, by human skin cells upon their exposure to ultraviolet-B radiation from sunlight and subsequent conversion to 7-dehydrocholesterol (provitamin D). After conversion, provitamin D is further metabolized, producing 25-hydroxycholecalciferol (calcidiol) and 1,25-dihydroxycholecalciferol (calcitriol). The 25-hydroxycholecalciferol, also called 25(OH)D3, is synthesized in the liver from cholecalciferol by 25-hydroxylase and reflects vitamin D intake and its endogenous production. It represents the pre-hormone and storage state of vitamin D, and—as a major circulating metabolite with a circulating half-life of 15 days—it is considered the best indicator of vitamin D status. Calcitriol (1,25-dihydroxycholecalciferol, also called 1,25(OH)2D3), is produced in the kidney by the action of 1α-hydroxylase, and it is considered to be the steroid's active form. In addition, vascular smooth muscle and endothelial cells have been shown to be able to convert 25(OH)D to

$1,25(OH)_2D$.[1] Calcitriol acts on vitamin D receptors (VDRs), which contain the hormones and DNA binding domains and thus control the genetic expression of several metabolic pathways. However, calcitriol serum levels are not considered clinically relevant when determining vitamin D status due to its short half-life of only a few hours.

Previously, vitamin D was mainly studied for its role in calcium/phosphorus homeostasis and bone health and metabolism. Since VDRs were identified on a large variety of cell types (including myocytes, cardiomyocytes, pancreatic beta-cells, vascular endothelial cells, neurons, immune cells, and osteoblasts[2]) and, more recently, were found in mitochondria,[3] there is increasing compelling evidence for the role of vitamin D levels at the extra-skeletal level. It has been reported that, directly or indirectly, $1,25(OH)_2D$ regulates the expression of over 200 genes, including those involved in renin production in the kidney, insulin production in the pancreas, the release of cytokines from lymphocytes, the production of cathelicidin in macrophages, and the growth and proliferation of vascular smooth muscle cells and cardiomyocytes.[2] Similarly, independent studies have provided evidence for the role of vitamin D and VDRs in obesity,[4] insulin resistance,[5] atherogenic dyslipidemia,[6] prothrombotic states,[7] inflammation,[8] and cardiovascular diseases.[9]

Factors affecting vitamin D levels. There is a great controversy regarding optimal biological circulating levels,[10–11] but the Endocrine Society's Clinical Guidelines[12] and the International Osteoporosis Foundation[13] defined vitamin D deficiency as a person's having a 25(OH)D level of <20 ng/ml and vitamin D insufficiency as a person's level ranging from 21 to 29 ng/ml. For most of the end points studied to date, the suggested optimal circulating levels of 25(OH)D is at least 30 ng/ml.[12–14] Besides the compelling evidence regarding the benefit of adequate vitamin D levels, there are reports worldwide (even in tropical countries where sun exposure is such that vitamin D levels are expected to be optimal) describing what is being called hypovitaminosis D. Despite the scaling system being used, there are two well-studied but non-modifiable factors that contribute to the lowering of vitamin D levels and that remain consistent among the reports: age and dark skin pigmentation. Older people show a reduced capacity for synthesizing UVB-induced cutaneous vitamin D synthesis; in addition, the kidneys in these individuals are less able to convert vitamin D to its active hormone form.[15–16] Americans aged 50 years or older are at increased risk of developing vitamin D insufficiency. After equal doses of sunlight exposure, a 70-year-old person produces 75% less vitamin D_3 than does a 20-year-old person.[17] Skin pigmentation also contributes to lower vitamin D levels because darker skin requires proportionally more sun exposure to synthesize equivalent amounts of vitamin D compared with that needed by people with lighter skin coloration.[2,18] Factors such as living away from the equator, lifestyles (remaining indoors as a habit, remaining indoors out of seasonal/weather considerations, and use of covering cloth for seasonal or cultural reasons), obesity, and diet have been shown to influence vitamin D levels. There are other factors including air pollution, smoking, mal-absorption syndromes, renal and liver diseases, and some medications that may also affect levels of vitamin D.

Population-based studies have shown that the prevalence of vitamin D deficiency increases proportionally with distance from the equator and has been attributed to the increased atmospheric filtering of UVB radiation at higher latitudes.[19] Similarly, indoor lifestyles are

suggested to cause the body to produce less cutaneous vitamin D because a reduced exposure to the sun blocks the first step in the synthesis, which is sun-dependent. Additionally, the increasing use of sunscreens is relevant. Sunscreens with a sun protection factor (SPF) of 15 block approximately 99% of cutaneous vitamin D production.[20–21] In contrast, Krause *et al.*,[22] and later Tangpricha *et al.*,[23] showed that increased exposure to UVB radiation in a tanning bed, three times per week for three months, led to a 180% increase in 25(OH)D levels (albeit while counteracting skin cancer prevention strategies). Obesity is also associated with vitamin D deficiency.[24] Individuals with a body mass index (BMI) greater than or equal to 30 kg/m^2 typically show low plasma concentration of 25(OH)D[24] and a decrease in these levels as obesity and body fat increase.[25] Even with orally administered vitamin D, the BMI is inversely correlated with peak serum concentrations. Compared with non-obese individuals after an equivalent exposure to UVB radiation or a bolus dose of vitamin D_2, obese individuals showed 50% lower blood levels of vitamins D_3 and D_2.[24,26] The epidermal cells present in obese people still retain their functional capacity to synthetize vitamin D, however, it has been proposed that the existance of larger amounts of subcutaneous fat sequesters the available vitamin D, decresasing the effective circulating levels.[26] The daily recommended intake of vitamin D has not yet been clearly defined.[10–11] However, the Institute of Medicine's current recommended dietary reference intakes (DRI) are: 400 IU for infants (0–12 months), 600 IU for people younger than 70 years of age, and 800 IU for those 70 years of age or older.[27] This is important because a meta-analysis of 57,000 individuals showed that a daily intake of less than 500 IU/day vitamin D decreases overall mortality, including cardiovascular (CV) disease mortality,[28] which is one of the leading causes of death in the U.S.

Vitamin D levels and the Puerto Rican population. Although the island of Puerto Rico (PR) is located in a tropical region, to our knowledge there are only two recently published studies addressing vitamin D levels in Puerto Ricans.[29–30] The first[29] was a cross-sectional study consisting of 1,292 Puerto Rican adults (379 men and 913 women, ranging in age from 45 to 75 years) and showed geometric means for plasma 25(OH)D concentrations of 15.8±1.5 ng/ml for men and 16.0±1.5 ng/ml for women. According to these results, 68.6% of the participating men and 65.5% of the participating women were deficient (cutoff of <20 ng/ml). However, these participants were Puerto Ricans living in the Greater Boston area. The second study consisted of a pilot investigation of 98 adults living in PR who were obese or overweight.[30] The authors reported median 25(OH)D levels of 30.7 ng/ml with 55% of the study population presenting adequate levels (>30 ng/ml). The study also revealed that 25(OH)D levels were significantly correlated to vitamin D intake, sun exposure, and percentage of body fat in this population.

Limited information is available on the vitamin D levels status of children and adults in Puerto Rico. Thus, to gain preliminary insights into the vitamin D status of people living in the island, we assessed the vitamin D status of Puerto Ricans living in Puerto Rico.

Methods

Population studied

Laboratory test results of 4, 090 individuals who had vitamin D levels assessed between February and April 2011 at the Immuno Reference Lab Inc. were retrospectively included in this study. These months were selected for controlling for seasonality, in this case variations on the exposure to solar UV radiation (UV index), which varies between months even in PR from a UV index of 7 in January and December of that year to a UV index of 14 in May and June (NOAA Center for Weather and Climate Prediction Center).

The Immuno Reference Lab is located in the Puerto Rico metropolitan area and serves all 78 municipalities of the island. The Ponce School of Medicine and Health Sciences approved an Exempt Review based on Section 6.4 of the Exemption Certification Form under Institutional Review Board Protocol #110523-ES. Identifiers were excluded from the reports provided by the lab oratory and only demographic information (sex, age, and living city) and vitamin D levels were collected. There were no exclusion criteria, as the data were provided if collected during the specified period. If any demographic information was not available, it was assumed as a missing value for the statistical analyses. Vitamin D levels were determined using the Diasorin Liaison platform. This method showed acceptable correlations (R^2=0.90)[31] when compared with the gold standard liquid chromatography tandem mass spectrometry and has been previously validated for routine clinical diagnoses.[32] The vitamin D levels were categorized as follows: deficient (<20 ng/ml), insufficient (20–30 ng/ml), and sufficient (>30 ng/ml).[33]

Statistical analysis

Summary statistics (mean ± standard deviation) and frequency distributions were computed for continuous and categorical variables, respectively. Student's t test was used to compare the distribution of serum 25(OH)D levels by gender. Analysis of variance was used to assess differences by age groups. Chi-square test statistic (linear-by-linear association) was used to compare vitamin D status (insufficient, deficient, or adequate) across age groups and genders. All statistical analyses were performed using Stata version 11 (Stata Corp LP, College Station, TX).

Results

Of the 4,090 individuals who underwent vitamin D status determination, more than two-thirds (86.3%) were 40 years old or older, 83.5% were females, and more than a third (35.1%) were from the San Juan region (Table 1). The distribution of serum 25(OH)D levels showed that 24.9% of individuals were vitamin D deficient, 43.6% had insuffi cient levels, and only 31.5% of the studied population had sufficient vitamin D levels (Table 2).

Vitamin D status differed significantly ($p<.05$) by age and gender (Table 3). Mean values of serum 25(OH)D were significantly ($p<.05$) higher for males (29.3±10.8 ng/ ml) than they were for females (26.9±12.3 ng/ml). Subjects who were 60 years or older also had higher mean values of serum 25(OH)D (28.8±11.7 ng/ml) than did individuals younger than 60

(Table 3). Low vitamin D (deficiency and insufficiency) was highest in the 18–39 year age group (76.9%) and in females (69.8%).

Discussion

Despite the lack of agreement regarding the most reliable determination method[34] and the optimal levels of vitamin D, most studies concur that a cut-off level of 30 ng/ml is adequate.[12–13] According to the Centers for Disease Control and Prevention (CDC),[35] based on the vitamin D levels reported by NHANES data (2001–2006), our results showed that only 31.5% of the studied population presented adequate levels of vitamin D compared with 67% of respondents to NHANES.[36] Similarly, in the NHANES, 8% of males and 12% of females were deficient compared with 26.5% and 16.5%, respectively by sex, in our study population, which represents a more than twofold difference. In the country of Jordan, there is a marked difference between men (5.1%) and women (37.3%) in terms of having insufficient levels of vitamin D (<30 ng/ml). This difference is attributed to the women's wearing of a traditional outfit called a *Hijab* or *Niqab*, with which much of the wearer's skin is covered.[36] Although levels in Puerto Rican women are similar to those found in Jordanian women, this phenomenon might not be explainable only by clothing coverage, because vitamin D levels in Puerto Rican men are also below adequate levels compared with *both* U.S. and Jordanian populations. In the Puerto Rican population, men are more likely to be overweight (BMI: 25–29.9 kg/m^2) with a prevalence of 40.4% *vs.* 33.4% in women. However, more women (43.7%) than men (37.6 %) are obese (BMI>30 kg/m^2).[38] The population studied in Jordan followed the same trend, with women showing a higher prevalence of obesity (53.1%) than men (28.1%). A recent study in overweight and obese Puerto Ricans that showed that vitamin D levels were inversely correlated with % of body fat.[30] Dietary habits and lifestyles should assessed in future epidemiologic studies for following up this observation.

Regarding age, contrary to what would be expected (a decrease with age having been previously demonstrated), people over 60 years old showed significantly higher mean vitamin D levels (28.8±11.7 ng/ml) than did those in younger age groups. This result might be explained if there is focused screening performed in this age group and an emphasis placed on supplement regimens prescribed by physicians for maintaining a balance for vitamins and nutrient intake. In addition, mean vitamin D levels (25.3±10.5 ng/ml) obtained in the young adult age group (18–39 years old) might be explained by inverse reasoning (i.e., people at this younger age in Puerto Rico are not usually screened in this way unless there is evidence of an underlying condition). The lack of monitoring and clinical indications for ingesting supplements is less likely in this age group. In most contexts, only people with calcium-related diseases are ordered to undergo testing to establish a baseline vitamin D level and then are monitored mainly for compliance and effectiveness of the prescribed treatment.[27] In the present study, members of the youngest age group (<18 years) showed a mean representing insufficient levels of vitamin D (27.7±11.3 ng/ml). It is unusual to monitor vitamin D levels in young people unless there is clinical suspicion of rickets (levels below 10). Despite the limited sample size (n=4), younger children (<3 years old) included in this analysis present with even lower mean levels (27.3 ±12.1), which is a matter for concern as bones are actively developing in such young children.

The present study has several limitations. By restricting our analyses to the clinical laboratories that sent the blood samples for vitamin D determination to this reference laboratory (Immuno Reference Lab Inc., Hato Rey, PR), our results may not be extrapolated to all municipalities in the island. Second, the possibility of selection bias cannot be excluded because we lack information about the medical diagnoses that lead physicians to order the test in the first instance. Third, due to the nature of the study, data on risk factors, including sun exposure, dietary vitamin D intake, and BMI, were not available. Despite these limitations, this is the first study assessing vitamin D status in a sample of individuals of all ages in Puerto Rico.

There is a trend towards change in vitamin D status worldwide, which may be explained by genetic, environmental, or behavioral factors or a combination of these. More importantly, independently of the selected cut-off level or method used, there is recent compelling evidence demonstrating an inverse association between vitamin D levels and the development and clinical manifestations of autoimmune, neoplastic, psychiatric, and CV conditions. For these reasons, it is important to promote awareness within the scientific, clinical, and general communities of the health impact and importance of maintaining vitamin D levels over 30 ng/ml.[39] It is imperative that each population establishes baseline levels of vitamin D because the existence of such data will contribute to perform further research discriminating and evaluating factors contributing to this worldwide deficiency. Questions that might be answered by monitoring these values over time may include, Are there problems with exposure to sunlight (duration and quality)? At the conversion or activation steps? During the absorption or metabolism stages?

Future studies are certainly warranted to understand the prevalence of vitamin D deficiency and factors influencing it in Puerto Rico and other areas rich in sunlight.

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Notes

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Table 1

DEMOGRAPHIC CHARACTERISTICS OF 4,090 PARTICIPANTS WHO UNDERWENT SERUM 25(OH)D DETERMINATION[a]

Characteristic	Number (N)	Percent %
Age in years		
<18	87	2.1
18–39	471	11.6
40–59	1,822	45.0
≥60	1,671	41.3
Mean ± SD	55.4±15.3	
Gender		
Female	3,414	83.5
Male	676	16.5
Region		
San Juan	1,440	35.1
Bayamón	671	16.4
Mayaguez	613	15.0
Caguas	532	13.0
Ponce	511	12.5
Arecibo	331	8.0

[a]Numbers may not add up to 4,090 due to missing values.

Table 2

SERUM 25(OH)D STATUS OF PERSONS AGED 1 YEAR OLD AND OVER (N=4,090)[a]

Vitamin D status (ng/ml)	Number (%)
Deficient (<20.0)	1,020 (24.9)
Insufficient (20.0–30.0)	1,790 (43.6)
Sufficient (>30.0)	1,293 (31.5)
Mean ± SD	27.3±12.1
Median (Percentiles 25 and 75)	25.4 (20.0, 32.2)

[a]Numbers may not add up to 4,090 due to missing values.

Table 3

COMPARISON OF VITAMIN D LEVELS BY AGE AND GENDER

Characteristic	n	25(OH)D levels (ng/ml) Mean ± SD	25(OH)D status			p value[a]
			Deficient (<20 ng/ml)	Insufficient (20–30 ng/ml)	Sufficient (>30 ng/ml)	
Age in years						<.001
<18	87	27.7±11.3	21 (24.1)	38 (43.7)	28 (32.2)	
18–39	471	25.3±10.5	152 (32.3)	210 (44.6)	109 (23.1)	
40–59	1,822	26.5±12.7	504 (27.7)	797 (43.7)	521 (28.6)	
≥60	1,671	28.8±11.7	326 (19.5)	725 (43.4)	620 (37.1)	
p value[b]		<0.001				
Gender						<.001
Female	3,414	26.9±12.3	906 (26.5)	1,477 (43.3)	1,031 (30.2)	
Male	676	29.3±10.8	109 (16.1)	308 (45.6)	259 (38.3)	
p value[b]		<0.001				

[a] p value to assess differences in distribution of 25(OH)D status across subgroups.

[b] p value to assess differences in mean levels of 25(OH)D across subgroups.