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ORIGINAL ARTICLE

Correlation between 25-hydroxyvitamin D levels and latitude in Brazilian postmenopausal women: from the Arzoxifene Generations Trial

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Abstract
Summary We investigated vitamin D status in Brazilian cities located at different latitudes. Insufficiency (<50 nmol/L) was common (17 %), even in those living in a tropical climate. Vitamin D insufficiency increased as a function of latitude. Mean 25-hydroxyvitamin D (25(OH)D) levels in each site and latitude correlation were very high (r=−0.88; p<0.0001).
Introduction Inadequate vitamin D, determined by low levels of 25(OH)D, has become very common despite the availability of sunlight at some latitudes. National data from a country that spans a wide range of latitudes would help to determine to what extent latitude or other factors are responsible for vitamin D deficiency. We investigated vitamin D status in cities located at different latitudes in Brazil, a large continental country.
Methods The source is the Brazilian database from the Generations Trial (1,933 osteopenic or osteoporotic postmenopausal women (60 to 85 years old) with 25(OH)D measurements). 25(OH)D below 25 nmol/L (10 ng/mL) was an exclusion criterion. Baseline values were between fall and winter. The sites included Recife, Salvador, Rio de Janeiro, São Paulo, Curitiba, and Porto Alegre. Mean and standard deviation of 25(OH)D, age, spine and femoral neck T-score, calcium, creatinine, and alkaline phosphatase were calculated for each city. Pearson correlation was used for 25(OH)D and latitude.
Results Insufficiency (<50 or <20 ng/mL) was common (329 subjects, 17 %). Vitamin D insufficiency increased as a function of latitude, reaching 24.5 % in the southernmost city, Porto Alegre. The correlation between mean 25(OH)D

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levels in each site and latitude was very high ($r=-0.88$, $p<0.0001$).
Conclusion There is a high percentage of individuals with vitamin D insufficiency in Brazil, even in cities near the equator, and this percentage progressively increases with more southern latitudes.

Keywords Brazilian postmenopausal · Latitude · Vitamin D insufficiency · Zenith angle

Introduction

Vitamin D, a major regulator of calcium and phosphate homeostasis, is critically important not only for normal bone metabolism, but also for the normal physiology of other tissues and systems, including immunomodulation, cell proliferation, and differentiation [1–3]. Skin is the natural source of vitamin D through activation steps initiated by UV-B sunlight radiation. Latitude, seasonal variation, as well as skin pigmentation and age are among the many factors that can influence this cutaneous step [4–6].

Solar radiation levels at the critical wavelength (290–315 nm) to convert 7-dehydrocholesterol to previtamin D reach the earth's surface as a function of the changing solar zenith angle, which decreases poleward [4]. Similarly, the effective incident radiation reaching the earth's surface is reduced during the fall and winter months when the sun is "lower in the sky" [5, 7]. Therefore, variation in cutaneous UV-B radiation exposure due to seasonal variation or geographical location can influence the amount of vitamin D_3 synthesized in the skin. Other factors are thought to contribute to vitamin D inadequacy, as determined by low levels of 25-hydroxyvitamin D (25(OH)D). In modern urban societies, indoor activities are more common, use of sunscreens in the context of outdoor activities, aging that diminishes the skin's capacity to respond to sunlight, atmospheric pollution in big cities, and changes in food consumption patterns are all important factors that have led some to describe a worldwide epidemic of vitamin D deficiency, even in countries known for its sun, like Brazil [7, 8]. Despite these key contributing factors, the importance of latitude and, consequently, the effect of sun radiation angle are not very well defined. National data from a country that spans a wide range of latitudes would help to determine to what extent latitude contributes to vitamin D inadequacy.

The large Brazilian database cohort from the Arzoxifene Generations Study [9] gave us the opportunity to investigate the relationship between 25(OH)D levels at a wide range of latitudes in this country.

Methods

The source of the data is the Brazilian database from the Phase 3 Arzoxifene Study (The Generations Trial) which included 1,933 osteopenic or osteoporotic postmenopausal women (60 to 85 years old) who had 25(OH)D measured. The following were exclusionary criteria: any condition associated with abnormal bone metabolism; 25(OH)D level <25 nmol/L (10 ng/mL); unexplained or abnormal vaginal bleeding within 6 months; history of breast cancer or estrogen-dependent neoplasia; any history of venous thromboembolism, stroke, or transient ischemic attack; liver disease; impaired kidney function; any endocrine disorders besides type 2 diabetes and hypothyroidism that required pharmacologic therapy; bisphosphonate therapy in the previous year if treatment had been less than 1 year or within 3 years if bisphosphonate therapy had lasted more than 1 year; and use of aromatase inhibitors, estrogens, calcitonin, or SERMs within the previous 3 months, sodium fluoride within the past 6 months, or more than 1 month of systemic corticosteroids in the previous 6 months [9]. Vitamin D supplement intake was not an exclusion criterion. In this study, 25(OH)D level was obtained during the screening visit which was prior to the elemental calcium and vitamin D supplementation being introduced. Nevertheless, when The Generations Trial began, vitamin D supplementation was not routine, and vitamin D supplements were not generally available in Brazil. Fasting blood samples were obtained during the fall and winter months. Sampling came from three regions in Brazil: Recife ($n=208$) and Salvador ($n=104$) located in the northeast; Rio de Janeiro ($n=514$) and São Paulo ($n=928$) located in the southeast; and Curitiba ($n=126$) and Porto Alegre ($n=53$) located in the south. Recife, Salvador, and Rio de Janeiro are coastal cities. São Paulo, Curitiba, and Porto Alegre are approximately 70 to 100 km inland.

25(OH)D was measured by the DiaSorin radioimmunoassay [formerly Incstar Corporation, Stillwater, MN] in which the intra-assay and inter-assay precision is 8.6–12.5 and 8.2–11 %, respectively. Other indices that were measured are ionized calcium (in millimoles per liter), creatinine (in micromoles per liter), and total alkaline phosphatase (in units per liter). Bone mineral density (BMD) at the femoral neck and lumbar spine was measured by dual-energy X-ray absorptiometry. Body mass index (BMI) was available for 967 subjects. We defined a 25(OH)D concentration equal or higher than 50 nmol/L (20 ng/mL) as vitamin D adequacy, as recommended by the Institute of Medicine [10].

The protocol was approved by the institutional review boards at each investigative site and by the Brazilian Ethics Committee for Clinical Research [9]. All patients gave written informed consent

All statistical analyses were performed using BioStat 2005 software. Descriptive statistics (mean, standard deviation) were evaluated within each subgroup. Parametric one-way ANOVA and Pearson correlation were utilized. Linear regression was calculated as needed. A p value of <0.05 two-tailed tests was considered significant.

Results

The main characteristics of this Brazilian cohort of postmenopausal women are shown in Table 1. There were no major differences in Table 1 indices except for age, BMI (footnote in Table 1), and 25(OH)D level. For 25(OH)D, there were significant differences between the sites ($p<0.05$). In the northernmost cities (Recife and Salvador), 25(OH)D was significantly higher than in Rio de Janeiro, São Paulo, Curitiba, and Porto Alegre.

For the entire population, 83 % had 25(OH)D higher than 50 nmol/L (20 ng/mL), using the definition of vitamin D sufficiency of the Institute of Medicine. On the other hand, only 31.7 % ($n=612$) had 25(OH)D higher than 75 nmol/L (30 ng/mL), the cutoff of vitamin D sufficiency recommended by The Endocrine Society [11]. The prevalence of vitamin D inadequacy (<50 nmol/L) increased with progressively more southern latitudes, reaching a peak prevalence of 24.5 % in Porto Alegre, the most southern city in this study (33°05′S). This prevalence was more than two times greater than in Recife and Salvador (Fig. 1). The correlation between all 25(OH)D measurements and latitude was significant ($r=-0.3$, $p<0.0001$). This correlation was still present if we analyze 25(OH)D and latitude in the different seasons of the year, being higher in the fall ($r=-0.22$, $p<0.0001$) than in the winter ($r=-0.14$, $p=0.0016$). By linear regression, using the mean 25(OH)D levels of each site and respective latitude, the correlation was very high ($r=-0.88$, $p=0.02$). On average, for each degree of latitude further south, there was a 0.7-nmol/L (0.28 ng/mL) reduction in 25(OH)D concentration (Fig. 2).

We found no correlation between 25(OH)D levels and age, calcium, creatinine, femoral neck BMD, lumbar spine BMD, or with total alkaline phosphatase. There was a weak but significant correlation between 25(OH)D and BMI among the 967 subjects for whom BMI was known ($r=-0.14$, $p<0.001$). Without specific knowledge of skin color or ethnicity in our participants, we resorted to data from the Brazilian Institute of Statistics and Geography (IBGE) [12]. Proportion of patients with white skin tone and insufficiency in vitamin D in each site is shown in Fig. 3.

Discussion

This is one of the only studies to evaluate serum 25(OH)D concentrations as a function of latitude in a country of the southern hemisphere. In Argentina, Ladizesky et al. showed lower photoproduction of previtamin D and vitamin D in Ushuaia (55° S) than in Buenos Aires (34° S) [6]. In a study based in North America, vitamin D levels were studied in three different latitude cutoffs (<35° N, between 35° and 42° N, or >42° N), a trend between vitamin D levels and latitudes was found, but the differences did not reach statistical significance [13].

We found an inverse correlation between mean serum 25(OH)D level site and latitude, with a mean reduction of 0.7 nmol/L (0.28 ng/mL) for each latitude's degree south of the equatorial line (Fig. 2).

The threshold value for 25(OH)D sufficiency is controversial, particularly in view of the Institute of Medicine's recent recommendations [10] and official disagreement

Table 1 Characteristics of 1,933 postmenopausal women from each city. The data are presented as mean (SD)

City (latitude)	Number of patients	Age (years)	BMI[a]	25(OH)D (nmol/L)	Lumbar spine T-score	Femoral neck T-score	Calcium (mmol/L)	Creatinine (μmol/L)	Alkaline phosphatase (U/L)
All	1,933	67.1 (5.2)	26.9 (4.4)	68.1 (21.5)	−2.5 (1.0)	−1.8 (0.7)	2.42 (0.09)	69.6 (13.4)	84 (22.7)
Recife (8°04′S)	208	67.6 (5.3)	28 (4.3)	74.6 (23)	−2.5 (1.0)	−1.9 (0.7)	2.44 (0.10)	67.8 (12.8)	87.9 (22.7)
Salvador (12°58′S)	104	66.5 (4.5)	26.4 (3.9)	82.4 (27.9)	−2.5 (1.0)	−1.8 (0.6)	2.41 (0.08)	69.6 (13.0)	80.6 (21.8)
Rio de Janeiro (22°53′S)	514	68.3 (5.4)	25.9 (3.8)	67.9 (20.8)	−2.6 (1.0)	−2.0 (0.7)	2.41 (0.08)	67.8 (14)	82.5 (23.1)
São Paulo (23°32′S)	928	66.5 (5.1)	27.2 (4.5)	66 (18.1)	−2.5 (1.0)	−1.7 (0.8)	2.43 (0.09)	70.5 (13.1)	83.8 (22.7)
Curitiba (25°25′S)	126	66.6 (5.5)	27.5 (4.9)	65.5 (31.4)	−2.3 (1.0)	−1.7 (0.4)	2.45 (0.09)	72.8 (13.8)	86.4 (19.1)
Porto Alegre (33°05′S)	53	66.8 (4.6)	26.5 (4.9)	61.8 (18.5)	−3.2 (0.8)	−2.0 (0.7)	2.42 (0.08)	71 (14.4)	85.2 (26)

The mean age from Rio de Janeiro showed a small but significant difference compared to Salvador, São Paulo, and Curitiba. One-way ANOVA showed that BMI was different in Rio de Janeiro compared to Recife and São Paulo ($p<0.01$)

[a] $n=967$ (104, 52, 258, 464, 62, and 27, respectively, in each site)

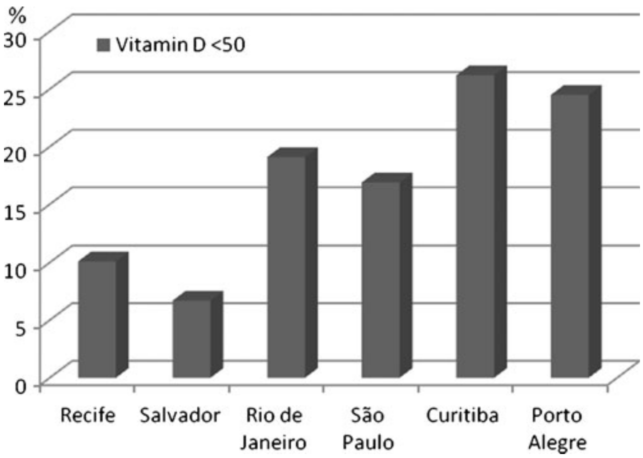

Fig. 1 Prevalence of vitamin D insufficiency (in nanomoles per liter) in each site. Patients with 25(OH)D below 25 nmol/L were excluded

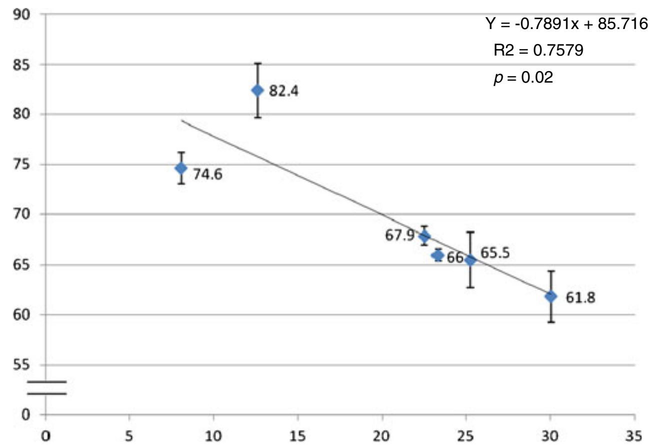

Fig. 2 Linear regression showing the relationship between mean ± SEM 25(OH)D (in nanomoles per liter) in each site and latitude (in degrees)

about those recommendations [11]. There is no debate, however, on the level of 50 nmol/L (20 ng/mL) below which vitamin D deficiency is clearly present. When this lower level is taken as the threshold value, vitamin D deficiency is appreciable, but not as frequently seen as it is when the higher threshold is used. Using either threshold, though, a latitude-dependent trend is clearly evident. Using the threshold value of 75 nmol/L for vitamin D sufficiency, as recommended by The Endocrine Society [11], less than 40 % of this postmenopausal Brazilian population was sufficient. Moving south, a sharper increase in insufficiency is seen, reaching 83 % in Porto Alegre (8°04′S). The Endocrine Society recommendations are consistent with another Brazilian study in which similar percentages for vitamin D deficiency (>75 nmol/L) were found among postmenopausal women [14].

In Brazil, the summer/winter seasonal ratio of UV-B irradiation reaching the earth's surface increases proportionally to latitude [more than three times higher in Porto Alegre (33°05′S) compared to Natal (5°84′S)] [15]. This difference in UV-B irradiation is due to latitude-dependent variability in the solar zenith angle as well as higher ozone columns far from the equator. This suggests that vitamin D production in the skin can vary by latitude. Another useful parameter is the UV index, an indication of UV radiation intensity. The UV index is higher in lower latitude cities, such as Natal (5°84′S), which is near Recife (8°04′S). Recife and Salvador, thus, have higher UV-B exposure during the day and season of the year than the more southern latitude cities of Curitiba and Porto Alegre [15]. Not unexpectedly, therefore, the proportion of patients with 25(OH)D below 50 nmol/L (20 ng/mL) clearly increases as a function of latitude (Fig. 1), being more than two times higher in cities located in the south (Curitiba and Porto Alegre) compared to the northeast ones (Recife and Salvador).

Skin color can interfere in vitamin D production. When individuals of different skin pigmentation are exposed to the same suberythemic, whole-body dose of UV-B radiation (27 mJ/cm^2), post-UV-B levels are significantly higher in whites than in Indians (South Asian) or blacks [16]. In Brazil, southern (Curitiba and Porto Alegre) cities have a higher proportion of white skin tone than the northeastern (Recife and Salvador) ones [12], as shown in Fig. 3. Nevertheless, we found higher levels of vitamin D in the northeastern cities, leading us to hypothesize that the amount of UV-B available is more important than skin color of our population. This is a speculation due to the fact that we had no access to the race or skin pigmentation of these patients.

Air pollution could be another confounding factor because it pollutants can efficiently absorb UV-B photons and thus reducing the cutaneous synthesis of precholecalciferol [5, 7]. Nevertheless, from 2002 to 2008, the maximal ozone concentration (in milligrams per cubic meter) data from the IBGE showed higher pollution in São Paulo and Rio de Janeiro than in Curitiba and Porto Alegre [17], where we

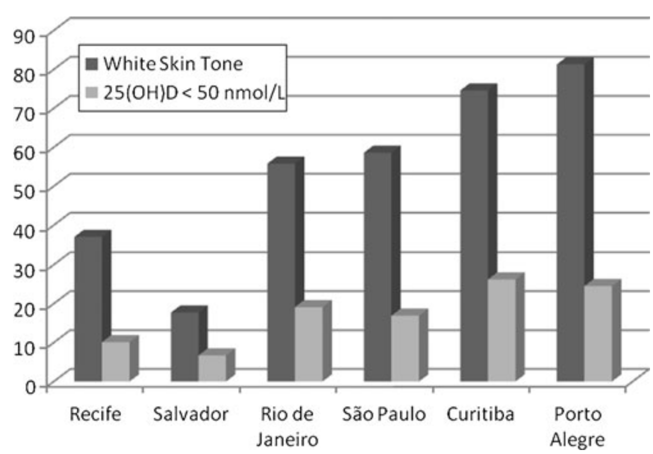

Fig. 3 Percentage of postmenopausal women with white skin tone in each city (data obtained from IBGE) and insufficient levels of 25(OH)D for the respective site

found the lowest levels of 25(OH)D. Air pollution, thus, cannot account in a major way for our observations.

In contrast to Northern European countries [18], where there is a high dietary consumption of fatty fish and cod liver oil, which may explain the lower prevalence of vitamin D deficiency in these countries compared to others, including Mediterranean ones, Brazil has no food fortification policy and vitamin D provided by diet is negligible [19]. Mean daily dietary intake of vitamin D in this study was estimated to be less than 2.2 μg [19], a value that is much lower than the Institute of Medicine's recommendations at the time this study was conducted, namely 5–15 μg [20]. We believe that the vast majority of the Brazilian women in this study were not taking anything more than trivial amounts of vitamin D supplementation due to the fact that at that time, vitamin D supplements were not routinely available in Brazil.

We found an inverse correlation between 25(OH)D and BMI, which is in agreement with the findings shown by Vimaleswaran et al. [21]. Mean BMI was very similar between most cities from this study, except for Rio de Janeiro, compared to Recife and São Paulo.

This study has limitations. We have no specific information about skin color, sun exposure, or sunscreen use among these patients. 25(OH)D measurement below 25 nmol/L was an exclusion criterion, so we cannot comment on severely deficient individuals, if they existed. This seems unlikely from the work of Kuchuk et al. [22] in which less than 1 % of the population in winter or summer had levels less than 25 nmol/L.

In conclusion, we have demonstrated that vitamin D status in postmenopausal women is latitude dependent in the southern hemispheric country of Brazil.

Conflicts of interest Dr. Alan Y. Chiang works at Eli Lilly and Company. The other authors have no competing interests.

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