Seasonal variation of vitamin D among healthy adult men in a subtropical region

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SUMMARY

OBJECTIVE: To evaluate seasonal variation of 25(OH)vitamin D [25(OH)D3] levels, and factors associated with it, in healthy adult men, who exercised outdoors for 50 min., at least twice a week, from 10AM to 4PM, in a Brazilian semitropical region.

METHODS: Blood samples were collected at the end of each season for 25(OH)D3, measured by liquid chromatography with tandem mass spectrometry. Ultraviolet irradiation was estimated by radiometer, calculating the daily photobiological response to vitamin D synthesis in human skin (D-VitD). The prevalence of 25(OH)D3 <20ng/mL changed with the seasons (p=0.000): 8.7% (n=6/69), 1.5% (n=1/66), 0 (n=0/64), and 21.7% (n=13/60), respectively, at the end of winter, spring, summer, and autumn. The prevalence, adjusted for multiple comparisons, was higher in winter than summer (p=0.026), and in autumn than spring (p=0.001) and summer (p=0.000). There were no associations of 25(OH) D3 levels with BMI (p=0.207), body fat (p=0.064), and phototype (p=0.485), in univariate analysis. It was associated with D-VitD in the 30 days before blood sampling (p=0.000), after adjustment to body fat. The prevalence of 25(OH) D3 <30ng/mL varied seasonally (p=0.000): 69.6% (n=48/69), 68.2% (n=45/66), 43.8% (n=28/64), and 88.4% (n=53/60), respectively, in winter, spring, summer, and autumn.

CONCLUSIONS: In a Brazilian subtropical region, a seasonal variation in 25(OH)D3 was observed in healthy adult males, although they spent at least 50 min outdoors twice a week, wearing shorts and T-shirts. 25(OH)D3 <20ng/mL was 21.7% in autumn; D-vitD 30 days prior to blood sampling was the only factor independently associated with 25(OH)D3 levels.

KEYWORDS: Cholecalciferol. Vitamin D. Vitamin D deficiency. Seasons. Ultraviolet rays.
INTRODUCTION

Vitamin D deficiency has been associated with several diseases\(^1\,^2\), and it has been observed in sunny areas, such as the semitropical region of the southern hemisphere\(^3\,^5\). Human vitamin D sources are food intake or ultraviolet B radiation (UVB)-induced skin production, which has been associated to its content of 7-dehydrocholesterol, UVB wavelength, phototype, sunblock use, latitude, season, time of the day, weather conditions, area and length of exposure, and age\(^2\,^6\). Our aim was to evaluate the seasonal variation of 25-hydroxyvitamin D3 [25(OH)D3] in Porto Alegre, RS, Brasil (30° 1'40" S and 51° 13'43" W), and its associated factors in healthy male adults who practiced regular outdoor activities.

METHODS

Ethics statement

All procedures were approved by the Ethics Committee in Research of the Hospital de Clinicas de Porto Alegre, under number 14-0173, and CAEE number 28822014.5.0000.5327. Written informed consent was obtained from all subjects. All authors declare no conflicts of interest.

Subjects

Male military police officers of Porto Alegre/RS/Brasil, aged ≥18 years to ≤55 years were invited to participate. The exclusion criteria were body mass index (BMI) ≥39 Kg/m\(^2\), travel within the last 3 months, use of vitamin D supplements or diuretics, anticonvulsants, glucocorticoids, anti-HIV or antifungal medications, history of bariatric surgery, and known diseases, which could interfere with vitamin D metabolism. As part of their professional duties, they performed outdoor physical activities wearing shorts and T-shirts at least twice a week from 10 AM to 4 PM.

Experimental design: four cross-sectional studies.

Logistics

Participants were evaluated by a trained healthcare professional, who measured their weight and height, while barefoot, on a standing stadiometer and a digital scale, and body fat content was calculated by the measurement of seven cutaneous folds with a caliper. They were divided into 3 groups, according to the Fitzpatrick phototype classification: 1= I+II, 2= III+IV, and 3=V+VI. All participants answered a questionnaire about current tobacco use, alcohol intake (frequent or not), use of prescription drugs, vitamin D supplements or sunblock, and known diseases.

Biochemical data

Blood samples were collected on the last day of each season for 25(OH)D\(_3\) measurements. In autumn, blood was collected after overnight fast to measure plasma PTH, and serum total calcium, creatinine, and albumin. Serum and plasma were kept at -70ºC. Total serum calcium, creatinine, and albumin levels were measured by routine assays. Intact PTH was measured by chemiluminescence (ARCHITECT, Abbott Diagnostics, Wiesbaden, Germany) with an intra-assay variation of 4.1%. All samples were measured in the same assay.

Plasma concentrations of 25(OH)D\(_3\) were measured by Liquid Chromatography with tandem mass spectrometry after protein precipitation. Briefly, 100 μL of plasma was transferred to a 2mL polypropylene tube with 200 μl of acetonitrile and the 20ng/mL internal standard of D6-25(OH)D\(_3\), and Vortex mixed for 1 minute. After centrifugation at 12,000g for 15 minutes, 15 μL of the supernatant was injected into an Ultimate 3000 XRS UHPLC system (Thermo Scientific, San Jose, USA). The separation was performed in an Acquity C18 column (150 × 2.6 mm, p.d. 1.7 μm) from Waters (Milford, USA), maintained at 40°C. The mobile phase was a mixture of 0.1% formic acid in water and methanol (20:80, v/v), eluted at a flow rate of 0.25mL min\(^{-1}\). Detection was performed in a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Scientific, San Jose, USA) with an atmospheric-pressure chemical ionization (APCI) probe. The MS settings were: positive ionization mode, corona discharge needle voltage 7 kV; sheath gas, nitrogen at a flow rate of 60 arbitrary units; auxiliary gas, nitrogen at a flow rate of 5 arbitrary units; collision gas, argon; vaporizer temperature at 390°C; and ion transfer capillary temperature at 202°C. The scan time was set to 0.3 seconds per transition. The following transitions were used for RM acquisition: m/z 401 → 365 (quantification), 401 → 159, and 401 → 105 (qualification) for 25(OH)D\(_3\); and m/z 407 → 371 (quantification), and m/z 407 → 105, and 401 → 91 (qualification) of internal standard. The method was linear from 5.0 to 100.0 ng mL\(^{-1}\) (r=0.999). Accuracy and imprecision were acceptable with an accuracy between 90.7 and 103.4%, and within and between assay coefficients of variation in the range of 2.8-7.5% and 3.9-7.8%,
respectively. Daily calibration curves were included in all analytical batches. Commercial quality control samples from Chromsystem® (Munich, Germany) were processed every 20 samples.

Two 25(OH)D cut-off levels were used to classify vitamin D status: <20ng/mL and <30ng/mL.

### Ultraviolet radiation measurement

UV-R was measured from solar radiation using the Model 501 calibrated radiometer of Solarlight (https://solarlight.com/product/uvb-biometer-model-501-radiometer/), which has a normalized spectral response for the 297nm unit, simulating skin response to the formation of erythema. The equipment was stabilized at 25°C in order to prevent changes in spectral response and in sensitivity to variations in ambient conditions. The irradiiances [Wm⁻²], weighted by the photobiological response to erythema formation (D-Ery), were collected at 1-second intervals, and the mean of these values was recorded every 10 minutes. Irradiances were integrated between 07:00 AM and 5:00 PM (local time) in order to evaluate the amount of UV-R accumulated in daily exposures, and total daily doses (D-Ery) [Jm⁻²] were determined. Since this photobiological response depends almost exclusively on UVB-R, a conversion factor based on the total ozone content and the position of the sun was used to determine it. This conversion factor aims to represent the UVB-R attenuation processes caused by both meteorological parameters.

### Statistical analysis

The distribution of data was evaluated by the Kolmogorov-Smirnov test. Mean 25(OH)D₃ levels for the 4 seasons and their associated factors were compared by the Generalised Estimating Equation method, adjusted to multiple comparisons by the Bonferroni test. Mean seasonal doses of D-VitD were compared by one-way analysis of variance (ANOVA), adjusted for multiple comparisons by the Tukey HSD test. The prevalence of 25(OH)D <20ng/mL and <30ng/mL at the end of each season was calculated by the likelihood-ratio chi-square test, adjusted for multiple pairwise comparisons by the Bonferroni test. The PTH and 25(OH)D correlation was evaluated by the Pearson test. All analyses were made in the SPSS (Statistical Package for Social Studies) software, version 18.0, except for the analysis of the prevalence of low vitamin D, which was conducted in the WINPEPI software, version 11.65.

### RESULTS

One hundred and ten men were invited to participate and 71 accepted. Two were excluded: 1 for using a vitamin D supplement, and 1 for having a BMI ≥39Kg/m², so 69 were included in the 1st evaluation. In the 2nd evaluation, 3 were excluded for traveling, so 66 were included; in the 3rd evaluation, 2 were excluded (1 suffered a gunshot wound and 1 gave up participating), so 64 were included; and in the 4th evaluation, 4 gave up participating, so 60 were included. The clinical characteristics of the participants are shown in Table 1.

### TABLE 1. CLINICAL CHARACTERISTICS OF THE PARTICIPANTS

| Age (years) | Mean±SD or n | n |
|-------------|--------------|---|
| BMI (Kg/m²) | 25.2±2.5     | 69 |
| Body fat (%) | 17.8±3.2     | 62 |
| Phototype*  | 1: 35, 2: 31, and 3: 3 | 69 |
| Use of sunscreen | 2 | 69 |
| Smoking     | 4            | 69 |
| Frequent alcohol intake | 0 | 69 |
| Chronic use of medications | 7 | 69 |
| Known diseases | 3 | 69 |

Phototype*: 1 = Fitzpatrick phototype I + II; 2 = Fitzpatrick phototype III + IV; 3 = Fitzpatrick phototype V + VI. Data are shown as mean ± SD or number (n)

### Plasma 25(OH)D³ levels

Mean 25(OH)D₃ levels changed with seasons (p<0.001), respectively, 27.2 ± 6.6ng/mL (n=69), 28.9 ± 6.1ng/mL (n=66), 31.7 ± 6.4ng/mL (n=64), and 23.3 ± 5.2ng/mL (n=60), in winter, spring, summer, and autumn. Pairwise comparisons were all different (p<0.000), adjusted by the Bonferroni test, except when comparing levels at the end of summer and spring (p=0.139), as shown in table 2. In autumn, mean serum albumin, total calcium, PTH, and creatinine levels were 4.4±1.3g/dL, 8.9±2.3mg/dL, 59.2±22.8pg/mL, and 1.03±0.24mg/dL, respectively.

There was a seasonal variation in the 25(OH)D₃ <20ng/mL prevalence (p<0.000): 8.7% (n=6), 1.5% (n=1), zero, and 21.7% (n=13), respectively, at the end of winter, spring, summer, and autumn. Prevalence was higher in winter than in summer (p<0.026), and
in autumn than in spring (p=0.001) and in summer (p=0.000); it was similar in winter and in spring (p=0.283), in winter and in autumn (p=0.227), and in spring and in summer (p=1). The prevalence of 25(OH)D3 <30ng/mL changed with the seasons (p=0.000), and it was 69.6% (n=48), 68.2% (n=45), 43.8% (n=28), and 88.4% (n=53), respectively, in winter, spring, summer, and autumn. It was higher in autumn than in spring (p=0.033) or in summer (p=0.000); and in winter than in spring (p=0.015) and in summer (p=0.029) than in summer. It was similar in winter and in spring (p=1.000), and in winter and in autumn (p=0.05). These data are shown in Figure 1.

**Measurement of UV-R**

There was a seasonal variation in mean D-vitD (p=0.000), which was 913.3±383.9, 1937.8±934.0, 1945.9±1180.0, and 903.6±507.2 Jm⁻², in winter, spring, summer, and autumn, respectively. D-vitD was higher in spring than in winter (p=0.000) and in autumn (p=0.000); and in summer than in winter (p=0.000) and in autumn (p=0.000); it was similar when comparing winter and autumn (p=0.000) and spring and summer (p=1.000). Mean D-VitD measured in periods prior to blood sampling are shown in Table 2.

**Factors associated with 25(OH)D3**

Correlations between mean 25(OH)D3 levels at the end of each season and mean D-VitD before blood sampling are shown in Table 2.

There was no association between 25(OH)D3 levels and BMI (p=0.207), body fat content (p=0.064), and phototype (p=0.485). In a multivariate regression model including mean D-vitD and body fat content, mean D-vitD in the 30 days before blood sampling was independently associated with 25(OH)D3 levels. These data are shown in Table 3. PTH and 25(OH)D 3 levels were inversely correlated (r=-0.308, p=0.019) at the end of autumn.

**TABLE 2.** MEAN 25(OH) VITAMIN D3 [25(OH)D3] LEVELS AND ITS CORRELATION WITH THE MEAN ULTRAVIOLET LIGHT RESPONSIBLE FOR VITAMIN D SYNTHESIS IN HUMAN SKIN (D-VITD) IN THE 30, 45, AND 90 DAYS BEFORE BLOOD SAMPLING

|          | 25(OH)D₃ (ng/mL)* | D-vitD 30 days (Jm⁻²) | D-vitD 45 days (Jm⁻²) | D-vitD 90 days (Jm⁻²) |
|----------|-------------------|-----------------------|-----------------------|-----------------------|
| Winter   | 27.2 ± 6.6        | 1038.5 ± 301.8        | 950.1 ± 165.9         | 626.1 ± 406.6         |
| Spring   | 28.9 ± 6.1        | 1488.4±1016.2         | 1485.8±1328.7         | 1036.7 ± 584.5        |
| Summer   | 31.7 ± 6.4        | 2192.2 ± 492.5        | 1876.0 ± 521.4        | 2326.9±1057.9         |
| Autumn   | 23.3 ± 5.2        | 606.7±285.5           | 630.9 ± 294.9         | 1779.3 ± 102.7        |
| r**      | 0.982; p= 0.018   | 0.972; p= 0.024        | 0.276; p= 0.723        |

*Mean 25(OH)D3 were compared by the Generalised Estimating Equation method: p=0.000, when comparing all four seasons, and all pairwise comparisons adjusted to multiple comparisons by the Bonferroni test, except between spring and summer (p=0.139). ** Pearson correlation test between mean 25(OH)D3 and mean D-VitD before blood sampling.

**FIGURE 1**

Prevalence of vitamin 25(OH)D3 changed at the end of seasons, for both cut-off points, <20 ng/mL (A) and <30 ng/mL (B), (p=0.000), by the likelihood-ratio chi-square test. The prevalence of vitamin 25(OH)D3 <30ng/mL was similar in autumn (21.7%) vs. winter (8.7%), p=0.227, in spring (1.5%) vs. summer (0%), p=1.000, and in winter vs. spring, p=0.285. The prevalence of vitamin 25(OH)D3 <30 ng/mL was 43.8%, in summer, it was similar in winter (69.6%) vs. spring (68.2%), p=1.000, and autumn (88.4%), p=0.050. Other multiple pairwise comparisons are shown in the panels, and all were adjusted by the Bonferroni test.

**TABLE 3. FACTORS ASSOCIATED WITH 25(OH)D3 LEVELS, MULTIVARIATE MODEL**

| Parameter             | B     | p   |
|-----------------------|-------|-----|
| Intercept             | 26.146| 0.000|
| Mean UVB 30 days before blood sampling | 0.004 | 0.000|
| Body fat (%)          | -0.212| 0.097|

Dependent variable: 25(OH)D3. Model (intercept), UVB 30 days before blood sampling, body fat content.
DISCUSSION

Our results have shown a seasonal variation of 25(OH)D$_3$ in healthy adult men, living in a semitropical region, which was associated with the mean D-VitD in the 30 and 45 previous days. These data are in line with studies conducted in subtropical areas with the elderly$^{15,16}$, and in two large studies, one including subjects aged from 2-95 years$^3$, and another with children$^{15}$, with vitamin D peaks in autumn and troughs in spring.

In our study, peak vitamin D was observed in summer and spring, and a quite unexpected trough in autumn. Nevertheless, these results are in agreement with the low levels of D-VitD measured at 30 and 45 days before blood sampling in the autumn. Besides, they are in accordance with the D-VitD, which peaked in summer and spring, and was lower in autumn and winter. As data were collected in just one year, we cannot exclude a shifting in D-vitD in the earth’s surface, in a given season, caused by meteorological variables as cloud coverage, aerosol pollution, or local ozone content variation.$^9$

A surprising aspect of our study was the high prevalence of 25(OH)D$_3$ <30ng/mL, which ranged from 43.8% in summer to 88.4% in autumn. There was no inflammation$^3$, nor hypoalbuminemia, which could have been implicated$^{15}$, and they exercised outdoors regularly with light clothing, exposing a skin area considered to provide enough vitamin D$^2$. Genetic factors, which appear to contribute 70% to the seasonal variation of 25(OH)D$_3$ levels, could have been implicated$^{13,16}$. Nevertheless, even with the cut-off of <20ng/mL, which has been deemed sufficient by the Institute of Medicine of the USA for practically all persons, when considering bone health$^7$, 21.7% of the subjects had low vitamin D at the end of autumn. Probably, at this time of the year, there was not enough D-vitD to provide the needed synthesis of vitamin D.

Several factors have been shown to influence the amount of solar UVB-R which reaches the surface of the earth, such as atmospheric dispersion of solar rays, air attenuation, absorption by molecular oxygen and ozone, and the line structure in the solar spectrum$^2$.

In addition to UVB-R indices, other factors might affect 25(OH)D levels, such as clothing and the time spent outdoors$^{17,18}$. Although our subjects exercised at least twice a week outdoors wearing light clothing, as part of their professional schedule, this was not enough to keep 25(OH)D in the recommended levels$^7$, so probably the amount of D-vitD was not sufficient to provide adequate vitamin D. Vitamin D has been shown to increase with exposed body area$^{21-23}$, although a plateau in its response has been suggested when more than 33% of body area was irradiated$^{19}$. Nevertheless, in a more recent study, a positive association was found between 25(OH)D$_3$ levels and exposed body area$^{20}$. In another study, biweekly exposure of 88% of body area to 1 Standard Erythemal Dose treatment was sufficient to maintain appropriate levels of 25(OH)D$_3$.$^{22}$

In our study, only two participants used sunscreen, so it was not possible to evaluate its association with vitamin D. Also, there was no association between 25(OH)D$_3$ levels and body fat content, which could have been due to the small sample, since low vitamin D levels have been consistently reported in obesity$^{3,23}$. As expected, PTH levels were inversely proportional to 25(OH)D$_3$ levels.

The strengths of our work were the prospective collection and measurements on the same individuals in the 4 cross-sections; its weaknesses were no individual UV-R measurements and no assessment of dietary factors, which could contribute to 25(OH)D$_3$ levels variability.

CONCLUSIONS

25(OH)D$_3$ levels changed seasonally in healthy adult males in Southern Brasil, which was strongly and independently associated with UV-R indexes 30 days before blood sampling. The prevalence of 25(OH)D$_3$ <20ng/mL in late summer and spring was nil or low; however, it increased in late autumn and winter, although our subjects spent at least 50 min outdoors twice a week with light clothing, from 10 AM to 4 PM. Therefore, probably, D-vitD at this time of the year was not sufficient to provide adequate vitamin D. The prevalence of 25(OH)D$_3$ <30ng/mL was high during all seasons of the year, especially in autumn.

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RESUMO

OBJETIVOS: Avaliar a sazonalidade da 25(OH)vitamina D3 [25(OH)D3] e fatores associados em homens adultos saudáveis, que se exercitavam ao ar livre pelo menos 50 min duas vezes por semana, das 10 às 16h, em uma região subtropical.

MÉTODOS: Sangue foi colhido no fim das estações para medir 25(OH)D3, por cromatografia líquida em tandem com espectroscopia de massas. A radiação ultravioleta foi estimada por radiômetro, calculando diariamente a resposta fotobiológica para sintetizar vitamina D no pele humana (D-VitD).

RESULTADOS: A prevalência de 25(OH)D3 <20ng/mL foi sazonal (p<0.000): 8.7% (n=6/69), 1.5% (n=1/66), 0% (n=0/64), e 21.7% (n=13/60), respectivamente, no final do inverno, primavera, verão e outono. A prevalência, ajustada para comparações múltiplas, foi maior no inverno do que no verão (p<0.026) e no outono do que na primavera (p<0.001) e verão (p<0.000). A 25(OH)D3 não se associou com o índice de massa corporal (p=0.207), gordura corporal (p=0.064) ou fototipo (p=0.485), na análise univariada. Associou-se à D-VitD nos 30 dias antes da coleta de sangue (p<0.000), ajustada para gordura corporal. Houve sazonalidade na prevalência de 25(OH)D3 <30ng/mL (n=48/69), 69.6% (n=45/66), 43.8% (n=28/64), e 88.4% (n=53/60), respectivamente, no inverno, primavera, verão e outono.

CONCLUSÕES: Em uma região subtropical, houve sazonalidade na 25(OH)D3 em homens adultos, saudáveis, embora se exercitassem ao ar livre pelo menos 50 minutos duas vezes por semana, usando shorts e camiseta. 25(OH)D3 <20ng/mL foi 21.7% no outono e a 25(OH)D3 <30ng/mL (p=0.000): 69.6% (n=48/69), 68.2% (n=45/66), 43.8% (n=28/64), e 88.4% (n=53/60), respectivamente, no inverno, primavera, verão e outono.

PALAVRAS-CHAVE: Colecalciferol. Vitamina D. Deficiência de vitamina D. Estações. Raio ultravioleta.

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