Seasonal variation in the serum 25-hydroxyvitamin D levels of young and elderly active and inactive adults in São Paulo, Brazil
The São Paulo Vitamin D Evaluation Study (SPADES)

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Abbreviations: 25(OH)D, 25-hydroxyvitamin D; UVR, ultraviolet radiation; PTH, parathyroid hormone; SPADES, The São PAulo Vitamin D Evaluation Study; UNIFESP, Universidade Federal de São Paulo (Federal University of São Paulo); IBGE, Instituto Brasileiro de Geografia e Estatística (Brazilian Institute of Geography and Statistics)

Objective: To evaluate the 25-hydroxyvitamin D [25(OH)D] concentrations in individuals in the city of São Paulo belonging to different age groups and exhibiting specific behavioral characteristics and to correlate the 25(OH)D concentration with the level of UV radiation (UVR).

Results: The UVR values varied throughout the year, following a sinusoidal-like pattern. Because of the Earth’s orbit, we hypothesized that there would be cyclic patterns for the 25(OH)D and UVR values that repeat every 12 mo. The general formula is represented by the equation

\[ P_1 + P_2 \cdot \sin\left(\frac{2 \cdot \pi}{12} (t - P_1)\right) \]

The mean 25(OH)D concentration and the amplitude of the variation were significantly higher for the YOUNG and ACTIVE groups than for the COMMUNITY and NURSING groups. The nadir for UVR was in June, whereas the nadir for the 25(OH)D concentration was in the spring, corresponding to a delay of one season.

Patients and Methods: A total of 591 individuals were included, distributed as follows: 177 were living in institutions (NURSING, 76.2 ± 9.0 y old), 243 were part of the community elderly (COMMUNITY, 79.6 ± 5.3 y old), 99 were enrolled in a physical activity program targeting the elderly (ACTIVE, 67.6 ± 5.4 y old) and 72 were young (YOUNG, 23.9 ± 2.8 y old). Blood samples from all individuals were collected throughout the year. UVR measurements were taken by an official meteorology institution.

Conclusions: There was seasonal variation in the 25(OH)D concentration for all the groups studied; however, the amplitude of the variation was higher for the groups of young and physically active people, possibly due to the higher level of sunlight exposure for these groups. The lowest 25(OH)D concentration was detected in the spring.

Introduction

The main vitamin D source is cutaneous production involving the conversion of 7-dehydrocholesterol into previtamin D₃ by solar UV radiation (UVR). Once formed, previtamin D₃ is converted into vitamin D₃ by a thermally induced rearrangement. The photosynthesis of vitamin D₃ is dependent on the latitude, the time of day, the season of the year and the concentrations of atmospheric ozone and pollutants.1–4 In addition, vitamin D synthesis decreases with age, diminished sunlight exposure, higher melanin content of the skin and greater use of non-translucent clothing and sunscreen; all of these factors have been extensively described in the literature.5–9

Seasonal variations in the 25-hydroxyvitamin D [25(OH)D] concentration have been demonstrated in temperate latitudes. Surprisingly, people in southern European countries had lower
Table 1 presents the means and standard deviations (SDs) of the UVR (mJ/cm²) and 25(OH)D (nmol/L) values grouped according to the season of the year and the study group. There were statistically significant differences in the UVR level between the four seasons, with the UVR level being higher during the summer and lower during the winter (p < 0.001). All the study groups exhibited significant differences in the 25(OH)D concentration according to the season of the year.

For the 25(OH)D measurements, samples were collected from the COMMUNITY group throughout the entire year, and therefore, it was possible to construct a sinusoidal curve using the same formula (Table 2; Fig. 2). For the COMMUNITY group, the lowest 25(OH)D concentration was measured in the spring. For the YOUNG group, samples were collected over a period of 6 mo (from August to February). Using these results, it was possible to construct a sinusoidal curve, and this curve also had the lowest value in the spring (Table 2; Fig. 2). Blood samples were drawn during only 2 mo for the NURSING and ACTIVE groups, and based on the assumption that the 25(OH)D levels of these groups cycle the same manner as observed for the COMMUNITY group and based on the UVR model, sinusoidal curves were created (Fig. 2).

For the COMMUNITY group, no correlations were found between the 25(OH)D level and the UVR level from the same season (r = −0.03). In contrast, a strong correlation was found between the current 25(OH)D concentration and the UVR levels from the previous season (r = +0.98, p < 0.001).

Figure 3 shows the curves for the mean 25(OH)D levels of each population group throughout the year along with the corresponding UVR means. The amplitude of the variation in the 25(OH)D concentration was highest for the YOUNG group, followed by the ACTIVE, COMMUNITY and NURSING groups (Table 2). Although the lowest value for the UVR level was measured during the winter, the lowest 25(OH)D concentration was measured during the spring, corresponding to an average delay of one season between UVR exposure and endogenous steroid production.

**Discussion**

Vitamin D₃ is a steroidal molecule synthesized in the skin by an UV light-catalyzed reaction, and therefore, the level of this
molecule closely related to the Earth’s orbit around the sun. The efficiency of this synthesis reaction is affected by environmental variations, such as the latitude and the concentration of pollutants in the atmosphere.\textsuperscript{12} The level of vitamin D found in food sources is extremely low in our environment, where an average of 140 IU/day or 3.5 (3.0–3.9) \( \mu \text{g/day} \) being ingested.\textsuperscript{13,14}

The city of São Paulo is crossed by the Tropic of Capricorn (23\textdegree S), and the winter is relatively mild, with temperatures rarely falling to 0\textdegree C (32\textdegree F). São Paulo is a large urban center and is the largest city in South America with a predominantly Caucasian population. There are more than 10 million inhabitants, and there is a significant concentration of tall buildings and industries.

The seasonal variation in the 25(OH)D blood level is a well-established phenomenon in countries with temperate weather.\textsuperscript{1,2,15} Correlations between the 25(OH)D concentration and weather parameters, such as the temperature, and the duration of sunlight exposure have also been previously demonstrated.\textsuperscript{16,17} However, the existence and biological relevance of this phenomenon in places with lower latitudes have been poorly documented to date. This study found a clear seasonal pattern for the UV radiation incidence in the city of São Paulo (Fig. 1). This seasonal pattern had an apparent influence on the 25(OH)D concentration, which followed a sinusoidal model, as previously described by Stryd et al.\textsuperscript{1} and Sherman et al.\textsuperscript{2} Moreover, different population groups were evaluated, and there were marked differences in the 25(OH)D level according to sunlight exposure and age.

The NURSING and COMMUNITY groups had very low levels of 25(OH)D, and 88.7% and 81.9% of the members of these groups, respectively, had values lower than 75.0 nmol/L. These low levels are most likely due to the short duration of exposure to sunlight, which created a smaller variation in the vitamin D status for these groups compared with the variation in the YOUNG and ACTIVE groups.

An interesting fact is that ACTIVE people had frequent sunlight exposure during their participation in outdoor physical activity. It has been demonstrated that elderly individuals living in institutions have lower vitamin D levels than elderly individuals living at home.\textsuperscript{20,21} Our data suggest that if they are exposed to sunlight more frequently, these people would achieve adequate 25(OH)D levels, even taking into consideration the fact that the synthesis capacity of the skin is lower for the elderly than for younger people, and this conclusion is consistent with previous observations.\textsuperscript{3,12,13}

In a study published by our group, there was an excellent correlation between the current mean 25(OH)D concentration and the mean UVR value from the previous season (\( r = +0.98 \)).\textsuperscript{24} In the present study, a one-season delay was observed between the nadirs of the UVR and 25(OH)D levels. This dyssynchrony has already been described by others and may be related to both the half-life and skin synthesis of vitamin D\(_3\). In the Geelong Osteoporosis Study, performed in Australia (38–39\textdegree S), the authors found a 1-mo delay between the lowest level of radiation and the lowest 25(OH)D concentration.\textsuperscript{15} In a study performed in Adelaide, Australia (36\textdegree S), Need et al.\textsuperscript{25} also found this dyssynchrony, which was correlated with the body mass index. These authors demonstrated that for the obese, the delay between the two lowest values was two months, whereas the delay was one month for the non-obese. No BMI differences were found among the ACTIVE, COMMUNITY and NURSING groups (Table 1). Olivieri et al.\textsuperscript{26} demonstrated that young individuals confined to Antarctica for one year, where they were totally deprived of sunlight exposure during the winter, had 25(OH)D concentration nadirs after four months of no sunlight exposure.

The positive aspects of this study include the inclusion of a large number of individuals living in the same city and the use of the same laboratory assays for 25(OH)D determination. The limitations include the lack of information on the duration and quantity of radiation that the individuals received. Most of the individuals evaluated were Caucasian, closely representing the composition of individuals in the city of São Paulo. However, our data cannot be extrapolated to the rest of the country because of the large area covered by Brazil. This country includes areas with different geographical characteristics related to the latitude (varies from 5\textdegree N to 33\textdegree S), different weather conditions and populations with different genetic backgrounds. In addition, there are

### Table 1. Body mass index, 25(OH)D for each population group, grouped according to the season of the year and UVR values

| Season of the year | BMI\(^{\text{a}} \) (kg/m\(^2\)) | UVR (mJ/cm\(^2\)) | Community (nmol/L) | Nursing (nmol/L) | Active (nmol/L) | Young (nmol/L) |
|-------------------|-----------------|-----------------|-------------------|-----------------|----------------|----------------|
| SUMMER            | 63.9 ± 3.3      | 12.5–190        | 54.6 (12.5–130.0) | 26.3 ± 5.6\(^{a}\) | 26.8 ± 4.4\(^{a}\) | 22.4 ± 3.0     |
| FALL              | 36.8 ± 12.3     | 12.5–117.3      | 58.0 (12.5–161.0) | 23.0            | 77.2           | 106.6 ± 35.0   |
| WINTER            | 25.7 ± 5.8      | 12.5–86.5       | 40.0 (12.5–190)  | 26.7 ± 4.3\(^{a}\) |               | 74.8 ± 28.2    |
| SPRING            | 53.2 ± 7.6      | 12.5–165.0      | 42.8 (12.5–165.0)| 26.7 ± 4.3\(^{a}\) |               | 68.0 ± 24.7    |
| P                 | <0.001\(^{\text{f}}\) | 0.016\(^{\text{e}}\) | 0.010\(^{\text{e}}\) | <0.001\(^{\text{f}}\) | 0.010\(^{\text{e}}\) | <0.001\(^{\text{f}}\) |

UVR values (mJ/cm\(^2\)) for each season of the year and body mass index (BMI, kg/m\(^2\)) and 25(OH)D concentration (nmol/L) for each population group, grouped according to the season of the year. The parameters with a normal distribution are summarized using the mean and standard deviation, and the data with a non-normal distribution are summarized using the median and variation. \(^{\text{Tukey test; *Mann–Whitney test; †Kruskal–Wallis test; \#not significant.}}\)
Figure 2. Models representing the sinusoidal curves for each group, which were created from the equation presented in Table 2. The months are represented numerically (1 – January, 2 – February, ...).
different sunlight exposure habits that are appropriate for each location, these habits are important determining factors of the vitamin D status.

Patients and Methods

Subjects. The study protocol was previously approved by the Ethics Committee of UNIFESP, and all volunteers gave written informed consent.

With the cross-sectional SPADES study, we aimed to compile data from different population groups living in the city of São Paulo with different ages and distinct behavioral patterns. The final size of the population included in the SPADES study was 591 individuals. In relation to ethnicity, the study population was predominantly Caucasian (84.4%). The data for the individual populations used in this study have already been published elsewhere, and the objective of the present study was to evaluate these populations used in this study have already been published else-

The first population studied, the NURSING group, was composed of individuals who lived in two nursing homes in the city of São Paulo. Most of the individuals who live in these nursing homes are from low-income families that have little access to health services, and for this reason, the health conditions of these individuals are generally poor when they are taken to nursing homes. Individuals with creatinine values above 2.0 mg/dL, hypercalcemia or hypocalcemia, and those who were confined to bed were excluded from the study. The final sample included 177 individuals (128 women and 49 men; mean age, 76.2 ± 9.0 y old). Blood samples were drawn during the months of April (fall; 37.3% of individuals) and July (winter; 62.7% of individuals).24,27

The second group, the COMMUNITY group, was formed from a cohort study (EPIDOSO) performed with people who lived in the district around UNIFESP (Universidade Federal de São Paulo). Data from 243 elderly members of this community were analyzed (168 women and 75 men; mean age, 79.6 ± 5.3 y old) after sample collection throughout the year.27,28

The third group, the ACTIVE group, consisted of 99 individuals (52 women and 47 men; mean age, 67.6 ± 5.4 y old), 88 of whom provided a second blood sample (88.9%) at a different time point. The first collection time point was in June (winter), and the second was in December (summer). This group was composed of people enrolled in a physical activity program targeting the elderly.29

The fourth group, named the YOUNG group, consisted of 72 individuals (40 women and 32 men) with a mean age of 23.9 ± 2.8 y old, ranging from 17 to 35. Blood samples were collected between February (summer) and August (winter).30

Methods. The blood samples were drawn after an 8 h fast, and the 25(OH)D level was measured. All serum samples were collected into refrigerated tubes, processed in refrigerated centrifuged, and frozen at -20°C before analysis. The 25(OH)D concentrations were determined using an immunoradiometric assay (Diagnostics Nichols Institute). The intra-assay coefficient of variation was 4.8%, and the inter-assay coefficient of variation was 16.0% for the lowest values (mean: 35.5 nmol/L) and 3.0% for the highest control (mean: 154.0 nmol/L).

The daily doses of UVR accumulated between 7 a.m. and 5 p.m. were determined using a UVB501 sunlight biometer properly calibrated and adjusted every 10 min.31 The data were obtained locally by the Institute of Atmospheric Sciences of the Universidade de São Paulo (23°34’S) during the same time period as that during which the blood samples were collected for the NURSING and COMMUNITY groups.

The level of UVR throughout the year exhibits a sinusoidal-like pattern that repeats every 12 mo (2π/12). In this study, we determined if the variation in the 25(OH)D concentration follows the same pattern. Sinusoidal formulas that predict the UVR and 25(OH)D values according to the month of the year were created using Origin 5.0 (Microcal Inc.). A nonlinear fitting method was used to fit the UVR intensity and the 25(OH)D concentration to the sinusoidal model

\[
P_t + P_2 \cdot \sin\left(-\frac{2 \cdot \pi}{12} \cdot (t - P_3)\right)
\]

where \(P_t\) is the mean value, \(P_2\) is the oscillation amplitude and \(P_3\) is the time phase parameter. This same program was used to create the graph shown in Figure 2.

Statistical analysis. The strategy used for the statistical analysis after ANOVA was performed to identify significant differences between more than two groups followed this protocol: when the experimental data had a normal distribution, the Tukey test was used (UVR and 25(OH)D in relation to the season of the year and BMI of the subjects). When the distribution was not normal, the Kruska-Wallis was used (differences in the 25(OH)D level within the COMMUNITY group). The Mann-Whitney test was used to evaluate the differences in the 25(OH)D concentration between the ACTIVE and NURSING groups in relation to the season of the year.

The correlation between the 25(OH)D level and the UVR level during the same season and the correlation between the current 25(OH)D level and the UVR level from the previous

| Radiation | Nursing | Community | Active | Young |
|-----------|---------|-----------|--------|-------|
| (Mean value) | (Amplitude) | (Time phase) | (Mean value) | (Amplitude) | (Time phase) |
| 44.9 ± 0.5 | 22.7 ± 0.7 | 39.2 ± 0.3 | 51.1 ± 1.0 | 10.5 ± 1.5 | 18.1 ± 0.3 |
| 85.2 ± 9.1 | 12.8 ± 0.8 | 35.1 ± 0.1 | 93.3 ± 1.8 | 25.4 ± 2.3 | 30.5 ± 0.2 |

P1 represents the mean value, P2 represents the amplitude and P3 is the time phase parameter. The delay represents the time in months between the lowest value for the UV radiation level and the lowest 25(OH)D concentration for each group.

Figure 2. Using the formula equations to predict the UVR level (mJ/cm²) and 25(OH)D concentration (nmol/L) for each group were developed.
season were evaluated using Pearson’s correlation coefficients. The parameters with normal distributions were summarized using the mean ± SD (standard deviation), and the data that were not normally distributed were summarized using the median and variation. Differences were considered significant when p < 0.05.

Conclusions

These data demonstrate the existence of a sinusoidal seasonal variation in the 25(OH)D concentration in all the groups studied. The amplitude of the variation was higher for the groups composed of young people and those who practice outdoor physical activities, possibly due to the higher level of sunlight exposure. The lowest 25(OH)D concentration was found in the spring, and the low level corresponds to the consumption of the 25(OH)D stores formed during the previous summer.

Disclosure of Potential Conflicts of Interests

No potential conflicts of interest were disclosed.

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References

1. Stryd RP, Gilberston TJ, Brunden MN. A seasonal variation study of 25-hydroxyvitamin D3 serum levels in normal humans. J Clin Endocrinol Metab 1979; 48:771-5; PMID:429522; http://dx.doi.org/10.1210/jcem-48-5-771.
2. Sherman SS, Hollis BW, Tobin JD. Vitamin D status and related parameters in a healthy population: the effects of age, sex, and season. J Clin Endocrinol Metab 1996; 71:405-13; PMID:2380336; http://dx.doi.org/10.1210/jcem-71-2-405.
3. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3 exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab 1988; 67:373-8; PMID:2839537; http://dx.doi.org/10.1210/jcem-67-2-373.
4. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr 1995; 61(Suppl):638S-45S; PMID:7879731.
5. McLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. J Clin Invest 1995; 95(Suppl):638S-45S; PMID:7879731.
6. Freaney R, McBriinn Y, McKenna MJ. Secondary hyperparathyroidism in elderly people: combined effect of renal insufficiency and vitamin D deficiency. Am J Clin Nutr 1993; 58:187-91; PMID:8338046.
7. Bell NH, Greene A, Epstein S, Oexmann MJ, Shaw S, Shary J. Evidence for alteration of the vitamin D-endocrine system in blacks. J Clin Invest 1985; 76:470-3; PMID:3839801; http://dx.doi.org/10.1172/JCI111995.
8. Matsuoka LY, Wortsman J, Dannenberg MJ, Hollis BW, Lu Z, Holick MF. Clothing prevents ultraviolet-B radiation-dependent photosynthesis of vitamin D3. J Clin Endocrinol Metab 1992; 75:1099-103; PMID:1328275; http://dx.doi.org/10.1210/jcem-75-4-1099.
9. Matsuoka LY, Ide I, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab 1987; 64:1165-8; PMID:3033008; http://dx.doi.org/10.1210/jcem-64-6-1165.
10. van der Wielen RPJ, Löwik MRH, van den Berg H, de Groot LCPGM, Haller J, Moreiras O, et al. Serum vitamin D concentrations among elderly people in Europe. Lancet 1995; 346:207-10; PMID:7616799; http://dx.doi.org/10.1016/S0140-6736(95)95126-6.

11. Lips P, Duong T, Oleksik A, Black D, Cammings S, Cox D, et al. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. J Clin Endocrinol Metab 2001; 86:1212-21; PMID:11283511; http://dx.doi.org/10.1210/jc.86.3.1212.

12. Manicourt DH, Devogelaer JP. Urban tropospheric ozone increases the prevalence of vitamin D deficiency among Belgian postmenopausal women with outdoor activities during summer. J Clin Endocrinol Metab 2008; 93:3893-9; PMID:18628525; http://dx.doi.org/10.1210/jc.2007-2663.

13. Pinheiro MM, Schuch NJ, Genaro PS, Ciconelli RM, Ferraz MB, Martini LA. Nutrient intakes related to osteoporotic fractures in men and women—the Brazilian Osteoporosis Study (BRAZOS). Nutr J 2009; 8:6; PMID:19178745; http://dx.doi.org/10.1186/1475-2891-8-6.

14. Peters BS, dos Santos LC, Fisberg M, Wood RJ, Martini LA. Prevalence of vitamin D insufficiency in Brazilian adolescents. Ann Nutr Metab 2009; 54:15-21; PMID:19194104; http://dx.doi.org/10.1159/000199454.

15. Pasco JA, Henry MJ, Kotowicz MA, Sanders KM, Seeman E, Pasco JR, et al. Seasonal periodicity of serum vitamin D and parathyroid hormone, bone resorption, and fractures: the Geelong Osteoporosis Study. J Bone Miner Res 2004; 19:752-8; PMID:15068498; http://dx.doi.org/10.1359/jbmr.2004.10.040125.

16. Melin A, Wilke J, Ringerth I, Sauf M. Seasonal variations in serum levels of 25-hydroxyvitamin D and parathyroid hormone but no detectable change in femoral neck bone density in an older population with regular outdoor exposure. J Am Geriatr Soc 2001; 49:1190-6; PMID:11559378; http://dx.doi.org/10.1046/j.1532-5415.2001.49236.x.

17. Ladizesky M, Lu Z, Oliveri B, San Román N, Díaz S, Holек MF, et al. Solar ultraviolet B radiation and photoproduction of vitamin D, in central and southern areas of Argentina. J Bone Miner Res 1995; 10:545-9; PMID:7610924; http://dx.doi.org/10.1002/jbmr.5650100406.

18. Brot C, Vestergaard P, Kolthoff N, Gram J, Hermann AP, Sørensen OH. Vitamin D status and its adequacy in healthy Danish perimenopausal women: relationships to dietary intake, sun exposure and serum parathyroid hormone. Br J Nutr 2001; 86(Suppl 1):S97-103; PMID:11520426; http://dx.doi.org/10.1079/BJN2001345.

19. Sedrani SH, Eldrissy AWTH, HI Arabi KM. Sunlight and vitamin D status in normal Saudi subjects. Am J Clin Nutr 1998; 68:92-8; PMID:9504937; http://dx.doi.org/10.1093/aje/156.NEJM199803193381201.

20. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch JT, et al. Hypovitaminosis D in medical inpatients. N Engl J Med 1998; 338:777-83; PMID:9504937; http://dx.doi.org/10.1093/aje/156.NEJM199803193381201.

21. Davies M, Mawer EB, Hann JT, Taylor JL. Seasonal changes in the biochemical indices of vitamin D deficiency in the elderly: a comparison of people in residential homes, long-stay wards and attending a day hospital. Age Ageing 1986; 15:77-83; PMID:3008525; http://dx.doi.org/10.1093/ageing/15.2.77.

22. Verth R, Ladak Y, Wallfisch PG. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. J Clin Endocrinol Metab 2003; 88:185-91; PMID:12519850; http://dx.doi.org/10.1012/jc.2002-021064.

23. Reid IR, Gallagher DJ, Bosworth J. Prophylaxis against osteoporosis in the elderly by regular sunlight exposure. Age Ageing 1986; 15:35-40; PMID:3953329; http://dx.doi.org/10.1093/ageing/15.1.35.

24. Saraiva GL, Cendoroglo MS, Ramos L, Araújo LM, Vieira JGH, Kunii IS, et al. Influence of ultraviolet radiation on the production of 25-hydroxyvitamin D in the elderly population in the city of São Paulo (23° 34’S), Brazil. Osteoporos Int 2005; 16:1649-54; PMID:16289786; http://dx.doi.org/10.1007/s00198-005-1895-3.

25. Need AG, Morris HA, Horowitz M, Nordin C. Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. Am J Clin Nutr 1993; 58:882-5; PMID:8249872.

26. Oliveri MB, Mautalen C, Bustamante L, Gómez García V. Serum levels of 25-hydroxyvitamin D in a year of residence on the Antarctic continent. Eur J Clin Nutr 1994; 48:397-401; PMID:7925221.

27. Saraiva GL, Cendoroglo MS, Ramos LR, Araújo LM, Vieira JGH, Maeda SS, et al. Prevalence of the deficiency, insufficiency of vitamin D and hyperparathyroidism in elderly institutionalized and non-aged subjects in the city of São Paulo, Brazil. Lancet 1994; 343:47-9; PMID:8249872.

28. Ramos LR, Toniolo Neta J, Cendoroglo MS, García JT, Najas M, Porracini M, et al. Two-year follow-up study of elderly residents in São Paulo, Brazil: methodology and preliminary results. J Public Health (Bangkok) 1998; 32:397-407.

29. Maeda SS, Kunii IS, Hayashi LF, Lazaretti-Castro M. Increases in serum serum 25-hydroxyvitamin D (25OHD) concentrations in elderly subjects in São Paulo, Brazil vary with age, gender and ethnicity. BMC Endocr Disord 2010; 10:12; PMID:20546572; http://dx.doi.org/10.1186/1472-6823-10-12.

30. Maeda SS, Kunii IS, Hayashi LF, Lazaretti-Castro M. The effect of sun exposure on serum 25-hydroxyvitamin D concentrations in young healthy subjects living in the city of São Paulo, Brazil. Braz J Med Biol Res 2007; 40:1653-9; PMID:17713647; http://dx.doi.org/10.1590/S0031-10562007000300012.

31. Corrêa MF, Dubuisson P, Flana-Farto A. An overview of the ultraviolet index and the skin cancer cases in Brazil. Photochem Photobiol 2003; 78:49-54; PMID:12929748; http://dx.doi.org/10.1562/0031-8655(2003)078<0049:AOOTUI>2.0.CO;2.