Association between vitamin D status and lifestyle factors in Brazilian women: Implications of Sun Exposure Levels, Diet, and Health

Keila Valente de Souza de Santana,*a Sofia Lizarralde Oliver,b Marcela Moraes Mendes,c Susan Lanham-New,c Karen E Charlton,d and Helena Ribeirob

aPrograma de Pós-Graduação em Saúde Global e Sustentabilidade, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, SP, Brazil
bDepartamento de Saúde Ambiental, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, SP, Brazil
cSchool of Biosciences and Medicine, Faculty of Health and Medical Sciences, Department of Nutritional Sciences, University of Surrey, Guildford, Surrey, United Kingdom
dSchool of Medicine, University of Wollongong, Wollongong, Australia NSW and Illawarra Health and Medical Research Institute, Wollongong, Australia NSW

Summary

Background Vitamin D deficiency has been documented to be prevalent, even in low latitude regions; and this may be related to sun exposure behaviors. The aim of the current study was to assess the association between serum 25-hydroxyvitamin D [25(OH)D] concentrations and lifestyle–related factors in a sample of Brazilian women living at latitude 21° 8’ S.

Methods A cross-sectional study was undertaken in 101 women aged 35 years or older in July 2019 to assess the association between 25(OH)D concentration and level of exposure to ultraviolet radiation (UVR), smoking habits, alcohol consumption, and physical activity levels. Age, body mass index (BMI), and postmenopausal status were investigated.

Findings According to the slope coefficient for individual daily UVR levels, the concentration of 25(OH)D increased by 5 nmol / L for each extra Standard Erythema Dose of UVR, regardless of age and BMI (p < 0.001). Postmenopausal women had a significantly higher mean concentration of 25(OH)D (p = 0.01), higher UVR exposure (p = 0.01) and lower BMI (p = 0.005) compared with younger women, independent of other confounders including smoking, alcohol, occupation and physical activity.

Interpretation Although postmenopausal women from Brazil had higher mean concentrations of 25(OH)D than younger women, more studies are necessary to understand how sun exposure and lifestyle variables interfere with these levels. These findings have important public health implications since they suggest that vitamin D deficiency in older age is not inevitable.

Funding This study was funded by an award received by Universities Global Partnership Network – UGPN. KVSS and SLO receive scholarship from CAPES, Brazilian Ministry of Education. HR receives a productivity grant from CNPq.

Copyright © 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Keywords: Vitamin D; Lifestyle; Women; Ultraviolet radiation

Introduction

Recent research has pointed specifically to the existence of a global ‘syndemic’; characterized by the interaction and synergy of three major pandemics — obesity, malnutrition and climate change.1 Regarding malnutrition, widespread research has highlighted the pandemic nature of vitamin D deficiency, essential for human health,2,3 due to its widespread prevalence in both high and low latitude countries.

Moderate skin exposure to ultraviolet B (UVB) radiation of the solar electromagnetic spectrum allows for

---

*eCorresponding author at: Av. Dr. Arnaldo, Cerqueira Cesar 715, ZIP 01246-904, São Paulo, SP, Brazil
E-mail address: keilla@usp.br (K.V.d.S.d. Santana).
However, to indicate supplementation, it is necessary and chronic diseases such as osteoporosis, and increased risk of acute respiratory tract infection over-nutrient intake (RNI) of insufficient sunlight, been reported to have intakes below the recommended insufficient to meet requirements. Most adults have rooms) and dietary intake of the vitamin is generally vitamin D are relatively few (e.g. oily fish, eggs, meat, mush-cosynthesis of vitamin D (cholecalciferol). Given sufficient sunlight, 7-dehydrocholesterol in the skin is converted to pre-vitamin D₃. Food sources of vitamin D are relatively few (e.g. oily fish, eggs, meat, mushrooms) and dietary intake of the vitamin is generally insufficient to meet requirements. Most adults have been reported to have intakes below the recommended daily allowance (RDA) of 15 μg/day and the reference nutrient intake (RNI) of 10 μg/day.

Vitamin D plays a crucial role in metabolic processes ranging from calcium and phosphorus metabolism to cell maturation and growth. There is evidence from observational studies regarding vitamin D deficiency/insufficiency and increased risk of acute respiratory tract infection overall and chronic diseases such as osteoporosis, hypertension, cardiovascular disease, myocardial infarction, cancer, diabetes, and more recently covid 19. However, to indicate supplementation, it is necessary to verify whether the patient has significant risk factors for vitamin D deficiency, such as osteoporosis, osteomalacia, malabsorption, use of drugs that can affect vitamin D metabolism, or institutionalization. In addition, studies using both experimental and non-experimental designs on vitamin D and mood disorders in women suggest that vitamin D may be an important nutrient for women’s mental wellbeing in addition to their physical health.

Latitude, season, and prevailing climatic conditions are some of the environmental factors that determine the availability of UVB for vitamin D production. Although UVB radiation is sufficient for vitamin D synthesis throughout the year, at least up to latitude 35° North/South, vitamin D deficiency has been reported to be high, even in low latitude regions. The explanation for this phenomenon may lie in sun exposure behavior. The type of clothing, the use of sunscreen, working in closed spaces and the lack of physical activity in open environments can prevent or impede the adequate synthesis of vitamin D.

Personal characteristics such as skin pigmentation and age are also limiting factors. Vitamin D synthesis decreases with increasing age, due in part to a drop in 7-dehydrocholesterol levels and in changes in skin morphology. In the case of skin color, darker skin has more melanin, acting as a filter for ultraviolet radiation (UVR) and therefore requiring about 6 times more sun exposure for vitamin D synthesis than white skin.

The migrations and high-speed transportation have brought many people into UVR regimes different from those experienced by their ancestors whose skin pigmentation evolved as a compromise between the conflicting physiological demands of protection against the deleterious effects of ultraviolet radiation (UVR) and photosynthesis of UVB-dependent vitamin D₃.

Vitamin D deficiency is more prevalent in females than males. It is now well established that women will lose significant amounts of bone during and after the menopause due to the lack of estrogen production; this can be exacerbated by hypovitaminosis D, characterizing postmenopausal osteoporosis. Concomitant with this, overweight and obesity have been shown to be associated with vitamin D insufficiency.

The aim of the current study was to assess the association between serum 25(OH)D concentrations in healthy adult women and lifestyle-related factors including sun exposure, physical activity levels, smoking habits, alcohol consumption, occupation, and dietary intake. In addition, the association between vitamin D status and distress score was also analyzed.

Methods

This study is part of the multicenter project Healthy Living Healthy Aging, developed by three universities (University of Sao Paulo, University of Surrey — England and University of Wollongong — Australia). It was conducted in cities of similar sizes, but with different latitudes and lifestyle habits. Sample size was...
conveniently agreed with partners of the study from other cities and latitudes. As the prevalence of insufficient 25(OH)D varies largely around the world, depending on population and on climate, the authors adopted a prevalence of 50% which would give a larger number of women, and considered 5% standard error.

The cross-sectional study was carried out in Araraquara (São Paulo), a medium-sized city located at latitude 23° 8’ S, known as the “Home of the Sun”, and is part of a larger research project in which cities from different continents and latitudes are being studied. Araraquara is 646 meters above sea level. Data was collected in July 2019 in the beginning of winter and in the midst of the dry season, which runs from May to October in the State of São Paulo, Brazil. Within the scope of the multicenter project, it was decided to collect samples in the winter season, considering the difficulty of synthesizing vitamin D during that period in countries like England. In the case of Brazil, it was defined the month of July for data collection, in the middle of the winter season in south hemisphere.

During the collection period, ozone (24-hour mean O3) level was around 65 µg/m³.26 Previously there was no record of daily averages higher than 100 µg/m³, recommended by WHO to prevent health problems. Regarding PM10, throughout the year the concentrations were below 50 µg/m³, but reached 70 µg/m³ in June and July.26 Even so, these values are within the recommended annual mean interim target 1 of WHO (2021).27 In these dry months with frequent atmospheric stability, the dispersion of pollutants is impaired but not enough to prevent UVB radiation.

It was undertaken in a total of 101 women aged 35 years or older to assess the association between serum 25 – hydroxyvitamin D [25 (OH) D] concentration and lifestyle factors (level of exposure to ultraviolet radiation, calcium and vitamin D intake, occupation, smoking habits, alcohol consumption, physical activity levels and distress score). Age, skin color, and postmenopausal status were also investigated.

The inclusion criteria were: healthy women of any race or color, live in the city of Araraquara or region, age 35 years or older. Exclusion criteria were factors likely to affect vitamin D metabolism, namely: taking vitamin D supplementation, therapy for osteoporosis, cancer treatment, diabetes, heart disease or hypertension.

Approval for the study was provided by the Research Ethics Committee of the Faculty of Public Health of the University of São Paulo according to CNS Resolution 196/96 (protocol number CAAE: 11939219.3.0000.5421; June 4, 2019). All volunteers provided written informed consent.

Several communication channels were used for participant recruitment, including local radio stations, social networks, and local TV interviews. Informative posters were posted in local Primary Care Health Centers, churches, universities, gyms, beauty salons, and sports clubs. A cell phone line was made widely available for scheduling the attendance of those interested in the study. The sample of participants can be considered representative of Araraquara’s women population regarding race and color (Table S1). White women were 72%, black and brown 26%.

To assess the vitamin D status in the research participants, the circulating serum 25(OH)D concentrations were measured by chemiluminescence method. The instrument was Centaur XP, manufacturer Siemens, City and country of supplier was São Paulo, Brazil; calibrated by essay of proficiency Controllab and PNCQ (Programa Nacional de Controle de Qualidade). The instrument’s measurements compared with mass spectroscopy measurements: ADVIA Centaur VitD = 0.93 (ID-LC/MS/MS) + 2.89ng/ml (7.23nmol/l), r = 0.99.

There is no global consensus on the optimal concentrations of 25(OH)D in serum. For evaluation, treatment, and prevention of vitamin D deficiency, the Endocrine Society Clinical Practice Guideline has been set a cut-point ≥ 75 nmol/L as an optimal cut-off point for bone health and fall prevention.29 The deficiency as < 30 nmol/L is associated with increased risk of metabolic bone diseases.4

In this study the cut-off point below 50 nmol/L has been used as an indicator of vitamin D insufficiency. The adoption of this cut-off point allows comparison with many epidemiological studies,10–12 either as an isolated measure or as a complement to other cut-off points. In the case of values < 50 nmol/l, participants were referred to a physician.

A polysulfone badge functioning as a dosimeter measured the level of UVR to which the participant was exposed. The dosimeter was attached to the participants’ outer clothing, either on the shoulder or on the upper chest, to be worn for four days, including weekends. Adherence to wearing the dosimeter was 95%, as indicated by the number of women who mailed the used dosimeter to the University. Two of the women who lost or got wet the badge called up the investigators and received new badges which were used for the four days. All dosimeters were manufactured and read at University Manchester (UK).31 The radiation absorbed by the dosimeters was measured using a spectrophotometer, and spectral response function at 330 nm, to detect change in absorbency.19 Exposure to UVR was expressed in Standard Erythema Dose (SED) units, with 1 SED being equivalent to 100 Jm⁻² of erythemal UVR, which is considered an acceptable daily dose of exposure. Although erythemal effective UVR is not identical to UVR that is effective for vitamin D synthesis, it provides an appropriate proxy for sun exposure for the purpose of this study.34

Participants’ food and nutrient intake, particularly calcium and vitamin D intake, was determined according to food diaries completed by participants for 4 consecutive days, including one weekend day.
were instructed by the research team on how to complete the diary correctly and asked to provide as much detail as possible of each meal, including portion size. Participants were provided with envelopes addressed to the investigators to return the dosimeter and the food diary, 95% of which were returned. Food intake data was analyzed using the Nutrition Data System for Research® (NDSR, Minneapolis, MN, USA), version 2014 that was based on the North American food composition database of the United States Department of Agriculture. In order to identify possible errors in data collection and processing, consistency analysis of dietary data was performed. The Brazilian Food Composition Tables of the University of Campinas and the University of São Paulo were used to verify the adequacy of the nutritional values of the foods present in the program. Only the foods that obtained percentages of agreement between 80% and 120% of the energy and macronutrient values were used. The nutrients calcium and vitamin D were corrected after exporting the NDSR data, according to the values available in the Brazilian national tables.

A self-administered questionnaire was completed to describe sociodemographic characteristics, menopausal status, smoking habits, alcohol consumption, frequency of sunbathing in the previous year (≥ 20 times or < 20 times), and use of sunscreen, skin color [self-reported skin color according to IBGE (Instituto Brasileiro de Geografia e Estatística) classification], weight, and height (self-reported or measured when the participant did not know), and waist circumferences were measured by trained researchers.

To characterize physical activity levels, the International Physical Activities Questionnaire (IPAQ) short form was used which comprises eight questions on time spent per week in categories of registered physical activities (walking and moderate and vigorous physical effort) and physical inactivity (sitting). The IPAQ has been validated for use in the adult Brazilian population. The level of physical activity was dichotomized into: active individuals (sufficiently active and very active IPAQ categories) and inactive individuals (insufficiently active IPAQ category) as shown in Box 1.

Distress was investigated by the 12-item version of the General Health Questionnaire (GHQ), a screening instrument conceived for use in general population surveys. The short version screening instrument, with 12-items, has been validated in the Brazilian population. According to the distress scores, the women have been classified in two groups [low, 0–3 and high, >4]. The GHQ is a screening instrument designed to identify non-psychotic psychiatric disturbance in primary care settings and in the community. Depression is probably the most well-known type of distress and more prevalent in women.

### Very active: one who has fulfilled the recommendations of
Vigorous activity
Vigorous activity
Active: one who has met the recommendations of
Vigorous activity
Moderate activity or walking
Any activity added together (walking + moderate + vigorous)

### Box 1: Classification of IPAQ according to categories

### Statistical Analysis

Statistical analysis of the data was performed in R 3.6.3 software for MacBook Pro. Standard descriptive statistics and linear regression were used throughout. The variables were tested for normal distribution using Shapiro–Wilk normality tests. The concentration of 25 (OH) D in the study population has a normal distribution.

Nonparametric tests were used when the log transformation did not normalize the data. Descriptive statistics were determined for all variables. Continuous variables are presented as mean ± standard deviation (SD) for normally distributed variables or as median (25%, 75% percentiles) for not normally distributed, and 95% confidence interval for numeric variables. For non-numerical variables, descriptive statistics are given by frequency.

Mean vitamin D levels were compared between participants who responded affirmatively or negatively to characteristics related to lifestyle habits (smoking, drinking, sunbathing, sunscreen use), postmenopausal, and low and high risk of distress (GHQ) using the student t-test, or Mann-Whitney U tests for non-normally distributed data. Comparison was also performed for age and SED categories, as well as mean SED for vitamin D sufficiency/insufficiency categories. Nonparametric ANOVA and corresponding Tukey’s test, or Kruskal-Wallis for non-normally distributed data was used to compare mean 25(OH) D concentrations between skin color, physical activity, and occupational category groups.

Pearson’s correlation was applied to investigate the correlation between 25(OH)D concentrations and UVR, body mass index (BMI), waist circumference, age, distress score, and vitamin D and calcium intake. The effect of each variable was tested using simple linear regression. Those with a significant effect were then tested in a multiple linear regression including all the above mentioned covariates. A P value < 0.05 was considered significant.

### Role of the funding source

This study was funded by the 2018 Award received from University Global Partnership Network, which did not have any role on the study subject, design, methods, or results. The sponsors value publication of research findings, but did not interfere in the decision to submit the
paper for publication. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
The BMI and waist circumference demonstrated normal distribution and showed that, on average, the study participants were overweight. The level of UVR did not have normal distribution, requiring logarithmic transformation. The mean age of participants was 51 years (SD = 3.3 years) with mean BMI of 27 kg/m² (SD = 4 kg/m²) and serum 25(OH)D concentrations within the normal range (mean = 64 nmol/L SD = 15 nmol/L) while 16% had vitamin D ‘insufficiency’ (< 50 nmol/L). Those who presented sufficient concentrations of 25(OH)D were 52% and ideal concentrations 32%.

Sociodemographic characteristics of participants are presented in Table 1, stratified by age group (>50 or ≤50 years). In the 95 participants who returned food diaries, mean dietary intake of vitamin D was 3.7 ± 2.5 µg/day and mean calcium intake was 650 ± 343 mg/day. There were no significant differences between age groups regarding vitamin D and calcium intake. Almost all participants (97%) had vitamin D intakes below the recommended 10 µg/day, with only three participants recording intakes above this threshold. For calcium, 87% and 94% had average intakes below the Recommended Dietary Allowance (RDA) (US) of 1000 and 1200 mg/day for women ≤50 years (n = 45) and >50 years (n = 50), in addition 80% and 84% had average intakes below the Estimated Average Requirement (EAR) (UK) [22] of 800 and 1000 mg/day for women ≤50 years and >50 years, respectively.

Surprisingly, older women had a significantly higher concentration of 25(OH)D than the younger women (P = 0.01) but also had higher UVR exposure (P = 0.01) and lower BMI (P = 0.003). There were no significant differences in any other characteristics according to age.

Exposure levels to ultraviolet radiation in different groups
We assessed whether there was a significant difference in UVR exposure dose, vitamin D status, lifestyle, and distress groups. This analysis aimed to verify a possible ideal dose of UVR. UVR exposure in winter is shown in Table 2 for: weekly alcohol consumption, smoking, number of times she sunbathed in the previous year (≥ 20 times or < 20 times), postmenopausal status, stress groups, skin color, level of physical activity and occupational category. The dose of personal exposure to UVR was significantly higher in postmenopausal women (P = 0.01) and lower in women classified as having vitamin D insufficiency (P < 0.001). Those who were overweight also had significantly lower levels of UVR exposure than those who were not overweight (P = 0.006). There was a significant difference between the occupational category groups (P < 0.001). The dose of exposure to UVR was significantly higher in the “housewife” group when compared with the “administrative” and “health and beauty” groups (P = 0.002 and P < 0.001, respectively). In the “other” group, consisting mostly of cleaning professionals, tradesmen, and self-employed, the dose of exposure to UVR was significantly higher when compared with the “administrative” and “health and beauty” groups (P = 0.006, P = 0.003, respectively). There were no significant differences between the other groups.

Circulating levels of 25 – hydroxyvitamin D in different groups
The 25 (OH)D concentration levels of the sample are shown in Table 3, stratified by group for: ultraviolet radiation (UVR) (≥ 2 sed or < 2 sed total over four days), weekly alcohol consumption, smoking, daily sunscreen use, sunbathing in the previous year (≥ 20 times or < 20 times), post menopause, distress score, skin color, physical activity level, and occupational category.

Vitamin D concentration was significantly higher in postmenopausal women compared to those who had

| Parameters                       | Total (n=95) | 35 – 50 (n=50) | 51 – 72 (n=45) | P value a |
|----------------------------------|-------------|----------------|----------------|-----------|
| Serum 25(OH)D (nmol/L)           | 64±15       | 60±14          | 67±16          | 0.01      |
| BMI (kg/m²)                      | 22±4        | 28±5           | 26±4           | 0.005     |
| Waist circumference (cm)         | 91±12       | 94±13          | 91±10          | 0.06      |
| Ultraviolet radiation (UVR)     | 1.8 (1.3 – 2.7) | 1.6 (1 – 2.5) | 2.2 (1.4 – 3.1) | 0.01 b |
| Vitamin D intake (µg/day)        | 3.5 (1.9 – 4.7) | 3.4 (1 – 4.4) | 3.8 (2 – 5) | 0.12 |
| Calcium intake (mg/day)          | 604 (412 – 801) | 590 (400 – 725) | 640 (429 – 851) | 0.23 |
| Sunbathing (n/year)              | 3 (0 – 10)  | 4 (0 – 10)     | 2 (0 – 9.5)    | 0.21 a |
| Distress score                   | 2 (0 – 7)   | 2.5 (0 – 9)    | 2 (0 – 6)      | 0.12 a |

Table 1: Characteristics of female residents of Araraquara (SP – Brazil) stratified by age group (>50 or ≤50 years)
aValues: mean ± SD or median (25th–75th percentile). b Statistical analysis: independent t-test, unless otherwise indicated; Mann–Whitney U-test.
not yet entered menopause (P < 0.001). Overweight women had significantly lower vitamin D concentrations than non-overweight women (P < 0.001). Women with average levels of UVR exposure greater than 2 SED had higher 25(OH)D concentrations. Women who had distress score had significantly lower vitamin D concentrations than those who did not have the symptoms (P = 0.001). There was a significant difference between skin color groups, with the average 25(OH)D concentration being lower in women with black or brown skin (P = 0.008). There were no significant differences in vitamin D concentrations between the other groups.

**Correlation between lifestyle behaviors and 25(OH)D concentration**

In Table 4, the participants’ lifestyle behaviors and sociodemographic characteristics are stratified according to vitamin D status. Women with vitamin D concentrations between 25 and 50 nmol/L had significantly lower levels of UVR exposure than women with concentrations between 50 and 75 nmol/L (P = 0.005) and than women with concentrations above 75 nmol/L (P = 0.007). The BMI of women with vitamin D concentrations greater than 75 nmol/L was significantly lower than in women with vitamin D concentrations between 25 and 50 nmol/L (P = 0.03). The distress score of women with vitamin D concentrations greater than 75 nmol/L was significantly lower than in women with vitamin D concentrations between 25 and 50 nmol/L (P = 0.03). There were no significant differences in vitamin D levels between the other groups.

To perform the correlation and linear regression tests, a logarithmic transformation of the UVR dose was required. There was a low, positive but significant correlation between age and UVR (r = 0.22; P = 0.03) and an inverse correlation between BMI and age (r = −0.21; P = 0.03) as well as with UVR (r = −0.23; P = 0.03). There was a positive correlation of distress score with BMI (r = 0.33; P < 0.001) and a weak inverse association between age and distress score (r = −0.263; P = 0.008).

An inverse association was found between 25(OH)D concentrations and BMI, even controlling for UVR (P = 0.009). Whereas the correlation between 25(OH)D concentrations and age of r = 0.22 was no longer significant after controlling for BMI and UVR (P = 0.1 and P = 0.09, respectively), there was also a trend toward a positive correlation of r = 0.29 between UVR and 25(OH)D concentrations, even after controlling for BMI.

### Table 2: Dose of exposure to ultraviolet radiation (UVR) of the Brazilian participants from Araraquara (SP) according to vitamin D status, lifestyle behaviors and distress groups.

| Variable                        | Class            | Median (IQR) | P – value |
|---------------------------------|------------------|--------------|-----------|
| Vitamin D insufficiency         | Yes              | 1.33 (0.77–1.47) | <0.001    |
|                                 | No               | 2.11 (1.34–2.87) |           |
| Postmenopausal                  | Yes              | 2.15 (1.37–3.08) | 0.01      |
|                                 | No               | 1.6 (0.93–2.45)  |           |
| Overweight                      | Yes              | 1.46 (0.94–2.47) | 0.006     |
|                                 | No               | 2.19 (1.6–3.11)  |           |
| Smoking                         | Yes              | 2.12 (1.24–2.6)  | 0.37      |
|                                 | No               | 1.77 (1.25–2.65) |           |
| Consumption of alcoholic beverages | Yes              | 1.44 (0.84–2.4)  | 0.04      |
|                                 | No               | 2.06 (1.36–2.66) |           |
| Sunbathing                      | Yes              | 1.76 (1.02–2.13) | 0.72      |
|                                 | No               | 1.75 (1.14–2.6)  |           |
| Distress groups (GHQ)           | High             | 1.75 (0.88–2.65) | 0.23      |
|                                 | Low              | 1.82 (1.31–2.63) |           |
| Skin color (self-determined)    | White            | 1.88 (1.32–2.7)  | 0.29      |
|                                 | Brown or Black   | 1.74 (0.85–2.6)  |           |
| Physical Activities             | Very active      | 2.17 (1.55–2.99) | 0.2       |
|                                 | Active           | 1.81 (1.13–2.6)  |           |
|                                 | Sedentary        | 1.47 (0.98–2.47) |           |
| Occupational groups             | Beauty and Health| 1.39 (0.95–1.76) | <0.001    |
|                                 | Education        | 2.05 (1.24–2.47) |           |
|                                 | Administrative   | 1.54 (0.83–2.06) |           |
|                                 | Housewife        | 2.77 (1.91–4.33) |           |
|                                 | Others           | 2.92 (1.9–4.2)   |           |

**Statistical analysis:** 1 Mann–Whitney and Kruskal–Wallis test. 2 Overweight is BMI >25 or women over 60 with BMI >27. 3Sunbathing was taking a sunbath at least 20 times in the previous year or so. 4 Interquartile range (IQR).
(P < 0.02). There was an inverse association (r = -0.31) between distress score and 25(OH)D concentrations, even after controlling for BMI and UVR (P = 0.03 and P = 0.004) (Figure 1). No significant correlations were found between 25(OH)D concentration and vitamin D intake, as well as, with other variables.

Homoscedasticity and the appropriateness of other linear regression assumptions were assessed for the models to ensure validity. The process of verifying the underlying assumptions in the R program was done graphically and it is included in supplementary material (Figure S1 and Figure S2). A multiple regression analysis was used to investigate the ability of individual daily UVR levels (SED) and BMI to predict 25(OH)D (nmol/L) after controlling for BMI and UVR.

BMI (P = 0.007) and UVR levels (P = 0.02) made statistically significant contributions to the prediction of the 25(OH)D concentrations and the total variance explained by the model was 15.7%. According to the slope coefficient for individual daily UVR levels, the concentration of 25(OH)D increased by 5 nmol / L for each extra SED of UVR, regardless of BMI (P < 0.001) (Table S2). A second multiple regression analysis was used to investigate the ability of BMI and 25(OH)D (nmol/L) to predict distress score. BMI (P = 0.009) and 25(OH)D concentrations (P = 0.02) made statistically significant contributions to the prediction of the distress score and the total variance explained by the model was 15.6% (P < 0.001) (Table S3).

**Discussion**

In tropical countries there is higher exposure to solar radiation that is favorable for vitamin D synthesis, either due to latitude or favorable atmospheric conditions, with less severe winters. This study provides novel information on the association between vitamin D status and health and lifestyle characteristics of a population from a medium-sized city in Brazil. Most studies to date on Vitamin D have been conducted in countries located in regions of high latitude.44-45

The prevalence of vitamin D insufficiency of 16% among white women and 25% among brown and black women observed in healthy women living in Araraquara (Brazil), a city characterized by high insolation and low latitude, indicates the relevance of sunlight. These

| Variable                  | Class               | n (%) | Mean±SD | IC 95%    | P — value<sup>1</sup> |
|---------------------------|---------------------|-------|---------|-----------|------------------------|
| Postmenopausal            | Yes                 | 45 (45) | 69±16   | 65 – 73   | <0.001 |
|                           | No                  | 55 (55) | 58±13   | 54 – 62   | |
| Overweight                | Yes                 | 59    | 60±15   | 56 – 63   | <0.001 |
|                           | No                  | 42    | 79±14   | 66 – 74   | |
| Skin Color                | White               | 74 (73) | 66±15   | 63 – 70   | 0.008 |
|                           | Brown or Black      | 27 (27) | 58±15   | 52 – 64   | |
| Ultraviolet Radiation     | ≥ 2 sed             | 45    | 70±14   | 66 – 74   | <0.001 |
|                           | < 2 sed             | 51    | 59±15   | 55 – 63   | |
| Distress group (GHQ)      | High                | 41 (41) | 58±16   | 54 – 63   | 0.001 |
|                           | Low                 | 60 (59) | 68±1    | 64 – 71   | |
| Smoking                   | Yes                 | 26 (26) | 60±18   | 53 – 68   | 0.26 |
|                           | No                  | 75 (74) | 65±14   | 63 – 68   | |
| Consumption of alcoholic beverages | Yes | 34 (34) | 66±17   | 60 – 72   | 0.29 |
|                           | No                  | 67 (66) | 63±15   | 59 – 66   | |
| Sunscreen daily           | Yes                 | 52 (52) | 63±17   | 58 – 68   | 0.28 |
|                           | No                  | 48 (48) | 65±14   | 61 – 69   | |
| Sunbathing ≥ 20 times in the last year or so | Yes | 12 (14) | 66±17   | 60 – 72   | 0.09 |
|                           | No                  | 76 (86) | 63±15   | 59 – 66   | |
| Physical Activity level   | Very Active         | 26 (26) | 66±13   | 61 – 72   | 0.51 |
|                           | Active              | 50 (50) | 64±16   | 59 – 68   | |
|                           | Sedentary           | 25 (25) | 61±16   | 52 – 68   | |
| Occupation                | Beauty and Health   | 21 (21) | 59±16   | 52 – 67   | 0.68 |
|                           | Education           | 19 (19) | 66±13   | 60 – 72   | |
|                           | Administrative      | 26 (6)  | 65±18   | 58 – 72   | |
|                           | Housewife           | 19 (19) | 65±14   | 59 – 72   | |
|                           | Others              | 16 (6)  | 64±15   | 56 – 72   | |

*Table 3: 25-Hydroxyvitamin D concentration of the Brazilian participants from Araraquara (SP) according to health risk factors and lifestyle behaviors.*

Statistical Analysis: ' independent t – test; * analysis of variance (Anova). Overweight is BMI >25 or women over 60 with BMI >27.
results are similar to those found by Brazilian Longitudinal Study of Aging (ELSI). The higher mean concentrations of 25(OH)D in older women in the current study was surprising and contradictory to other studies. However, in the multivariate regression model, age was not associated with serum 25(OH)D in older postmenopausal women, as the variable lost significance when UV radiation and BMI were included. More studies are needed to explain how higher exposure to UV radiation and lower BMI in this age help with ideal optimal concentrations of 25(OH)D.

BMI was an important confounding factor, explaining 10-6% of the regression model for vitamin D concentrations. The inclusion criteria of participants being generally healthy and not undergoing treatment for chronic diseases, including osteoporosis, may have favored the result of higher vitamin D levels in the older age group compared to other studies. It is well known that vitamin D deficiency in postmenopausal women is associated with increased prevalence of bone diseases such as osteoporosis and osteomalacia, therefore the exclusion of these pre-existing conditions may have limited the generalizability of our findings.

Recent studies point to high rates of vitamin D insufficiency related to low consumption of vitamin D-containing foods, as well as a high prevalence of overweight and obesity. In a study conducted among Brazilian women, the mean serum 25(OH)D concentration in the older age group was found to be significantly higher compared to younger age groups. This is in contrast to other studies, where no significant difference in 25(OH)D levels was observed between age groups. The inclusion of BMI and UV radiation as confounding factors in the regression model may explain these discrepancies.

Table 4: Participants’ parameters by vitamin D status

| Parameters                        | 25 – 49.99 (n=16) | 50 – 74.99 (n=58) | >75 (n=27) | P value
|-----------------------------------|-------------------|-------------------|-----------|---------|
| Serum 25(OH)D (nmol/L)            | 41±6              | 61±6              | 83±8      | <0.001  |
| Age (years)                       | 46 (39 – 57)      | 50 (42 – 57)      | 56 (47 – 61) | 0.07    |
| BMI (kg/m²)                       | 28±4±4 8*         | 27±4±4 5         | 24±9±3 3* | 0.02    |
| Waist Circumference (cm)          | 94±2±10 5         | 94±2±12 9        | 87±7±9 5  | 0.05    |
| Ultraviolet Radiation (UVR)       | 1±3 (0.8 – 1.5)   | 1±8 (1.4 – 2.7)  | 2±2 (1.5 – 3.1) | 0.007  |
| Vitamin D intake (µg/day)         | 2±9 (2.2 – 4.2)   | 3±9 (2.3 – 4.9)  | 2±9 (1.8 – 4) | 0.23    |
| Calcium Intake (mg/day)           | 554±54 (469 – 669)| 654±54 (362 – 886)| 604±54 (437 – 713) | 0.53    |
| Sunbathing (times in the last year)| 3±1 (1 – 7)      | 3±1 (0 – 10)     | 3±1 (0 – 10) | 1.00    |
| Distress score                    | 6±5 (1.8 – 10)    | 2±1 (7)          | 1±0 (3.5) | 0.03    |

*Values: mean ± SD or median (25th,75th percentile). **Statistical analysis: Kruskal – Wallis, unless otherwise stated; †Anova. Values in the same row with the same superscript letters are significantly different (p <0.05).

Figure 1. Scatterplot diagram with correlation line between the independent variables (Age, Distress score, BMI and RUV) and the dependent variable 25(OH)D concentration of the Brazilian participants.
women that were living in either Goiás (Brazil) or Surrey (England) (n = 114), mean vitamin D dietary intake was 2·4 ± 1·9 μg/day.50 Brazil does not have food vitamin D fortification policies. In addition, the low intake of vitamin D in natural foods may have made it difficult to analyze the association with 25 (OH) D concentrations in the blood.

Skin pigmentation is known to be a limiting factor to vitamin D production, especially for migrants and descendants of darker—pigmented skin living in northern countries where there is low sun exposure, or due to dress cover and sun avoidance behavior.54 In our study, women who had white skin, had higher concentrations of 25(OH)D than those that were of black or brown skin.

In the UK, studies have been conducted that specify the weighted dose of UVR for vitamin D synthesis in summer, in order to ensure sufficient vitamin D production to prevent deficiencies in winter months.43,54 The desirable dose of UVR depends on skin type, and the exposure times required differ for different skin types.43 A light—skinned person who does not tan easily would show a very mild burn after absorbing 2–3 SED in a short period of time.54 The target dose of UVR throughout the summer for Caucasian adults living in high latitudes such as the UK remains 38 SED in a small and frequent exposure regime, i.e. a short, daily exposure.54 Studies such as this are needed for residents of tropical countries. In the present study, women with a dose of UVR greater than or equal to 2 SED, over four consecutive days, had 25 (OH) D concentrations of 70 nmol/L on average, while those who had doses less than 2 SED had a mean concentration of 59 nmol/L, which is borderline sub-optimal.

In a study conducted by Mendes et al in Brazilian women living in either England or Brazil, an increase of 20–25 nmol/L of 25 (OH) D concentration was observed for each extra SED of UVR measured by the same method.55 However, in our study, an increase of 5–15 nmol/L of 25 (OH) D concentration was observed for each extra SED of UVR measured by the same method. In that study, because it included participants from England, 25 (OH) D concentrations were very low, which led to a larger within—sample variance. Such results suggest that individual countries need specific sunlight exposure recommendations due to differences in solar radiation, as well as differences in sociodemographic characteristics such as age, skin color, and BMI, resulting in region—specific recommendations.

An individual’s work environment is directly related to the time of skin exposure to the sun. Long hours of indoor work can contribute to vitamin D deficiency in the adult population, as shown in studies of health care workers in hospitals and clinics.44,55 In our study, lower levels of exposure to UVR were found in certain occupation categories that work indoors, including health professionals which suggests that health promotion messaging to prevent suboptimal vitamin D status could be targeted in various workplaces. Even though there are no significant differences in 25(OH)D concentrations in the occupational categories analyzed, other studies have shown lower rates of vitamin D insufficiency in those who work outdoors.56

Cultural differences in clothing and other cultural habits of avoiding sun exposure with constant use of sunscreen and staying indoors all day may interfere with the process of endogenous vitamin D production.27,55 Enlarging the area of fully exposed skin (reduced clothing, no sunscreen) increases the skin’s supply of vitamin D. Experts recommend exposing as much skin as temperature and social and cultural customs allow, accepting that this may vary from day to day.59

In the present study, no associations were found between 25(OH)D concentrations and lifestyle behaviors such as smoking, alcohol consumption, physical activity levels, sunscreen use, and sunbathing (more than 20 times a year). The lack of association between daily use of sunscreen and 25(OH)D concentration should be evaluated in conjunction with other behavioral variables typical of tropical city dwellers, such as staying in shaded places that offer better thermal comfort. In Brazil, the population has the habit of sunbathing during high heat intensity periods on vacations or long holidays. Evidence reinforces that large and frequent exposure of skin to the sun are inefficient for vitamin D synthesis due to the complexities of skin photochemistry and may also increase the risk of sunburn and further skin damage.60 The failure to observe differences between physical activity groups assessed using IPAQ on mean vitamin D concentration needs to be further evaluated. Introduction of questions related to physical activity and/or walking outdoors is recommended for further studies.

In the present study, an association of distress score and lower 25 (OH) D concentrations was observed. A possible relationship of vitamin D in the pathophysiology of depression is currently speculative, and more rigorous research is needed to evaluate this association in large adult populations. Prospective associations between vitamin D status and depression have already been found suggesting that both vitamin D deficiency and insufficiency may be risk factors for the onset of depression in middle—aged adults.51

The analysis from the global syndemic perspective may lead to a greater understanding of the vitamin D deficiency pandemic. The relationship between lower vitamin D levels and higher body mass index, observed in the present study, brings yet another challenge for reducing overweight and malnutrition. The optimal level of vitamin D, being directly associated with ultraviolet radiation, may be impacted by climate change that is occurring globally.62 In countries with a tropical climate, these changes can lead to avoidance of sun
exposure because of high temperatures. In addition, the urban lifestyle, characterized by staying indoors, inside buildings, or spending long hours in transportation, are not conducive to outdoor activities.

Among the limitations of the study, we mention: A. The sample size, which was not based on the population size of the city of Araraquara. Nevertheless, it was representative of the census population regarding skin color. As part of a multicenter study, the sample was standardized for different population sizes. B. The potential for bias in the self-reported answers to questionnaires, and adherence to the use of radiation dosimeter badge without direct monitoring by the researchers. C. The results cannot be generalized for other Brazilian or tropical cities as it was undertaken in a middle-size urban area, with mild winter, prevalence of single-family homes, scarce high rise-buildings, and good level of tree shaded streets.

This cross-sectional study of healthy, community-dwelling Brazilian women has found the average concentration of 25 (OH) D to be in the normal range, but 15% had vitamin D insufficiency or deficiency using Endocrine Society Clinical Practice Guideline and 25% among brown and black women (<50nmol/L). Even in a tropical country such as Brazil, those with a darker skin color had lower vitamin D levels.

Our findings suggest that studies on the optimal exposure dose to UVR for each season to prevent vitamin D deficiency are needed in tropical countries, especially in highly urbanized areas that are inland. The higher 25(OH)D mean concentration observed in the older, postmenopausal women in the non-parametric analysis can be explained by their relatively higher sun exposure compared to those younger than 50 years. Nevertheless, multivariate analysis showed no association between age and vitamin D. More studies are necessary to understand how sun exposure and healthy eating habits interfere with these levels. Our findings have important public health implications since they suggest that vitamin D deficiency in older age is not inevitable. The challenge of developing public health policies to prevent and cope with vitamin D deficiency and its numerous health effects is more complex and interdisciplinary as life and work habits associated with climate change have limited healthy exposure to UV radiation, even under humid tropical climates characterized by mild and sunny winters.

Contributors
Keila Valente de Souza Santana - Literature search, methodology, figures, data collection, data analysis, data interpretation, underlying data, writing – original draft.
Helena Ribeiro - Literature search, Study design, methodology, funding acquisition, data collection, data interpretation, underlying data, supervision, writing – review & editing.
Sofia Oliver Lizarralde - Literature search, Data collection, writing – review & editing.
Marcela Moraes Mendes - Literature search, data interpretation, writing – review & editing.
Karen E Charlton - Study design, methodology, data interpretation, funding acquisition, writing – review & editing.
Susan Lanham-New - Study design, conceptualisation, methodology, funding acquisition, supervision, writing – review & editing.

Data sharing statement
The datasets are not subject to restrictions or embargo. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of interests
We declare no competing interests.

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2022.101400.

References
1 Swinburn BA, Kraak VI, Allender S, et al. The global syndemic of obesity, undernutrition, and climate change: the Lancet Commission report. Lancet. 2019;393(10173):791–846.
2 Holick MF. The vitamin D deficiency pandemic: a forgotten hormone important for health. Public Health Rev. 2010;32(3):267.
3 Van Schoor N, Lips P. Worldwide Vitamin D Status. Vitamin D. Elsevier; 2018:15–40.
4 Institute of Medicine [A. Catharine Ross CLT eds editor]. Vitamin D and Health. London, UK: Scientific Advisory Committee on Nutrition (SACN).
5 Swinburn BA, Blackbourn DJ, Ahmadi KR, Lanham-New SA. Vitamin D supplement use and associated demographic, dietary and lifestyle factors in 8024 South Asians aged 40-69 years: analysis of the UK Biobank cohort. Public Health Nutr. 2018;21(4):2678–2688.
6 Darling AL, Blackbourn DJ, Ahmadi KR, Lanham-New SA. Vitamin D supplement use and associated demographic, dietary and lifestyle factors in 8024 South Asians aged 40-69 years: analysis of the UK Biobank cohort. Public Health Nutr. 2018;21(4):2678–2688.
7 Martineau AR, Jolliffe DA, Hooper RL, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ. 2017;356.
8 Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266–281.
9 Vimalaswaran KS, Cavadino A, Berry DJ, et al. Association of vitamin D status with arterial blood pressure and hypertension risk: a
mendelian randomisation study. Lancet Diabetes Endocrinol. 2014;2 37:719–720.

10 Mirhosseini N, Vatanparast H, Kimball SM. The association between serum 25 (OH) D status and blood pressure in participants of a community-based program taking vitamin D supplements. Nutrients. 2017;9(11):1244.

11 Zhou A, Selvanayagam JB, Hyppén-ен E. Non-linear Mendelian randomisation analyses support a role for vitamin D deficiency in cardiovascular disease risk. Eur Heart J. 2022;1–10.

12 Acharya P, Dalia T, Ranka S, et al. The effects of vitamin D supplementation and 25-hydroxyvitamin D levels on the risk of myocardial infarction and mortality. J Endocr Soc. 2021;5(3):e2b124.

13 De Sousa Almeida-Filho B, et al. Vitamin D deficiency is associated with poor breast cancer prognostic features in postmenopausal women. J Steroid Biochem Mol Biol. 2017;174:278–289.

14 McDonnell SL, et al. Breast cancer risk markedly lower with serum 25-hydroxyvitamin D concentrations 60 vs < 50 nmol/L: pooled analysis of two randomized trials and a prospective cohort. PLoS One. 2018;13(9):e0209265.

15 Dawson-Hughes B, et al. Intratrial exposure to vitamin D and new-onset diabetes among adults with prediabetes: a secondary analysis from the vitamin D and type 2 diabetes (D2D) study. Diabetes Care. 2020;43(11):2916–2922.

16 Orstrell J, Oliva C, Casado E, et al. Vitamin D supplementation and COVID-19 risk: a population-based, cohort study. J. Endocrinol. Invest. 2021;1–11.

17 Manson JE, Brannon PM, Rosen C, et al. Vitamin D deficiency is there really a pandemic? N Engl J Med. 2016;375(9):837–840.

18 Murphy PK, Wagner CL. Vitamin D and mood disorders among women: an integrative review. J Midwifery Womens Health. 2008;53(5):440–446.

19 Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr. 2004;79(1):167–177.

20 Bandeira F, Griz L, Dreyer P, Eufrazino C, Bandeira C, Freese E. Vitamin D deficiency: a global perspective. Arq Bras de Endocrinol Metabol. 2005;49(5):640–646.

21 MacLaughlin J, Holick MF. Assessing the adequate skin-to-human skin to produce vitamin D3. J Clin Invest. 1991;78(4):1536–1538.

22 Clemens TN, Henderson SL, Adams J, Holick MF. Increased skin pigment reduces the capacity of skin to synthesize vitamin D3. Lancet. 1982;319(8263):74–76.

23 Jablonski NG, Chaplin G. Human skin pigmentation, migration and disease susceptibility. Philos Trans R Soc B Biol Sci. 2012;367(1600):565–572.

24 Sarazia GV, Cendoroglo MS, Ramos LR, et al. Prevalence of vitamin D deficiency, insufficiency and secondary hyperparathyroidism in the elderly inpatients and living in the community of the city of São Paulo, Brazil. Arq Bras de Endocrinol Metabol. 2007.

25 Darling AL, Hart KH, Arber S, et al. 25-hydroxyvitamin D status, light exposure and sleep quality in UK dwelling South Asian and Caucasian postmenopausal women. J Steroid Biochem Mol Biol. 2019;189:265–273.

26 CETESB. Relatordo de Qualidade do Ar no Estado de São Paulo - 2019. Secretaria do Meio Ambiente, Série Relatorios - ISSN 0103-4103. São Paulo, 2020; https://cetesb.sp.gov.br/apweb/content/uploads/site/2020/07/Relatorio%20de%20Qualidade%20do%20Ar-2019.pdf.

27 World Health Organization. Particulate Matter (PM2.5 and PM10). Ozone, Nitrogen dioxide, Sulfur Dioxide and Carbon Monoxide. Guidelines. W.G.A Q. Geneva: World Health Organization; 2021. Licenci: CC BY-NC-SA 3.0 IGO.

28 IBGE. Instituto Brasileiro de Geografia e Estatística. Sistema IBGE de Recuperação de Dados Autônomos SIDRA 2021; https://sidra.ibge.gov.br/topo/20190410163243.

29 Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911–1930.

30 Lima-Costa MF, Mambirini JVM, de Souza-Junior PRB, et al. Nationwide vitamin D status in older Brazilian adults and its determinants: the Brazilian Longitudinal Study of Aging (ELSI). Sci Rep. 2020;10(1):1–9.

31 Mendes MM, Hart KH, Lanham-New SA, et al. Exploring the impact of individual UVB radiation levels on serum 25-hydroxyvitamin D levels in women living in high vs low latitudes: a cross-sectional analysis from the RIO-D3 SOL study. Nutrients. 2020;12(12):3805.
57 Buckley AJ, Hannoun Z, Lessan N, Holick MF, Barakat MT. Environmental determinants of previtamin D synthesis in the United Arab Emirates. Dermatoendocrinol. 2017;9(1):e1267079.

58 Lee DH, Park KS, Cho MC. Laboratory confirmation of the effect of occupational sun exposure on serum 25-hydroxyvitamin D concentration. Medicine. 2018;97(27). (Baltimore).

59 Lanham-New SA, Webb AR, Cashman KD, et al. Vitamin D and SARS-CoV-2 virus/COVID-19 disease. Br Med J Nutr Prev Health. 2020;3(1):106.

60 Webb AR, Holick MF. The role of sunlight in the cutaneous production of vitamin D3. Annu Rev Nutr. 1988;8(1):375–399.

61 Ronaldson A, de la Torre JA, Gaughran F, et al. Prospective associations between vitamin D and depression in middle-aged adults: findings from the UK Biobank cohort. Psychol Med. 2020;1–9.

62 Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change IPCCMasson-Delmotte V, Zhai P, Pirani A, Connors SL, Pöschl C, Berger S, Huang M, Leitzell K, Lonnoy E, Matthews JBR, Maycock TK, Waterfield T, YelekÖi O, Yu R, Zhou B, et al. Summary for policymakers. Climate Change 2021: The Physical Science Basis. Cambridge University Press. 2021. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change(EDS.)In Press.