

Does the Access to Sun Exposure Ensure Adequate Levels of 25-Hydroxyvitamin D?

A exposição ao sol assegura níveis adequados de 25-hidroxivitamina D?

Ênio Luis Damaso¹ Francisco José Albuquerque de Paula² Silvio Antônio Franceschini¹
Carolina Sales Vieira¹ Rui Alberto Ferriani¹ Marcos Felipe Silva de Sá¹ Lucia Alves da Silva Lara¹

¹Department of Obstetrics and Gynecology, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil

²Department of Internal Medicine, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Address for correspondence Ênio Luis Damaso, Rua Romeu Engrácia de Faria, 325, Apartment 34, Nova Aliança, Ribeirão Preto, SP, Brazil (e-mail: eniodamaso@yahoo.com.br).

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Abstract

Objectives To assess the prevalence of hypovitaminosis D, altered arterial blood pressure, and serum levels of glucose and lipids in community-dwelling women in the city of Ribeirão Preto, in the southeast of Brazil.

Methods This was a cross-sectional study of women aged 40–70 years old. Calcium intake and level of sun exposure were assessed by means of a questionnaire. A blood sample was used to determine glucose, lipid profile and 25-hydroxyvitamin D (25[OH]D) concentration.

Results Ninety-one women were enrolled (age = 54.2 ± 7.1 years). The mean serum 25(OH)D concentration was 25.7 ± 8.9 ng/mL. A total of 24 (26.4%) women had 25(OH)D levels < 20 ng/mL. Seventy women (76.9%) had 25(OH)D levels < 30 ng/mL. Seventy-five women (90.4%) had inadequate calcium intake, and 61 women (67%) had appropriate sun exposure, 49 of whom (80.3%) had serum 25(OH)D levels < 30 ng/mL.

Conclusion This study indicates that even in community-dwelling women, living in a city with high sun exposure, serum levels of 25(OH)D > 30 ng/ml are hardly reached. Thus, it is probable that other intrinsic factors besides sun exposure may regulate the levels of vitamin D.

Keywords

- ▶ Female Gender
- ▶ 25-hydroxyvitamin D
- ▶ vitamin D deficiency
- ▶ sun exposure

Resumo

Objetivos Estimar a prevalência de hipovitaminose D, hipertensão arterial, e níveis séricos de glicose e perfil lipídico em uma comunidade de mulheres de Ribeirão Preto, no Sudeste brasileiro.

Métodos Estudo transversal com mulheres de 40 a 70 anos de idade, submetidas a um questionário para determinar ingestão diária de cálcio e nível de exposição solar, e coleta de sangue para determinar glicose, perfil lipídico e concentração de 25-hidroxivitamina D (25[OH]D).

Resultados Noventa e uma mulheres foram incluídas (idade = $54,2 \pm 7,1$ anos). O nível sérico médio de 25(OH)D foi $25,7 \pm 8,9$ ng/mL. Um total de 24 (26,4%) mulheres

Palavras-chave

- ▶ sexo feminino
- ▶ 25-hidroxvitamina D
- ▶ deficiência de vitamina D
- ▶ exposição ao sol

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teve níveis de 25(OH)D < 20 ng/mL. Setenta mulheres (76.9%) tiveram níveis de 25(OH)D < 30 ng/mL. Setenta e uma mulheres (90.4%) tiveram uma ingestão inadequada de cálcio e 61 mulheres (67%) tiveram exposição solar adequada; 49 das quais (80.3%) tiveram níveis séricos de 25(OH)D < 30 ng/mL.

Conclusão Este estudo indica que mesmo morando em uma cidade com exposição solar adequada, níveis séricos de 25(OH)D > 30 ng/mL dificilmente são atingidos por mulheres climatéricas. Logo, é provável que outros fatores intrínsecos podem regular o nível de vitamina D.

Introduction

Vitamin D, classically, was considered a hormone specialized in the control of bone and mineral metabolism. However, the role of vitamin D on human physiology has been completely re-dimensioned due to new evidences indicating that vitamin D is a pleiotropic hormone able to participate in the endocrine control of several systems, such as: cardiovascular, energy metabolism and immune.¹ Vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol) are the two main forms of vitamin, and act as prohormones. The ultraviolet radiation in sunlight promotes vitamin D₃ synthesis in the skin, and this accounts for ~ 60–80% of the required amount.¹ The non-enzymatic conversion of pro- to pre-, and subsequently to vitamin D formation in response to solar UVB radiation, leads thereafter to the production of the active hormone, through hydroxylation in the liver (forming 25-hydroxyvitamin D [25-OHD])¹ (and then 1 α hydroxylation in the kidney (synthesizing 1,25-dihydroxyvitamin D [1,25(OH)₂D])). The active form of vitamin D, 1,25(OH)₂D, binds to nuclear receptors of cells in the intestine and kidney, and stimulates calcium and phosphorus absorption. Vitamin D also binds to osteoclasts and stimulates bone reabsorption.² However, the synthesis of this secosteroid in the skin is tightly controlled to avoid vitamin D intoxication, even during continuous and excessive sun exposure. For instance, melanin production in the skin is stimulated by the sunlight and compete for UVB rays. In addition, inert compounds are synthesized by a thermos-regulated process during the steps of pre- and post-vitamin D formation synthesis within the skin.¹

There is controversy regarding the healthy range of serum vitamin D. The Institute of Medicine (IOM) proposed that circulatory levels of 25(OH)D above 20 ng/mL are adequate for the most part of the population. Moreover, the IOM indicated that severe vitamin D deficiency occurs only when the 25(OH)D levels are below 12 ng/mL.³ On the other hand, the Endocrine Society dictates that vitamin D sufficiency is achieved at levels above 30 ng/mL, while the range from 20 to 30 ng/mL indicates vitamin D insufficiency and levels below 20 ng/mL indicate deficiency.^{2,4} Thus, the prevalence of hypovitaminosis D depends on which recommendations are accepted.⁵

Vitamin D deficiency induces abnormal calcium, phosphorus, and bone metabolism. A reduced level of vitamin D

decreases intestinal calcium absorption, thereby increasing the level of parathyroid hormone (PTH), which in turn stimulates the renal synthesis of 25(OH)D-1 α -hydroxylase, the renal enzyme that converts 25(OH)D into 1,25(OH)₂D. The elevation of PTH also increases osteoclast activity, causing bone loss and, ultimately, leading to osteopenia and osteoporosis. Vitamin D deficiency has also been associated with muscle weakness, and increased fall risk in adults and elderly individuals,^{2,6} as well as with metabolic diseases,^{7,8} and insulin resistance.⁹

In most individuals, the serum level of vitamin D is higher in the summer than in the winter.¹⁰ Previous research estimated that a large number of adult men and women suffer from hypovitaminosis D, and that the elderly are the most affected.^{2,11,12} Even though Brazil is situated in the tropics and subtropics, previous study indicated a high prevalence of hypovitaminosis D in some subpopulations from certain regions.¹³

The metabolic consequences of the vitamin D status are shown in recent studies; hypertension and endothelial dysfunction,¹⁴ as well as dyslipidemia¹⁵ are associated with low levels of 25(OH)D. Supplemental vitamin D and calcium are frequently recommended for preservation of bone mass,¹⁶ although regular sun exposure is the simplest method to attain an adequate level of vitamin D. In particular, exposure to 5 to 15 minutes of sunlight between 10 AM and 3 PM in the spring, summer, and fall is sufficient for maintenance of adequate levels of vitamin D.¹⁷ Although Brazil has a continental size, most of his territory has abundant incidence of sun light, supposedly, precluding a high incidence of D hypovitaminosis. The main objective of the present study was to assess the prevalence of hypovitaminosis D in community-dwelling women from Ribeirão Preto, a sunny city in the southeast of Brazil

Methods

Women aged 40 to 70 years old who came to the Climacteric Outpatient Clinic of the Centro de Saúde Escola Sumarezinho from January to December of 2013 for routine gynecological visits were eligible for participation in this cross-sectional study. The nursing staff informed all women about the study, and invited them to participate. Institutionalized women, and women who had limited movement without the help of devices (orthoses, prostheses, wheelchairs, etc.) or of other

people were excluded because such individuals are known to have low serum levels of vitamin D.¹⁸ A total of 150 women were invited to participate in this study, 110 of whom agreed to it. Nineteen of these women were excluded because they did not undergo blood collection, and 91 women were ultimately enrolled.

In a private office, a doctor evaluated each participant using a semi-structured interview that asked about the use of medications (hormones, supplemental vitamin D and/or calcium), consumption of calcium-rich foods, duration of sun exposure, and duration of weekly physical activity.

Body mass index was estimated in all subjects and they were classified in accordance with the World Health Organization classification: underweight (BMI < 18.5 kg/m²), normal weight (BMI = 18.5–24.9 kg/m²), grade I overweight (BMI = 25–29.9 kg/m²), grade II overweight (BMI = 30–39.9 kg/m²), or grade III overweight (BMI ≥ 40 kg/m²).

A blood sample was collected between March and June of 2013 (fall and winter in Brazil) for measurement of serum 25(OH)D levels, ionized calcium, fast glucose, and lipid profile. Also, creatinine was measured as it is negatively correlated with vitamin D levels.¹⁹ Renal function was assessed by measurement of serum creatinine by automated spectrophotometry (Wiener Laboratory, Rosario, Santa Fe, Argentina). Serum 25(OH)D was determined by a chemiluminescence immunoassay using the LIAISON 25 OH Vitamin D TOTAL assay (DiaSorin, Saluggia, Italy). Glucose was determined by automated spectrophotometry using the AA liquid enzymatic glycaemia kit (Wiener Laboratory, Rosario, Santa Fe, Argentina). Ionized calcium was determined by automated spectrophotometry using the Arsenazo III AA kit (Wiener Laboratory, Rosario, Santa Fe, Argentina). Serum total cholesterol, HDL, and triglycerides were determined by automated spectrophotometry (Wiener Laboratory, Rosario, Santa Fe, Argentina).

Consumption of calcium was estimated according to the milk and dairy products data in the food composition table suggested by the US Department of Agricultural Research Service.²⁰

Sun exposure was estimated based on self-reported outdoor time and based on the professional and life habits of each subject. Exposure was considered adequate if at least 20% of the body surface (face, neck, arms, and legs) was exposed for at least 10 minutes, 3 or more times per week between 10 AM and 3 PM,¹⁷ and inadequate when the exposure was below this mark. The duration of physical activity (walking) was classified based on the time recommended for the prevention of osteoporosis by the American College of Sports' Guidelines for Exercise Testing and Prescription²¹: 20 to 40 minutes of exercise, 2 to 4 times per week.

Based on indications of the Endocrine Society, serum 25 (OH)D concentration was classified as deficient (< 20 ng/mL), insufficient (21–29 ng/mL), or sufficient (30–100 ng/mL).²⁴

Statistical Analysis

All statistical procedures were performed using the PROC MEANS and PROC FREQ features of the SAS 9.0 (Cary, NC,

USA) software. For the description of quantitative variables, mean and standard deviation were calculated. For qualitative variables, the absolute and relative frequencies were calculated. Fisher exact test was used to determine the association between two qualitative variables. Women with vitamin D deficiency (< 20 ng/ml) and adequate vitamin D levels were compared in terms of qualitative variables (systemic hypertension, diabetes mellitus, hypothyroidism, alcoholism, smoking, physical activity and sun exposure) and quantitative variables (age, BMI, serum calcium, blood glucose, HDL, total cholesterol, triglycerides and creatinine). Exploratory data analysis was performed through central position measurements and dispersion. Data distribution was checked by normal graphics. A non-parametric Mann Whitney test was used to compare quantitative variables between groups. The chi-square test was used to compare the distributions of qualitative variables in the groups. The variable sun exposure was dichotomized with cutoff of three times a week as adequate exhibition. A model of logistic regression was performed to verify the influence of the variables age, creatinine and sun exposure in relation to vitamin D concentration. Significance was set at $p < 0.05$.

Results

Ninety-one women participated in the study. ► **Table 1** shows the demographic, laboratory and clinical characteristics of the enrolled women.

Mean serum 25(OH)D was 25.7 ± 8.9 ng/mL. A total of 24 (26.4%) women had 25(OH)D levels < 20 ng/mL. Seventy women (76.9%) had 25(OH)D levels < 30 ng/mL (18 of 20 black women [90%] and 54 of 71 white women [76%]) and 21 (23.1%) women had sufficient 25(OH)D levels (≥ 30 ng/mL). Serum 25(OH)D concentration was dichotomized as < 20 ng/ml and ≥ 20 ng/ml. ► **Table 2** shows the distribution of the studied qualitative variables and the comparison between groups regarding the incidence of these variables according to the vitamin D levels. There was no difference between women with vitamin D deficiency regarding the incidence of hypertension, diabetes mellitus, hypothyroidism, and habits of smoking and drinking. Also, there was no difference between women with adequate amount of physical activity and adequate sun exposure (► **Table 2**).

► **Table 3** shows the distribution of the studied quantitative variables and the comparison between groups of the incidence of such variables. There was no significant difference between women with vitamin D deficiency regarding age, BMI, and dosage of calcium, fast glucose, lipid profile, and creatinine.

Based on recall, average calcium consumption was 560.4 ± 435.8 mg/day, and 75 women (90.4%) had inadequate calcium intake (< 1200 mg/day). Sixty-one women (67%) reported adequate sun exposure and 49 of these women (80.3%) had serum 25(OH)D levels < 30 ng/mL.

There was no significant difference in the serum 25(OH)D concentration for women with adequate and inadequate sun exposure ($p = 0.88$, ► **Fig. 1A**). In addition to this, there was

Table 1 Anthropometric, laboratory, and clinical characteristics of the enrolled women (n = 91)

Variable	Mean ± SD or n (%)
Age (years)	54.2 ± 7.1
BMI (kg/m ²)	27.1 ± 4.8
25(OH)D (ng/mL)	25.7 ± 8.9
Calcium ion (mmol/L)	1.09 ± 0.05
Glucose (mg/dL)	90.4 ± 18.5
Total cholesterol (mg/dL)	187.1 ± 38.5
HDL-C (mg/dL)	46.6 ± 11.8
Triglycerides (mg/dL)	115.2 ± 59.9
Creatinine (mg/dL)	0.80 ± 0.11
Diseases	
Arterial hypertension	30 (32.9)
Hypothyroidism	11 (12.0)
Diabetes mellitus	7 (7.6)
Dyslipidemia	15 (16.4)
Other	25 (27.4)
None	28 (30.7)
Race	
White	71 (78.0)
Mulatto/Black	20 (21.9)
Habits	
Smoking (Yes)	11 (12.0)
Alcohol ingestion (Yes)	20 (22)
Physical activity	
≤ 150 minute/week	76 (83.5)

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; n, number of subjects; SD, standard deviation

no significant difference in serum concentration of ionized calcium for women with adequate and inadequate calcium consumption ($p = 0.73$, ► **Fig. 1B**).

Finally, we used multiple linear regression analysis to determine the effect of sun exposure, age, and serum creatinine on serum 25(OH)D concentration (► **Table 4**). The results show that a normal level of serum creatinine was significantly and independently associated with a higher level of serum 25(OH)D. The other variables had no significant effect on the levels of serum 25(OH)D.

Discussion

The present study of women living in a sunny subtropical region indicated a high prevalence (76.9%) of vitamin D insufficiency (< 30 ng/mL), even in women whose self-reported sun exposure was considered to be adequate. Moreover, 26.4% of the women had a deficiency of vitamin D (serum 25(OH)D < 20 ng/mL). These findings confirm previous studies conducted in other Brazilian cities at dif-

ferent latitudes.^{18,22} The frequency of arterial hypertension and dyslipidemia in our study were similar in women with sufficient and deficient 25(OH)D levels. In fact, vitamin D supplementation in hypertensive patients with low 25(OH)D had no significant effect on blood pressure (BP) and several cardiovascular risk factors.²³ Also, long-term vitamin D supplementation, which increased mean 25(OH)D concentration to 30 ng/mL or higher, had no effect on BP.²⁴ Furthermore, oral vitamin D supplementation to correct vitamin D deficiency does not improve the lipid profile.²⁵ It is worth to emphasize that the relationship between vitamin D and cardiovascular disease, as well as other clinical conditions, comes from observational studies, while further controlled studies do not show such a relationship.

In the southeast region of Brazil, 43.7% of postmenopausal women have 25(OH)D levels < 20 ng/mL, and 25(OH)D deficiency increases significantly with age.²² The São Paulo Aging & Health Study evaluated individuals older than 65 years old in the city of São Paulo and reported 25(OH)D deficiency in more than half of this population, and this deficiency was particularly notable in women, subjects with type 2 diabetes, and during the winter and spring,²⁶ seasons when ultraviolet radiation and 25(OH)D concentrations reach a nadir in the city of São Paulo.²⁷ World data demonstrate that 5 to 25% of the independent elderly population and 60 to 80% of institutionalized patients have vitamin D deficiencies.²⁸ Likewise, 20 to 100% of elderly subjects in North America, Canada, and Europe,^{2,11} as well as postmenopausal women living in southeastern and central Europe,²⁹ are believed to have vitamin D deficiencies. We did not expect a high prevalence of 25(OH)D insufficiency/deficiency in the present study because these women live in a region that is sunny throughout the year, and wear warm clothing for only short periods of time. Furthermore, this region has high agricultural activity, and the population is frequently exposed to sunlight. Nevertheless, only a minority of these women had sufficient levels of 25(OH)D (> 30 ng/mL).⁴ This is clinically significant because previous observational studies demonstrated that hypovitaminosis D is associated with some clinical conditions, such as cardiovascular diseases, secondary hyperparathyroidism, osteoporosis, and fractures.^{18,30,31}

A previous population-based study of osteoporosis in Brazil (BRAZOS) indicated that vitamin D levels did not affect the risk for fractures,³² possibly because fractures are associated with polymorphisms of the vitamin D receptors. However, several previous studies failed to establish this correlation in Brazilian and British populations.^{33,34} Moreover, associations between 25(OH)D levels with other clinical conditions were reported in some but not all observational studies,³⁵ and the results from randomized and controlled clinical trials are not yet available. These observational trials were also limited due to small sample sizes and short duration, which affected the quality of the investigations.^{36,37} On this basis, the current recommendation is to measure serum 25(OH)D levels only in situations in which there is a risk for hypovitaminosis D due to certain morbidities.⁴ This implies that the information given by patients

Table 2 Distribution of qualitative variables according to vitamin D cutoff classification as deficient [< 20 ng/mL] and adequate [≥ 20 ng/mL] vitamin D levels.

	Vitamin D levels		p
	< 20 ng/mL (n = 24)	≥ 20 ng/mL (n = 67)	
Arterial hypertension			
Yes	7 (29.1%)	23 (34.3%)	0.64
No	17 (70.8%)	44 (65.6%)	
Diabetes Mellitus			
Yes	2 (8.3%)	5 (7.4%)	0.91
No	22 (91.6%)	62 (92.5%)	
Hypothyroidism			
Yes	2 (8.3%)	9 (13.4%)	0.51
No	22 (91.6%)	58 (86.5%)	
Alcohol ingestion			
Yes	6 (25.0%)	14 (20.9%)	0.68
No	18 (75.0%)	53 (79.1%)	
Smoking			
Yes	5 (20.8%)	6 (8.9%)	0.13
No	19 (7.1%)	61 (91.0%)	
Physical activity			
None	2 (8.3%)	3 (4.6%)	0.14
≤ 150 minute/week	21 (87.5%)	48 (73.8%)	
> 150 minute/week	1 (4.1%)	14 (21.5%)	
Sun exposure			
Adequate	17 (7.8%)	51 (7.2%)	0.53
Inadequate	7 (29.1%)	15 (2.7%)	

Abbreviations: n, number of subjects; p- value.

about their supply of vitamin D (from exposure to sunlight and use of supplements) and clinical observations are needed for estimation of vitamin D status.

The present study indicated no significant difference in serum vitamin D levels of women classified as having

adequate or inadequate sun exposure and no correlation between serum 25(OH)D levels and reported duration of sun exposure. Information on sun exposure was self-reported, and may have been biased due to imprecise recall^{38,39} and misinterpretation by the interviewer of the information

Table 3 Distribution of quantitative variables according to vitamin D cutoff classification as deficient [< 20 ng/mL] and adequate [≥ 20 ng/mL] vitamin D levels.

	Vitamin D levels		p
	< 20 ng/mL (n = 24) Median (IQR)	≥ 20 ng/mL (n = 67) Median (IQR)	
Age (years)	54.5 (47.5–57.0)	54.0 (49.0–60.0)	0.36
BMI (kg/m ²)	28.9 (24.9–31.9)	26.4 (23.8–28.8)	0.15
Calcium ion (mmol/L)	1.09 (1.08–1.11)	1.1 (1.06–1.13)	0.97
Glucose (mg/dL)	93.5 (80.0–101.0)	89.3 (79.0–94.0)	0.55
Total cholesterol (mg/dL)	200.9 (176.5–218.0)	182.1 (158.0–203.0)	0.03
HDL-C (mg/dL)	44.3 (37.0–50.5)	47.5 (37.0–56.0)	0.30
Triglycerides (mg/dL)	129.5 (78.5–172.0)	110.1 (73.0–130.0)	0.25
Creatinine (mg/dL)	0.77 (0.70–0.83)	0.81 (0.70–0.90)	0.22

Abbreviations: IQR, interquartile range; n, number of subjects; p- value.

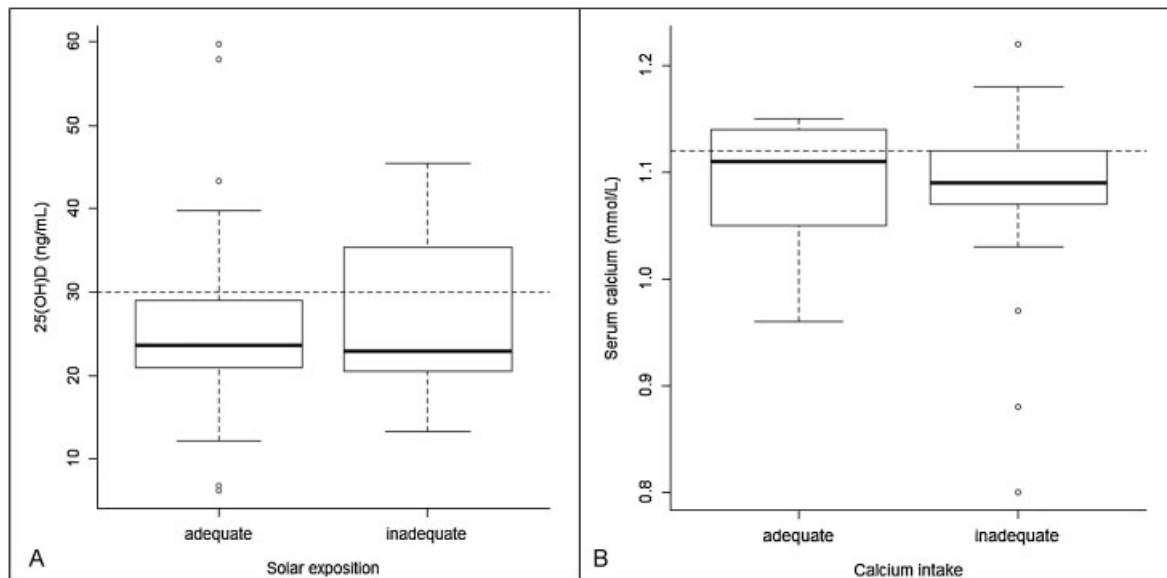


Fig. 1 Serum 25(OH)D concentrations in women with adequate and inadequate sun exposure (A) ($p = 0.88$), and serum ionized calcium concentrations (B) ($p = 0.73$) in women with adequate and inadequate calcium consumption ($n = 91$).

given by the interviewee. Consequently, the method of quantifying exposure to sunlight based on self-reporting has questionable accuracy, and more accurate methods are needed so that clinicians can provide guidelines to patients. However, it is also necessary to consider that other biological factors may interfere with 25(OH)D concentration, such as skin color.^{40,41} We did not thoroughly evaluate skin color in the present study by use of phototyping.⁴² It should be noted that dark skin interferes with vitamin D synthesis, and there is extensive racial mixing in the Brazilian population.⁴¹ A previous study conducted in São Paulo reported a high prevalence of hypovitaminosis D and secondary hyperparathyroidism in the sample population after the end of winter, and skin color significantly and independently correlated with this condition.⁴³

Most subjects in the present study had a self-reported calcium consumption of less than 1200 mg/day, the minimum recommended by the Institute of Medicine in the dietary reference intake of calcium.⁴⁴ A previous study had reported similar results (calcium consumption less than the minimum) for people residing in the state of São Paulo: an average of only 448 mg/day of calcium; although reported intake did not correlate with blood level of calcium, although intake did

Table 4 Multiple linear regression analysis of the effects of sun exposure (less than three times per week versus three or more times per week), age, and serum creatinine on serum 25(OH)D concentration ($n = 91$)

Variable	Odds Ratio	95% CI	<i>p</i>
Intercept	5.63	-14.09, 25.34	0.57
Sun exposure	0.36	-4.16, 4.87	0.88
Age	-0.03	-0.32, 0.26	0.83
Serum creatinine	28.11	10.36, 45.86	<0.01

Abbreviations: CI, confidence interval *p*-value.

not correlate with reported intake or blood level of calcium.⁴⁵ We also found no correlation between serum calcium and recall of calcium consumption or between serum calcium and the presence of 25(OH)D deficiency/insufficiency. It is known that calcium sensors in the parathyroid glands sense a change in circulating calcium, and this leads to increased PTH synthesis, and maintenance of blood calcium within the normal range.⁴⁶ This may partially explain the absence, in the present study, of a correlation between self-reported calcium consumption and serum level of calcium ion.

Our multiple linear regression analysis demonstrated that a serum creatinine level in the normal range had a significant and independent association with higher serum 25(OH)D concentration. Previous research indicated that in the absence of renal insufficiency, the renal production of 1,25(OH)₂D, the hydroxylation product of 25(OH)D, is normal,⁴⁷ but that renal insufficiency, which is associated with abnormally high serum creatinine, is associated with a low level of serum 25(OH)D.^{48,49}

Finally, the present findings support the need for a more objective evaluation of calcium and vitamin D supply, possibly by means of a 24-hour home diary.⁵⁰ Patient recall about the duration of sun exposure and calcium intake was not effective in assessing the actual levels of these substances in the present population. Although most of our study population had 25(OH)D insufficiency, there was no higher prevalence of arterial hypertension in women with insufficient 25(OH)D levels. However, the present study was limited due to the sample allocation being restricted to a climacteric outpatient clinic, which can potentially mean that our outcomes may not be applicable to other populations.

Conclusion

This study indicates that even in community-dwelling women living in a city under high sun exposure, levels of serum

25(OH)D > 30 ng/ml are hardly reached, leading to the conclusion that other intrinsic factors besides sun exposure may regulate vitamin D levels.

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