

Seasonal variations in 25-hydroxy vitamin D3, parathormone and alkaline phosphatase in school-aged children

Variación estacional de 25-hidroxi-vitamina D3, hormona paratiroidea y fosfatasa alcalina en niños escolares

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What do we know about the subject matter of this study?

Vitamin D deficiency is a common condition, especially during the darker months in the more extreme regions of the world, and it can have serious consequences for bone health as well as manifestations in other systems.

What does this study contribute to what is already known?

Almost 50% of school-aged children are deficient in vitamin D in non-extreme latitude areas, such as Santiago, Chile, showing a significant decrease in concentrations of 25-OH-vitamin D compared with the concentrations observed in summer.

Abstract

The main role of Vitamin D is to regulate calcium metabolism, whose main source is vitamin D3 obtained mostly from the action of ultraviolet (UV) light on the skin. **Objective:** To evaluate the seasonal differences in the concentrations of 25-hydroxy-vitamin D3 (25OHVitD3), parathyroid hormone (PTH), alkaline phosphatase (ALP), and calcium in school-age children. **Subjects and Method:** The concentrations of 25OHVitD3, PTH, ALP, and calcium were measured in children from Santiago, Chile (latitude -33.4372), aged 5 to 8 years, without Vitamin D supplementation, in different seasons of the year. VitD status was defined as sufficient with concentrations of 25OHVitD3 > 20 ng/mL (50 nmol/L), insufficient 12-20 ng/mL (30-50 nmol/L) and deficient < 12 ng/mL (30 nmol/L) based on the recommendations of the expert group of the "Global Consensus for the Prevention and Management of Nutritional Rickets". **Results:** 133 children participated (89 preterms under or equal to 32 weeks), 41 during summer, 28 in fall, 35 in winter, and 29 in spring. The difference of means between summer and winter was 9.6 ng/mL for 25OHVitD3 ($p < 0.0001$), -11.1 pg/mL for PTH ($p < 0.0001$), and -47.5 IU/mL for ALP ($p = 0.01$). There were no differences in calcium concentrations. In summer, 97.6% of the subjects were classified with sufficiency status (> 20 ng/mL), which decreased

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significantly in winter to 54.3% ($p < 0.0001$). **Conclusions:** In winter, 25OHVitD3 concentrations decreased in approximately half of the children, which was associated with an increase in PTH and ALP, and normal calcium concentrations. According to our results, children may need VitD supplementation during fall and winter.

Introduction

Vitamin D (VitD), which includes forms D2 and D3, is one of the main factors stimulating calcium absorption in the intestine and maintaining calcium and phosphate balance. VitD deficiency has been associated with both acute and chronic diseases, such as pre-eclampsia, autoimmune disorders, infectious diseases, cardiovascular diseases, cancers, and type 2 diabetes, among others¹.

Vitamin D3 or cholecalciferol is synthesized in the skin from exposure to ultraviolet (UV) light and is the most important source of VitD, especially in the summer months. It can also come from animals, mainly fish with high fat content, and in contrast, the main source of vitamin D2 or ergocalciferol is vegetal².

Bone metabolism is highly regulated by several factors, such as VitD, parathyroid hormone (PTH), alkaline phosphatase (ALP), calcium, and phosphate. Childhood and adolescence are critical periods for establishing lifelong bone health. VitD deficiency is associated with increased PTH secretion due to low serum calcium and 1.25-(OH)₂-vitamin D3 concentrations, resulting in increased bone resorption and, consequently, decreased bone mass. Severe VitD deficiency causes rickets in children and osteomalacia in adults. Worldwide, it is estimated that approximately one billion people have deficiency or insufficiency of VitD³.

VitD3 synthesis in the skin is influenced by different factors, such as age, skin pigmentation, use of sunscreen, and clothing. Risk factors for VitD deficiency include obesity, liver disease, chronic kidney disease or malabsorption, nutritional deficiencies, darker skin pigmentation, long-term parenteral nutrition, institutionalization, anti-epileptic treatment, and low sun exposure^{2,4}.

Geographical and climatic variables such as latitude, altitude, season, and time of day also influence VitD levels and affect VitD synthesis⁵. The increase in the prevalence of VitD deficiency and insufficiency during winter is related to different factors, and the decrease in UV radiation is the most important⁶. Santiago, Chile, located between 32°55' and 34°19' south parallels, has four distinct seasons during the year, with a reduction in solar radiation during fall and winter. During this period of the year, the solar zenith angle

is more oblique, so the absorption of UV radiation by ozone increases and decreases the radiation reaching the Earth's surface⁷. Another relevant factor affecting VitD synthesis is environmental pollution since it makes it difficult for UV radiation to reach the Earth's surface, which is associated with a reduction in VitD synthesis in the skin⁸. Relatedly, several reports show a higher incidence of VitD deficiency in areas with higher pollution^{8,9}.

The objective of this study is to determine if there are differences in the concentration of 25OHVitD3 during the seasons in school children in Santiago and to associate them with serum concentrations of PTH, ALP, and calcium, as well as with UV radiation and environmental pollutants.

Subjects and Method

Design

Cross-sectional association study with convenience sampling.

Subjects

In this study, prepubertal subjects aged between 5 and 8 years participated who attended the well-child checkups at the UC-Christus Health Network and the polyclinic of premature children at the *Complejo Asistencial Dr. Sótero del Río*. This sampling was carried out for the project FONDECYT 1160863. Subjects with VitD supplementation or with a history of undiagnosed chronic pathologies were excluded.

Protocol

A paediatrician conducted a complete physical examination of all subjects at the Pontifical Catholic University of Chile between January 2016 and August 2018. Height was measured using a stadiometer (Health or meter model 402 KL, Illinois, US) with 0.1 cm accuracy, and weight was determined using a precision scale (Omron model HBF-510, Japan). Waist circumference was measured with an inextensible tape measure, according to the recommendation of the World Health Organization (WHO)¹⁰. Height, body mass index (BMI), and waist circumference are expressed in standard deviation Z-scores (Z-scores) according to WHO references.

Biochemical analysis

Using blood samples taken after an overnight fast, the concentration of 25OHVitD3 was measured by liquid chromatography-mass spectrometry, as well as PTH (ECLIA, Cobas, Roche), ALP (enzymatic colorimetric assay, Roche, Cobas), and calcium (colorimetric, Roche, Cobas).

VitD status was defined as sufficient with concentrations of 25OHVitD3 > 20 ng/mL (50 nmol/L), insufficient 12-20 ng/mL (30-50 nmol/L), and deficient <12 ng/mL (30 nmol/L) according to the Global Consensus Recommendations for the Prevention and Management of Nutritional Rickets, 2016¹¹.

Environmental variables

We obtained the Santiago UV index from the database of the Chilean Meteorological Office, which is in charge of the registration and publication of this information¹². In this study, we selected the environmental pollutants most frequently described in the literature as affecting UV radiation⁹ and that were available from the National Air Quality Information System (SINCA)¹³, ozone (O3), carbon monoxide (CO), and particulate matter 10 (PM10).

Statistical analysis

The analysis of the normal distribution of variables was performed using the Kolmogorov-Smirnov test. The variables are expressed as medians and interquartile ranges. For the variables with a normal distribution, we used one-way ANOVA and Tukey's test for those without a normal distribution, the Kruskal-Wallis test, and later the Mann-Whitney U test (between two independent samples). The data were analysed using GraphPad® Prism software version 8.0.0 for Mac OS X (GraphPad Software, San Diego, California USA).

Ethics

The study was approved by the Ethics Committee of the Faculty of Medicine of the Pontifical Catholic University of Chile. One of the parents or legal guardians signed informed consent before any procedure.

Results

A total of 147 children were recruited, and 14 of them were excluded. One hundred thirty-three school-age children participated (65 girls, 48.9%); 89 were very premature newborns (less than or equal to 32 weeks of gestational age), and 44 were full-term. Among them, no statistically significant differences were observed in distribution by sex ($p = 0.231$), age ($p = 0.264$), BMI Z-score ($p = 0.568$), and 25OHVitD3 ($p = 0.165$), so

we considered the total group for the analysis. The concentrations of 25OHVitD3 measured during different times of the year were not correlated with sex ($p = 0.643$), chronological age ($p = 0.158$), gestational age ($p = 0.165$), waist circumference ($p = 0.294$), or BMI Z-score ($p = 0.545$).

Seasonal variation

When separating the data according to the seasons, there were significant variations in the average concentrations of 25OHVitD3 ($p < 0.0001$), ALP ($p = 0.001$), and PTH ($p < 0.0001$) but not in the calcium concentrations ($p = 0.356$) (table 1 and figure 1). Table 2 shows the differences between summer and other seasons for 25OHVitD3, ALP, and PTH.

Classification of 25OHVitD3 status

Among the 133 subjects, two presented deficiency (1.5%), 27 insufficiency (20.3%), and 104 sufficiency (78.2%). These three statuses showed seasonal variation (Pearson's chi-square, $p = 0.001$), where the percentage of sufficiency was significantly higher in summer (97.6%) than in fall (82.1%; $p = 0.0368$), winter (54.3%; $p < 0.0001$), and spring (75.9%; $p = 0.0072$).

Association between 25OHVitD3 concentrations and PTH, ALP, and calcium

The 25OHVitD3 concentration presented an inverse correlation with PTH ($r = -0.383$, $R^2 = 0.15$, $p < 0.0001$, figure 2A), and at the same time, it correlated directly with ALP ($r = 0.240$, $R^2 = 0.06$, $p = 0.0054$, figure 2B). In this study, we did not observe a statistically significant association between calcium and 25OHVitD3 ($p = 0.113$) or PTH ($p = 0.32$).

Association between 25OHVitD3, PTH, UV index, and environmental pollutants

The 25OHVitD3 concentration was directly proportional to the UV index ($r = 0.531$, $R^2 = 0.28$, $p < 0.0001$) and inversely proportional to the CO concentration ($r = -0.407$, $R^2 = 0.17$, $p < 0.0001$); however, there was no association with PM10 ($p = 0.703$). After monitoring by the UV index, the association between the 25OHVitD3 concentration and CO was not significant ($p = 0.381$). The PTH concentration was inversely related to the UV index ($r = -0.427$, $R^2 = 0.18$, $p < 0.0001$) and directly related to CO ($r = 0.248$, $R^2 = 0.06$, $p = 0.004$), but again, there was no association with PM10 ($p = 0.52$).

The correlations between the 25OHVitD3 concentrations and UV radiation of very premature and full-term subjects were identical, and there were no differences between the slopes ($F = 0.1538$, $DFn = 1$, $DFd = 129$, $p = 0.6956$) (Supplementary figure 1).

Table 1. Clinical and biochemical of newborn period by season

	Spring (n = 29)	Summer (n = 41)	Fall (n = 28)	Winter (n = 35)	p-value
<i>Newborn characteristics</i>					
Gestational age (weeks)	29 [26 - 31]	38 [30 - 40]	30 [28 - 32]	31 [28 - 38]	< 0.001*
Newborn weight (Z-score)	0.06 [-1.08 - 0.65]	0.42 [-0.04 - 1.02]	0.56 [-0.29 - 1.02]	0.04 [-1.09 - 0.87]	0.02**
Newborn length (Z-score)	-0.54 [-1.60 - -0.03]	0.34 [-0.22 - 1.22]	0.44 [-0.37 - 0.93]	-0.24 [-1.58 - 0.62]	< 0.001**
Very preterm (%)	26 (89.7)	16 (39.0)	22 (78.6)	25 (71.4)	< 0.001
<i>Clinical characteristics at the moment of the study</i>					
Women (%)	14 (48.2)	21 (51.2)	9 (32.1)	21 (60.0)	0.174
Age (years)	6.6 [5.8 - 7.6]	6.6 [5.9 - 7.2]	6.6 [5.8 - 7.3]	6.3 [5.3 - 6.9]	0.422*
Height (Z-score)	-0.1 [-0.49 - 0.49]	0.1 [-0.59 - 0.66]	-0.09 [-0.77 - 0.39]	-0.47 [-0.86 - 0.47]	0.466**
BMI (Z-score)	0.58 [-0.6 - 1.53]	0.58 [-0.29 - 1.02]	0.69 [-0.07 - 1.38]	0.45 [-0.7 - 1.64]	0.962**
Waist circumference (cm)	58.0 [54.0 - 62.0]	56.0 [54.0 - 60.0]	56.5 [54.0 - 63.3]	55.0 [52.0 - 62.0]	0.620*
<i>Calcium-Phosphate metabolism characteristics</i>					
25OHVitD3 (ng/mL)	26.3 [21.3 - 30.9]	30.7 [26.4 - 34.2]	24.1 [21.1 - 26.5]	21.4 [17.4 - 25.3]	< 0.001**
PTH (pg/mL)	33 [29 - 47]	24 [21 - 30]	33 [29 - 41]	35 [28 - 45]	< 0.001*
AP (IU/L)	236 [222 - 283]	213 [155 - 247]	260 [203 - 294]	239 [208 - 281]	0.001**
Calcium (mg/dL)	10 [9.8 - 10.1]	9.9 [9.7 - 10.2]	9.9 [9.7 - 10.1]	9.9 [9.7 - 10.1]	0.356*

The results are presented as median and interquartile range. The proportion of very preterm newborns (< 32 weeks of gestational age) and women were compared with Pearson's χ^2 . The p-value was obtained by Kruskal-Wallis test (*) for variables without normal distribution and with ANOVA test for variables with normal distribution (**). 25OHVitD3: ng/mL * 2.496 = nmol/L y PTH: pg/mL * 0.1060 = pmol/L. BMI, body mass index; PTH, parathormone; AP, Alkaline Phosphatases.

Table 2. 25OHVitD3, alkaline phosphatases, and PTH concentration differences are comparing the summer and the other seasons

	Summer versus fall	Summer versus winter	Summer versus spring
25OHVitD3 (ng/mL)	6.9 [2.8 - 11.1]***	9.6 [5.7 - 13.5]****	5.4 [1.3 - 9.5]**
AP (IU/L)	-54.7 [-96.5 - -12.8]**	-47.5 [-86.8 - -8.2]**	-49.9 [-91.4 - -8.5]*
PTH (pg/mL)	-9.9 [-12.0 - -5.0]***	-11.0 [-14.0 - -7.0]****	-10.0 [-16.0 - -6.0]****

The 25OHVitD3 and FA and concentration differences were analyzed with Tukey's multiple comparison test and are displayed as mean, with a 95% confidence interval. The PTH concentration differences were analyzed with the Mann-Whitney test, and the results are displayed as median differences, with a 95% confidence interval. AP, Alkaline Phosphatases; PTH, parathormone.

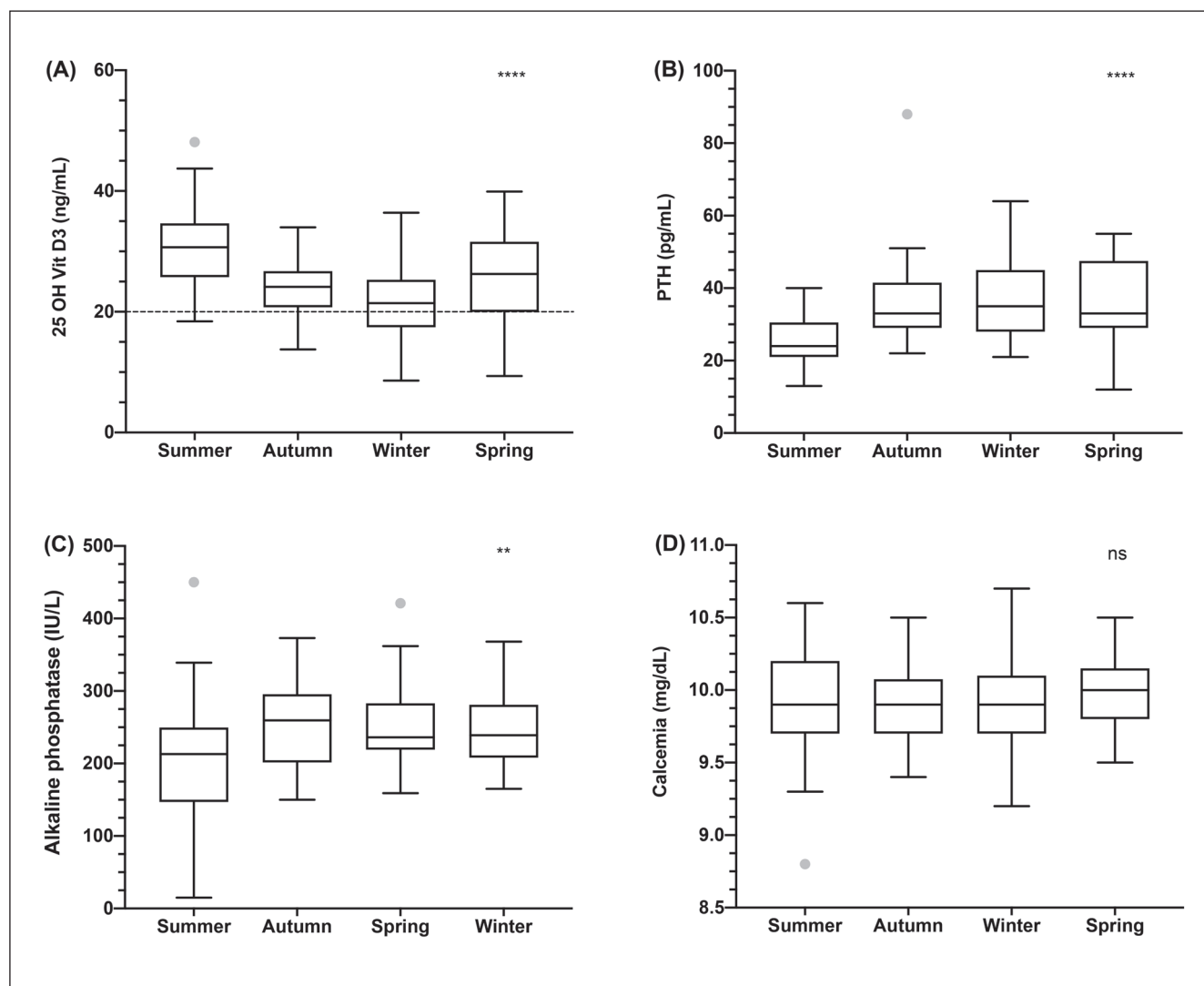


Figure 1. Seasonal variations and differences in mean concentration in 25-OH-Vitamin D3 (A), PTH (B), Alkaline Phosphatase (C) and Calcemia (D). The results are expressed as median and interquartile ranges. The segmented line (A) indicates Vitamin D's status: sufficient over 20 ng/mL and insufficient/deficient under 20 ng/mL. AP, alkaline phosphatase; PTH, 25OHVitD3 parathormone: ng/mL * 2.496 = nmol/L; PTH: pg / mL * 0.1060 = pmol/L.

Discussion

The 25OHVitD3 concentration showed a significant variation between the different seasons in prepubertal school-age children in Santiago and a significant increase in the percentage of deficiency status of subjects in winter and fall. It should be noted that this was associated with a significant increase in PTH and ALP concentrations. These results provide evidence to promote discussion about the need for VitD supplementation during winter in Chile.

In our study, we found no association between 25OHVitD3 and age, sex, or BMI, probably because the study group was very homogeneous since all sub-

jects were prepubertal and within a narrow age range, but the sample size could also explain this. Other studies have shown differences in the prevalence of VitD deficiency related to age, but they were conducted in populations with a wider age interval, including prepubertal children, adolescents, and/or older adults¹⁴⁻¹⁶. Differences by sex and nutritional status have also been described, explained by variables such as body composition that varies with age and pubertal development (body fat "sequesters" VitD), sociocultural characteristics, and lifestyle (e.g., fish consumption, outdoor activities)¹⁴⁻²⁵.

There are few studies on the prevalence of VitD deficiency in Chile, and only two of them have been

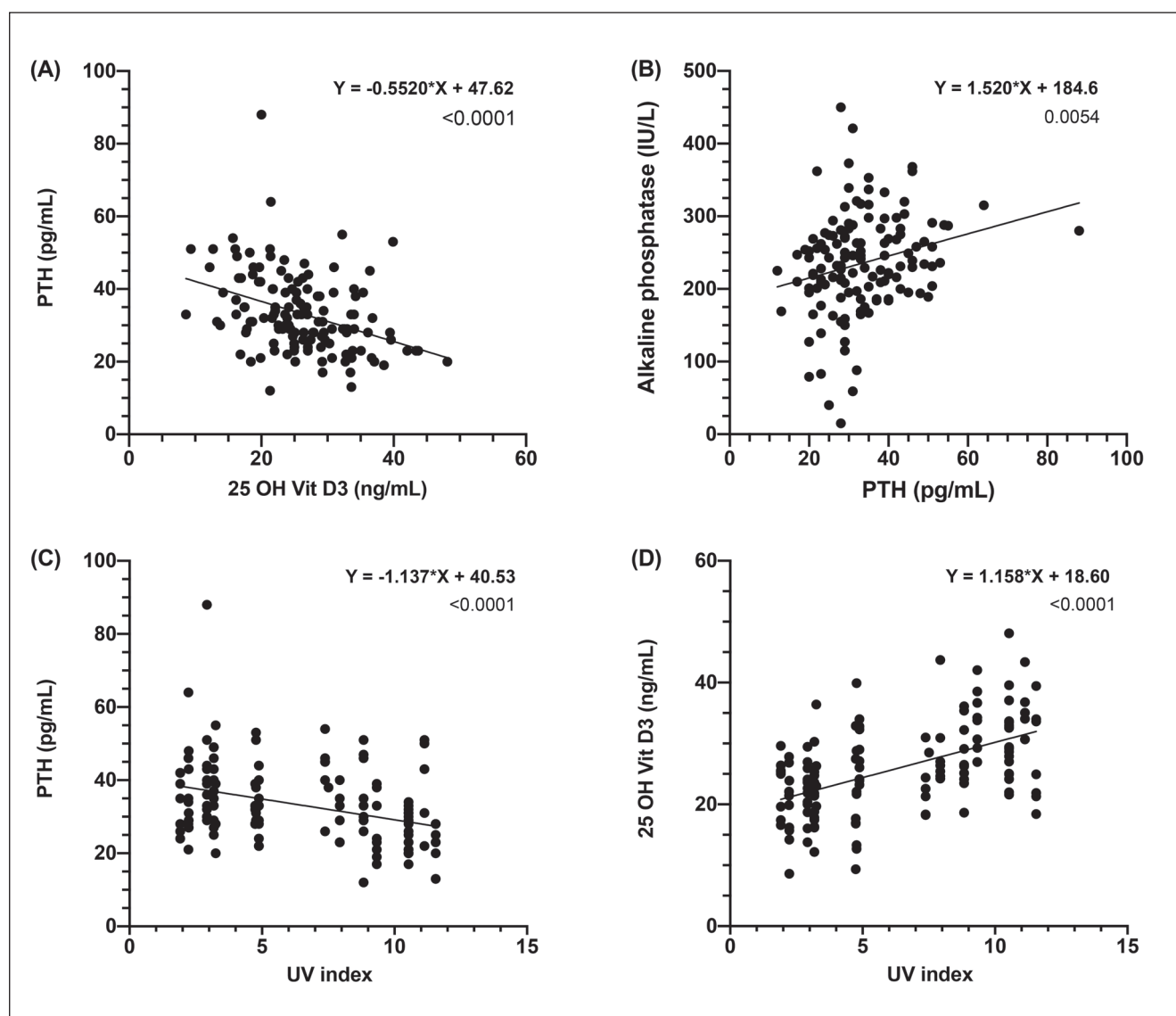


Figure 2. Association between parathyroid hormone, 25OHvitD3, alkaline phosphatase and UV index. AP, alkaline phosphatase; PTH, parathyroid hormone; UV index, ultraviolet index. 25OHvitD3: ng/mL * 2.496 = nmol/L; PTH: pg/mL * 0.1060 = pmol / L.

conducted in the paediatric population^{26,27}. However, it is difficult to compare them with our results because different definitions were used for VitD status and other laboratory tests.

In a study conducted in 60 children in Coyhaique, Chile (45°35'S), 16.4% had normal VitD concentrations (measured by radioimmunoassay) during fall (30 to 75 ng/mL), 20% presented insufficient status (20 to 29 ng/mL), and 63.6% presented deficient status (< 20 ng/mL)²⁶. Another study conducted in Punta Arenas, Chile (53°10'S), in which the VitD concentration and PTH were measured by ELISA, showed that of the 108 children studied, 96.3% presented deficiency (< 20 ng/mL) and 3.7% insufficiency (20 to 29 ng/mL)²⁷.

Seasonal variations in VitD concentration are ob-

served not only in regions far from the equator but also in countries such as Greece, where VitD levels in school-aged children are significantly lower in spring and higher in fall¹⁴. Similar results were observed in Denmark, where a higher prevalence of VitD deficiency was also observed in the spring²⁸.

Based on the definition of VitD status of the Global Consensus Recommendations for the Prevention and Management of Nutritional Rickets, our results show that in Santiago, most subjects present sufficient VitD status (> 20 ng/mL) during summer (97.6%). This could be explained by the greater solar exposure and higher levels of UV radiation that occur during this period of the year in this area. However, in winter, there is a significant drop in the percentage of subjects

with sufficiency (54.3%) since there is a decrease in the UV index and an increase in air pollutants in Santiago (12.13%). We observed an inverse correlation between the 25OHVitD3 concentrations and CO (not so with PM10), but the association did not persist after measuring UV radiation. Consequently, air pollutants act as blockers of UV radiation, as described above¹².

The decrease in the 25OHVitD3 concentration observed during the dark months was associated with a physiological response in markers of bone metabolism, as evidenced by the increase in PTH and consequently of ALP, to maintain homeostasis in calcium and phosphate.

The inverse proportional behaviour between 25OHVitD3 concentrations and PTH has been reported in both adult and paediatric populations. In adults, it has been described that there is a *plateau* in PTH concentration with VitD concentrations higher than 30 ng/mL (75 nmol/L)^{22,29,30}. However, the VitD concentration that leads to the PTH *plateau* is highly variable in the paediatric population, probably due to differences in sex, skin colour, and mainly calcium intake in the studies conducted^{31,32}. We did not observe a *plateau* in the association of 25OHVitD3 and PTH, probably due to the absence of extreme values of VitD and the number of subjects in this study.

The cost of maintaining calcium homeostasis by increasing bone resorption may lead to secondary hyperparathyroidism and hypocalcaemic rickets in children with significant VitD deficiency. Although none of the subjects in our study had these conditions, we observed a decrease in 25OHVitD3 concentrations of almost 10 ng/mL in winter, with an increase in PTH of 11 pg/mL and 47.5 IU/L of ALP, suggesting increased bone remodelling.

Even larger decreases have been observed in other regions of the Northern Hemisphere, with winter mean differences in VitD of -12.3 ng/mL in northern Sweden³³ and -15 ng/mL in Canada³⁴, as examples. We cannot rule out that changes in these markers of bone metabolism have a negative effect on bone health in at least some children, as other authors have previously described^{33,35-37}.

To date, there is no consensus on the cut-off value for defining VitD status; therefore, comparisons between studies are difficult. For instance, when classifying deficiency status according to the definitions of the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets (< 12 ng/mL)¹¹, the American Academy of Pediatrics (< 15 ng/mL)³⁸, and the Endocrine Society (< 20 ng/mL)²⁵, the overall frequency of VitD deficiency in our sample would be 1.5%, 5.3%, and 21.8%, respectively. Considering that the first two recommendations are for the paediatric population, in our study, we considered <20

ng/mL as the cut-off value to define deficiency status. Another aspect to consider for the interpretation of results is the variety of trials available to measure VitD that are not always comparable, despite efforts to standardize them.

In a comparison of different methods to measure an external quality control sample with a concentration of 19 ng/mL, the average varied between 15 and 22 ng/mL, differences that can be even greater when analysing patient samples³⁹. In our study, the samples were analysed by mass spectrometry, which is considered the reference method. However, different immunoassays are routinely used.

The usefulness of sun exposure has been described among the alternatives for optimizing VitD levels in dark months. A study in the United Kingdom showed that exposure of the face, neck, and hands at midday for one hour, before the appearance of erythema on the skin, would be enough to maintain normal VitD levels and would be equivalent to supplementation with 400 IU of vitamin D³⁴⁰. However, sun exposure without adequate skin protection has immediate and long-term risks, such as burns and skin cancer.

Lack of exposure to UV light can also be balanced with VitD supplementation, both from food and medications. Universal supplementation is a common practice in children under one year of age, but above this age, there is no consensus. In the United States, since 2003, the American Academy of Pediatrics has recommended supplementation in children and adolescents who do not consume fortified dairy products or multivitamins, as well as in other risk groups⁴¹, similar to what is recommended by the European Academy of Paediatrics⁴ and the Endocrine Society in the U.S.²⁵. Other scientific societies, such as the Nutrition Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN), do not recommend this supplementation as a protocol due to the lack of evidence of benefit⁴².

In some regions, such as Ushuaia, Argentina (55°S), and Paris, France (48.8°N), which are at more extreme latitudes than Santiago, children receive supplementation of 100,000 IU of vitamin D at the beginning of the winter and 3 months later^{43,44}. In the case of Argentina, the 18 subjects evaluated presented a sufficient level of VitD before and after supplementation without presenting intoxication levels.

The Endocrine Society in the U.S. recommends a daily intake of VitD of 400 IU, 600 IU, and 600 to 800 IU per day for children aged under one year, children from 1 to 18 years old, and all adults with risk factors, respectively, to prevent VitD deficiency²⁵. In Chile, no standards are regulating VitD supplementation in

children over one year of age. However, according to our results in children from 5 to 8 years old, supplementation may be necessary during the fall and winter months, at least in Santiago, as well as in regions of similar or more southern latitudes, especially in children with risk factors for VitD deficiency.

Locally, our work represents initial evidence on the decrease in 25OHVitD3 (measured by mass spectrometry) and its effect on bone remodelling markers in the winter months. In order to make local recommendations for VitD supplementation, we consider it necessary to carry out longitudinal studies with a greater number of subjects from different age groups.

Conclusion

In the prepubertal children in this study, we found no differences in 25OHVitD3 concentrations by sex, age, or nutritional status. During the months with a lower UV radiation index, there was a lower 25OH-VitD3 concentration and a physiological response with increased PTH and ALP, suggesting higher bone resorption to maintain calcium homeostasis. In winter, the proportion of subjects with sufficient levels of VitD decreased by approximately half. The decrease in VitD was associated with a lower rate of UV radiation, and environmental pollutants were only indirectly related to VitD levels through UV radiation.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

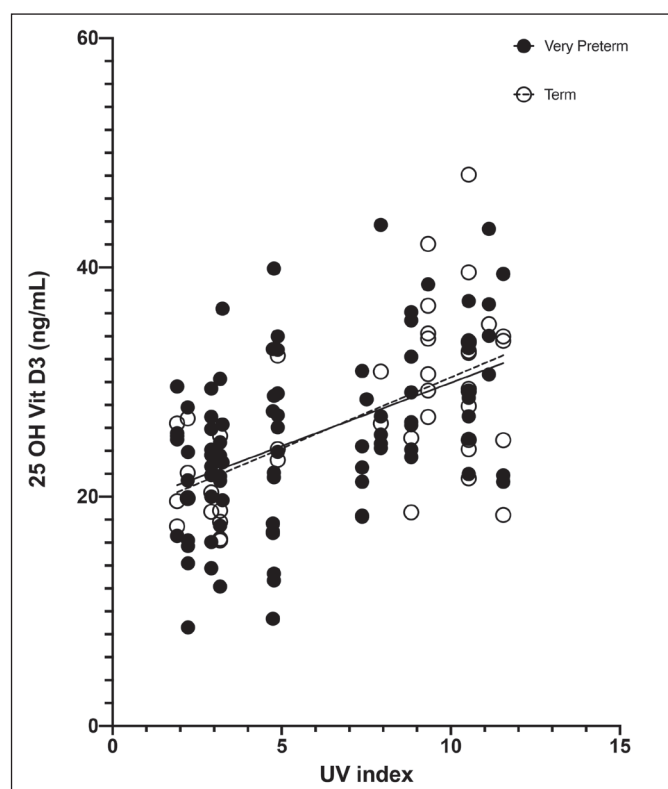
Authors declare no conflict of interest regarding the present study.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

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Supplementary Figure 1. Association between UV index and 25OHVitD3 distributed by born very prematurely or at term. Children born very preterm are represented by closed circles and their linear relationship by a solid line. Term-born children are represented by open circles and their linear regression by a segmented line.

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