

Vitamin D in a pediatric population after the end of home lockdown: a prospective study

Vitamina D en pediatría tras el fin del confinamiento domiciliario: estudio prospectivo

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

The SARS-CoV-2 pandemic led to a severe lack of sunlight exposure. So far, cross-sectional studies have shown no differences in pre-pandemic 25-OH-VD levels versus levels in the different waves of the pandemic.

What does this study contribute to what is already known?

This study explores the variation in 25-OH-VD levels after the end of home confinement due to the SARS-CoV-2 pandemic, demonstrating an upward slope of such levels. The different factors related to 25-OH-VD synthesis, such as sunlight exposure during confinement, use of photoprotection, and body mass index, among others, are also investigated.

Abstract

Home confinement during the onset of the SARS-CoV-2 pandemic decreased sunlight exposure, the main source of vitamin D in the body. **Objective:** To evaluate the impact of SARS-CoV-2 confinement on 25-hydroxyvitamin D (25-OH-VD) levels in a pediatric population. **Patients and Methods:** Observational study in a Spanish pediatric population between June and October 2020. 25-OH-VD levels were measured by electrochemiluminescence and several related variables were collected (anthropometry, sex, skin phototype, date, calcium level, inorganic phosphorus, parathormone, and alkaline phosphatase). The child's companion answered a survey that included the following aspects: access to open air in the house where the confinement took place, hours of sunlight per day received by the child after the end of the confinement, regular use of sunscreen with outdoor exposure, skin phototype of the child, type of milk the child usually drinks, 25-OH-VD supplementation intake,

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and if so, dosage and adherence to treatment. **Results:** 123 children participated, mean age 8.15 years (95%CI 7.52-8.79), and 56.1% were female. The median 25-OH-VD was 27.70 ng/ml (RIC 22.75-33.60), and 14% presented 25-OH-VD insufficiency (< 20 ng/ml). 25-OH-VD levels presented an ascending correlation slope as the date moved away from the end of confinement (Rho 0.467; $p < 0.001$), being related to sunshine hours (Rho 0.368; $p < 0.001$). 25-OH-VD levels were higher in patients with photoprotection (median 29.9 vs 23.5 ng/ml, $p = 0.005$), with differences according to skin phototype ($p = 0.032$), but were not related to age, weight z-score, height z-score, body mass index z-score, or the presence of a balcony or garden at home. **Conclusion:** The rate of 25-OH-VD insufficiency at the end of confinement was not higher than in previous studies. 25-OH-VD levels increased progressively in relation to the hours of sunlight exposure and to the summer months. Interestingly, 25-OH-VD levels were higher in children using phot

Introduction

Vitamin D is a prohormone whose main action is the regulation of phosphocalcic metabolism, allowing adequate bone mineralization. Its deficiency is associated with rickets and osteomalacia, however, and although it is not only responsible for bones, it also contributes to cell division and differentiation, acts on the immune system, etc.¹.

The main source of vitamin D synthesis is the interaction of the skin with UVB light. This interaction is influenced by factors such as skin color, latitude, season, time of day, and sunscreen use, among others¹.

The actual prevalence of vitamin D deficiency is unknown, and there are two main reasons for this. The first one is the controversy that exists in determining what is an insufficient vitamin D value. The Nutrition Committee of the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) proposes for clinical and scientific purposes the use of a plasma 25-hydroxyvitamin D (25-OH-VD) concentration of less than 20 ng/ml to define insufficient levels, and less than 10 ng/ml to define severe deficiency². The Spanish Association of Pediatrics, the United Kingdom Consensus, and the American Academy of Pediatrics all agree with these cut-off points; but there are countries such as Italy where the proposal is to define 25-OH-VD insufficiency values < 30 ng/ml³. Secondly, there are no large population-based studies in the literature that determine this prevalence. In Spain, several observational studies have been published, in which the prevalence of 25-OH-VD deficiency varies according to the areas of sunlight exposure, being between 0 and 45% in sunny areas such as Cordoba or Cadiz, reaching up to 60% in areas with less sunlight exposure such as La Coruña or Navarra⁴⁻¹³ (Table 1).

The first wave of the SARS-CoV-2 pandemic caused the Spanish government to decree a state of alarm on March 14th, 2020, limiting the freedom of movement of people¹⁴, forcing all Spanish children to be

confined to their homes. Later, Order SND/370/2020 of April 25th stipulated that children under 14 years of age could leave their homes for one hour a day maximum¹⁵; but it was not until May 4th, 2020, that Spain as a whole began a de-escalation period in which it was finally allowed to leave home freely¹⁶. In short, Spanish children experienced home isolation for almost two months. One of the direct consequences of this fact was the lack of sunlight necessary for an adequate synthesis of 25-OH-VD.

For this reason, we hypothesized that this home confinement due to the state of alarm between March and April 2020, decreased 25-OH-VD levels in the pediatric population. The primary objective was to determine both the levels and the rate of 25-OH-VD deficiency after home confinement and to subsequently evaluate its evolution over time. The secondary objective was to evaluate factors that could interfere with 25-OH-VD levels such as hours of sunlight per day, sunscreen use, race, skin phototype, etc.

Patients and Method

Observational, prospective, and descriptive study on a pediatric population in which 25-OH-VD levels and other associated variables were determined from the end of confinement (June) to October 2020 (5 months).

The target population of the study was the pediatric population of the province of Salamanca (Spain). This province is located in the central-western part of Spain, at a latitude of 40° (coordinates N40°58'7.75" W5°39'49.97") and at an altitude of 7985 masl (meters above sea level). The sample population of the study was children in the province of Salamanca (Spain) who underwent a blood test for another reason, from June to October 2020.

The inclusion criteria for the study were age 1 to 14 years, having a blood test for another reason in the

Complejo Asistencial Universitario de Salamanca or the healthcare centers of the province of Salamanca, and having an informed consent form signed by the guardian/legal representative. The exclusion criteria were the presence of severe malnutrition (previously diagnosed by a physician) and following a vegan or vegetarian diet.

Data were collected by consecutive sampling during the 5 months of the study. The sample size was not calculated beforehand due to the health emergency scenario and to speed up the study.

The participating physician collected anonymized data on the following variables: patient anthropometry (weight in kilograms, height in centimeters), date of birth, sex (male or female), patient skin phototype (from 1 to 6 according to Fitzpatrick types)¹⁷, date of blood test collection, and the data obtained from the analysis (25-OH-VD level in ng/ml measured by electrochemiluminescence, calcium and inorganic phosphorus level in mg/dl, parathyroid hormone level in pg/ml, and alkaline phosphatase level in IU/ml). Additionally, the child's guardian filled out a survey reporting: the presence of access to open air in the house where the confinement is spent (garden, balcony, or nothing); hours of sunlight exposure per day after the end of confinement; usual use of sunscreen with exposure to open air (yes/no); skin phototype of the child; what type of milk the child usually drinks (breast, animal, vegetable, other); 25-OH-VD supplementation (yes/no) and, if taken, indicate the dose taken and adherence to treatment (yes/no).

To determine 25-OH-VD sufficiency/insufficiency, the ESPGHAN proposal was used, which indicates

sufficient 25-OH-VD values ≥ 20 ng/ml, insufficient values < 20 ng/ml, and severe deficit < 10 ng/ml².

Regarding the statistical data analysis, for quantitative variables, mean or median were used as measures of position; and the interquartile range or 95% confidence interval as measures of dispersion, depending on whether they met the normality evaluated by the Kolmogorov-Smirnov test. For categorical variables, relative frequencies were used. For hypothesis testing, the Chi-square or Fisher test was used to compare nominal variables, Pearson's or Spearman's correlation coefficient to compare continuous variables, and the Student's t-test, ANOVA, Kruskal Wallis, or Mann Whitney U-test to compare nominal and continuous variables. The type I and II errors chosen were 0.05 and 0.20, respectively. The data were analyzed with the IBM® SPSS® Statistics software, version 25.0.

The study was approved by the Clinical Research Ethics Committee of the *Complejo Asistencial Universitario de Salamanca*.

Results

Data were collected from 123 patients between June and October 2020. The sample population consisted of children aged 1-14 years with a mean age of 8.15 years (95%CI 7.52-8.79) and 56.1% were female. Table 2 shows the remaining anthropometric and related variable results.

The main objective of the study was to determine 25-OH-VD values after home confinement and their subsequent evolution over time. In this sample, 86%

Table 1. Observational studies carried out in Spain regarding the prevalence of 25-hydroxyvitamin D deficiency in children

City of study	Age (Years)	N	Year	Season	25(OH) Vit D levels		
					< 30 ng/ml	< 20 ng/ml	< 10 ng/ml
Madrid ⁴	9 - 13	102	2007-2008			51%	8%
Gerona ⁵	< 5	307	2008-2010	Whole year		24.5% (8.2% caucasian)	1.6% (non caucasian)
Cádiz ⁶	10 - 14	146	2014*	March		45.2%	
Valencia ⁷	< 2	169	2014*	Whole year	24.2%	8.3%	
Aragón ⁸	1 - 15	107	2014-2016	Whole year	72.9%		
Coruña ⁹	5 - 15	153	2018	Whole year		60.1%	5.9%
Asturias ¹⁰	4	283	2004-2007	Whole year		51.4%	
Navarra ¹¹	3 - 15	602	2014	Whole year		60.4%	
Coruña, Barcelona, Madrid, Sevilla y Valencia ¹²	8 - 12	314	2014		47.1%	29%	6%
Córdoba ¹³	7 - 10	56	1994		25%	0%	

25 (OH)VitD: 25-hydroxyvitamin D. *Published that year, there is no record of the year the study was carried out.

of the patients had sufficient 25-OH-VD values (≥ 20 ng/ml) and 14% had insufficient 25-OH-VD values (< 20 ng/ml), of which only one patient (0.80%) had a severe deficit (< 10 ng/ml). The median 25-OH-VD level was within the sufficient level (27.70 ng/ml; IQR 22.75 - 33.60). The 25-OH-VD levels showed a moderate upward correlation slope with the value ascending as time progressed after the end of confinement (Spearman's Rho 0.467 $p < 0.001$) (Figure 1). This finding also reflected a higher rate of 25-OH-VD insufficiency at the end of the confinement ($p = 0.023$) (Figure 2).

When we analyzed 25-OH-VD levels as a function of specific months, there were differences between groups ($p < 0.001$) (Figure 3). When comparing 25-OH-VD levels, depending on the month of measurement, there were differences in the pairs in June (after the end of confinement) compared with July ($p < 0.001$), August ($p < 0.001$), and September ($p = 0.045$), maintaining similar figures in the comparison between the rest of the months.

As a secondary objective, we raised the possible relationship of certain variables in the levels of 25-OH-VD and/or its sufficiency. To assess sunlight exposure during home confinement, we asked about the characteristics of the house in which the participant lived (Table 2), without finding differences in the values of 25-OH-VD or its sufficiency rate depending on whether the house had a balcony, a garden, or neither. In the survey conducted with the families, we asked them to estimate the hours per day that the participant spent outdoors once the confinement was over (median 2 hours; IQR 1 - 4); the fewer hours per day of sunlight exposure, the lower the level of 25-OH-VD the participant had, with a moderate correlation (Spearman correlation coefficient 0.407; $p < 0.001$) (Figure 4), without reflecting differences in the rate of insufficiency.

During sunlight exposure, almost a quarter of the children (23.70%) did not routinely wear sunscreen; and children who did use it had higher 25-OH-VD levels (median 29.9 vs. 23.5 ng/ml, $p = 0.005$), without reflecting a higher percentage of 25-OH-VD sufficiency.

Both participating physicians and parents matched children according to skin phototype (Table 2), with an interobserver Kappa index of 0.579, translating a moderate agreement between them ($p < 0.001$). The 25-OH-VD values were significantly different when comparing the different phototypes ($p = 0.032$ for the physicians' group and $p = 0.044$ for the parents' group), but we did not observe a clear distribution of the differences, nor did it result in a different rate of insufficiency. The phototype with the lowest level of 25-OH-VD was type I.

We compared the regular application of photoprotection with the children's skin phototype (according to the parents or the physician) with no significant variation. There were also no differences in the administration of photoprotection according to the average number of hours spent in the sun by the participants.

Within the anthropometric variables of the participants (weight, height, and BMI z-score), there were no differences in the level or presence of 25-OH-VD insufficiency. In terms of sex, females had less 25-OH-VD insufficiency than males (7.4% vs. 22.6%, $p = 0.016$).

The survey also asked about the child's main source of dairy products and 25-OH-VD supplementation at the time of the study (Table 2), finding no significant relationship in either of these two variables with the level of 25-OH-VD or its higher rate of sufficiency.

Discussion

The main objective of the study was to determine 25-OH-VD values after the end of home confinement and to evaluate their evolution in the following months. The 25-OH-VD insufficiency rate of the sample collected was 14% and both raw and sufficiency 25-OH-VD values took an upward slope from the end of confinement. We cannot determine whether confinement influenced the percentage of 25-OH-VD insufficiency as we do not have control data in our population. However, when compared with other data on the Spanish population in the literature, we can infer that there is a low incidence of 25-OH-VD insufficiency in our sample⁴⁻¹³. On the other hand, the end of the confinement coincided with the end of spring; in the summer months, the days have more hours of sunlight and an incidence of UVB rays more favorable for the synthesis of 25-OH-VD^{18,19}, which causes that less sunlight exposure is needed for the same level of 25-OH-VD²⁰. This is an important confounding factor that, by itself, could lead to a progressive rise in 25-OH-VD levels as the summer months progress^{19,20}. Because of these two reasons, we believe that it cannot be concluded that home confinement during the first wave of the SARS-CoV-2 pandemic in Spain influenced 25-OH-VD levels in children from Salamanca.

Previous studies have been carried out to analyze the influence of the first wave of the SARS-CoV-2 pandemic on 25-OH-VD levels. In these studies, the period that the first wave lasted was compared with the same period in previous years, all of them concluding that there was no relationship between the pandemic and the levels of 25-OH-VD because they were at similar levels. These are cross-sectional studies that give the results as an average level of 25-OH-VD without considering the variation of it throughout the months that

Table 2. Continuous and categorical variables collected in the study and their results

Demographic variables						
Sex	Women n = 69 (56.1%)			Men n = 54 (43.9%)		
Anthropometric variables						
Weight	23.10 kg [RIC 19.10 - 36.00]					
Weight Z – score	- 0.20 [IC 95% -0.41 - +0.15]					
Height	126.04 cm [IC 95% 121.88 - 130.20]					
Height Z – score	- 0.09 [IC 95% -0.37 - +0.19]					
BMI	16.48 kg/m² [RIC 14.90 - 18.27]					
BMI Z – score	- 0.47 [RIC -1.03 - +0.43]					
ANALYTICAL VARIABLES						
Calcium	10.00 mg/dl [RIC 9.80 - 10.30]					
Phosphorus	4.80 mg/dl [IC 95% 4.69 - 4.91]					
Parathyroid hormone	20.70 pg/ml [RIC 15.60 - 28.07]					
Alkaline phosphatase	228.77 U/L [IC 95% 217.18 - 240.36]					
VARIABLES SURVEYED						
Hours of sunlight exposure	2 [RIC 1 – 4]					
Home open air access	No 16.4%		Balcony 46.7%		Garden 36.9%	
Photoprotection	Yes 75.8%			No 24.2%		
Main source of dairy	Animal 90.5%	Vegetal 3.4%		Breast 1.7%	Others 4.3%	
Oral supplementation with vitamin D	No 91.3%			Prophylaxis dose 22.2%		
	Yes 8.7%			Treatment dose 77.8%		
Skin phototype by doctor	I 0.8%	II 15.4%	III 42.3%	IV 35.0%	V 6.5%	VI 0%
Skin phototype by parents	I 3.3%	II 14.8%	III 41.0%	IV 33.6%	V 7.4%	VI 0%

BMI: body mass index. SPF: Sun Protection Factor. Variables with a normal distribution are expressed as mean and 95% confidence interval [95%CI]. Non-normally distributed variables are expressed as median and interquartile range 25-75 [IQR].

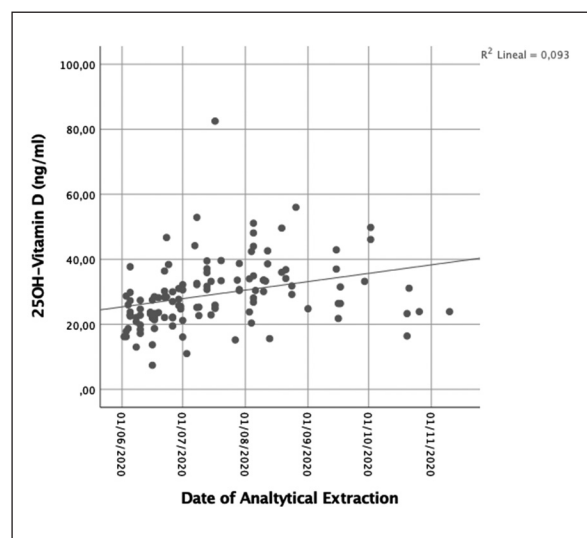


Figure 1. Scatter plot. Moderate positive correlation between the analytical level of 25-hydroxyvitamin D (ng/ml) and the date of analytical extraction (Spearman's rho 0.467 p < 0.001).

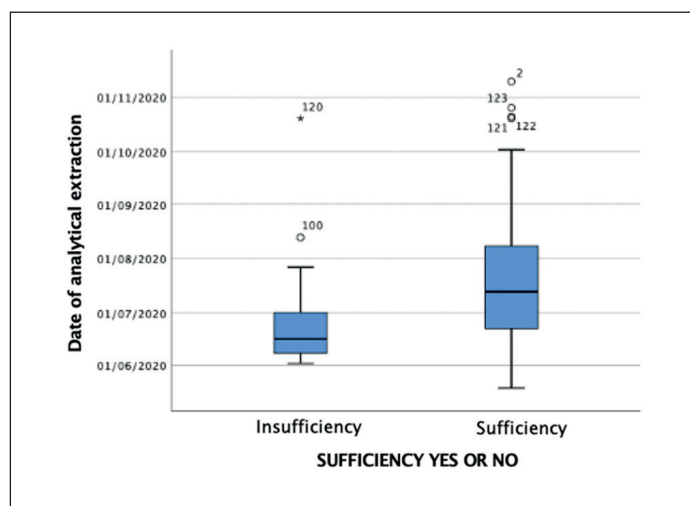


Figure 2. Box plot. Comparison between the sufficiency/insufficiency of 25-OH-VD and the date of analytical extraction (p = 0.023). Values of 25-OH-VD ≥ 20 ng/ml are taken as sufficiency and values < 20 ng/ml as insufficiency.

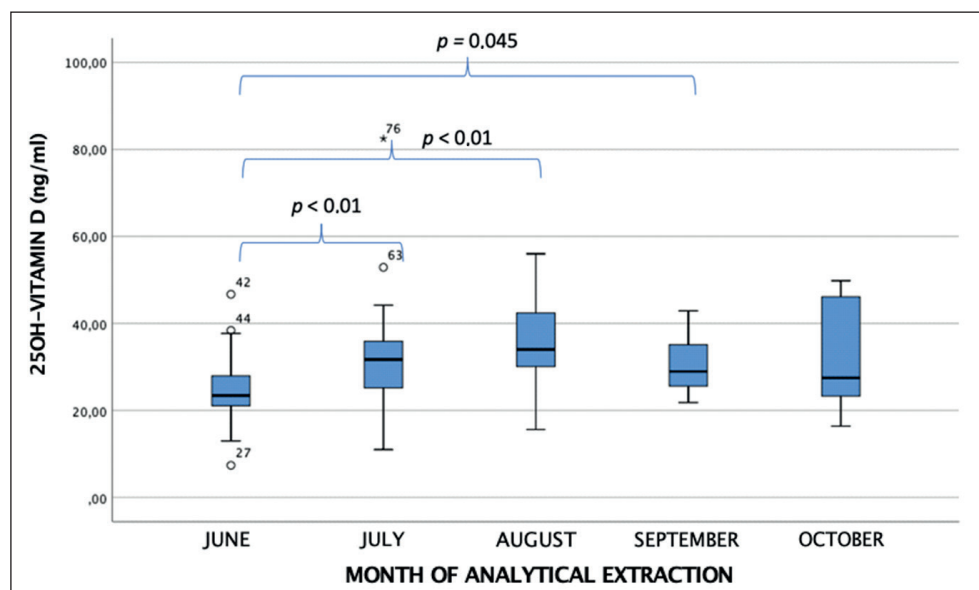


Figure 3. Box plot. Comparison between the month of analytical extraction and the level of 25-hydroxyvitamin D (ng/ml). The months in which the comparison has statistical significance are grouped using braces and the corresponding p value is noted.

the first wave lasted (they took data from January to the summer months of 2020)^{18,21-23}. To our knowledge, this is the first published study to analyze the temporal variation of 25-OH-VD prospectively after the end of home confinement.

As a secondary objective of the project, we aimed to demonstrate the relationship between certain variables and 25-OH-VD sufficiency. It is known that the greater the sunlight exposure, the higher the 25-OH-VD levels¹. In relation to this, we wanted to evaluate whether the presence of outdoor access in the home during confinement (balcony, garden) would improve the level of 25-OH-VD and its sufficiency, but the differences were not statistically significant. However, it did show that the more hours of sunlight exposure, the highest 25-OH-VD levels.

Previous literature suggests that the application of photoprotective creams either hinders or does not influence the synthesis of 25-OH-VD^{20,24,25}. In this study, the opposite idea is observed where patients who usually wear sunscreen had higher levels of 25-OH-VD. The research team considered two possible explanations. The first one was that patients with darker phototypes (who have more difficulty synthesizing 25-OH-VD)^{20,26} may be less aware of the need for sunlight protection; however, there was no difference in the administration of photoprotection according to skin phototypes. The second possible explanation suggested that patients who spent fewer hours in the sun did not usually use photoprotection and, therefore, synthesized less 25-OH-VD. There was also no evidence of a relationship between hours in the sun and regular use of sunscreen.

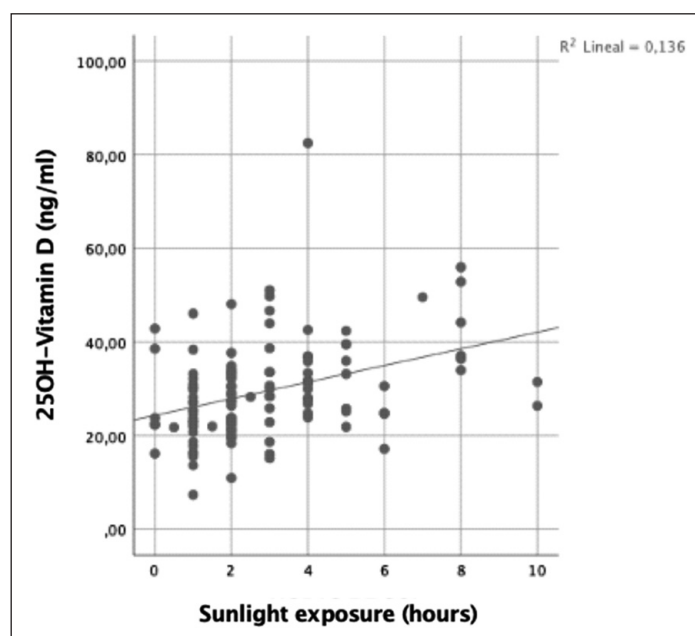


Figure 4. Scatter plot of the correlation between daily sunshine hours and the level of 25-hydroxyvitamin D (Spearman correlation coefficient 0.407 with $p < 0.001$).

In this study, females had a higher rate of 25-OH-VD sufficiency than males; in the literature reviewed, it is males who have a higher level of 25-OH-VD^{21,22}.

There are several limitations to this study. The most relevant limitations are a small sample size (123 patients) and a short follow-up time (5 months). There is a possible selection bias due to the collection of

data from patients who “needed” an analytical sample collection for another cause, as well as the absence of a previously determined sample size; thus, the sample may not be a reliable representation of the target population. Also, the end of home confinement in Spain was at the beginning of May 2020; since data collection began in June 2020 and the half-life of 25-OH-VD is 2-3 weeks, the insufficiency rate may be underestimated²⁷. Another limitation is that the study included patients with chronic diseases of all types, and some of these pathologies may be related to a variation of 25-OH-VD levels in blood¹. Patients taking medications that could influence 25-OH-VD levels (corticosteroids, antiepileptics, etc.) were not excluded from the study². Lastly, the collection of certain data through a survey provided subjectivity (average hours of sunlight per day, regular use of sunscreen, etc.).

In conclusion, after home confinement in Spain, children in Salamanca had a 25-OH-VD insufficiency rate of 14%. This value is not higher than in previous studies, so we cannot conclude that after home confinement and deprivation of sunlight exposure, there is a lower level of 25-OH-VD. Once confinement was over, 25-OH-VD values took an upward slope, a fact that may be altered by the confounding factor of the onset of summer and increased sunlight exposure. Photoprotection was related to a higher level of 25-OH-VD.

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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