

Original Research

Vitamin D levels in pregnant women in relation to solar radiation and the season of the year. analytical cross-sectional study

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Abstract

Background/objectives: Vitamin D deficiency is a health problem for pregnant women. Currently, there is controversy about the optimal levels of 25(OH) D, despite knowing its relationship with possible adverse maternal-fetal outcomes. The objective of this study was to determine the serum concentration of 25(OH)D during the first trimester of gestation in relation to solar radiation and the season of the year in women treated at the Virgen de las Nieves University Hospital in Granada, Spain. **Methods:** A cross-sectional descriptive study was carried out for 2 years, in which 500 pregnant women were recruited following the STROBE methodology, and from whom a blood sample was extracted during the first trimester of pregnancy. Descriptive statistics were carried out for the entire population, compared between groups and, finally, a multivariate analysis was carried out. **Results:** The results obtained show an average concentration of 25(OH)D, higher in summer (25.7 ng/ml), although 91.5% of pregnant women did not achieve optimal levels in that season. A significant association was also observed between vitamin D levels and season (0.001); however, there was no association with higher average monthly maximum radiation and increased probability of having optimal vitamin D levels, showing an irrelevant association with solar radiation. Furthermore, the results show that the risk of sun exposure is not associated with the probability of achieving optimal vitamin D levels. **Conclusions:** These results indicate a generalized vitamin D deficiency in pregnant women; therefore, it is advisable to evaluate the current recommendations for daily sun exposure, as well as to evaluate a standard supplementation protocol in these women.

Keywords: Vitamin D, Vitamin D deficiency; Solar radiation and Pregnant women.

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INTRODUCTION

Vitamin D also known as calciferol, is a prohormone that is part of the fat-soluble vitamin D group. It plays a key role in the maintenance of bone metabolism, modulation of immune responses and inflammation. Vitamin D deficiency appears to be widespread worldwide and is currently considered an emerging public health problem¹. It has a high prevalence and puts the health of the individual at risk, especially with a high severe deficiency, causing alterations in musculoskeletal metabolism. Also, extra-skeletal benefits in relation to vitamin D have been recently discovered²⁻⁴.

In Europe, the situation is similar to the rest of the world. Several studies have reported a high prevalence of vitamin D deficit and deficiency⁵⁻⁷. Vitamin D deficiency is very present in the European population, even considering the countries of the Mediterranean basin, with a mean population serum concentration of 26 nmol/L. In the Nordic countries, the reported average concentration of serum vitamin D levels is 45 nmol/L, despite their lower sun exposure⁷. The "Mediterranean paradox" becomes evident, in which serum vitamin D concentrations do not correspond to solar availability^{8,9}.

Vitamin D is endogenously generated by sunlight exposure, through dermal synthesis. It takes place through a photochemical reaction, a process by which the ultraviolet B (UVB) component of solar radiation converts 7-dehydrocholesterol (7-DHC) in the skin



into pre-vitamin D; this pre-vitamin spontaneously isomerizes into vitamin D¹⁰.

The availability and fluctuation of environmental UVB during the different seasons seems to be a determining factor in dermal vitamin D production, so it is an essential factor to predict the prevalence of vitamin D deficit in the population¹¹. In Spain, general population has shown low levels of vitamin D during all seasons of the year; however, a trend for higher serum vitamin D concentrations in summer and autumn has been reported¹².

In the Mediterranean region, it has been reported that, despite the elevated levels of solar exposure, maternal hypovitaminosis D during pregnancy is quite common¹³.

Increased calcium and adequate vitamin D levels are required during pregnancy, so pregnant women are at increased risk of vitamin D deficiency¹⁴. There are several biological pathways through which vitamin D can influence maternal and fetal health during this period. Vitamin D plays a key role in placental decidualization and embryo implantation in early pregnancy. It regulates the production of hormones, such as chorionic gonadotropin, placental lactogen, estradiol, and progesterone, which are relevant in the prevention of fetal rejection, in addition to important immunomodulatory properties¹⁵. A modulatory effect of the active form of vitamin D in immune system cells, particularly T lymphocytes, has been described, which improves the maternal immune response of the placenta and reduces the obstetric risks associated with vitamin D deficiency¹⁶.

During pregnancy, the definition of vitamin D deficiency (25 OH D) remains unclear. So, at present, there is controversy about the optimal levels of 25 OH D during pregnancy. Several institutions consider levels below <20 ng/mL to be indicative of vitamin D deficiency, although the Endocrine Society and other expert groups consider levels between 20-29 ng/mL as "insufficiency". Other authors propose reaching levels of ≥30 ng/mL. The Spanish Society of Gynecology and Obstetrics (SEGO) maintains that, although the optimal level of 25-hydroxyvitamin D is unknown, serum levels above 20 ng/mL are recommended to prevent complications during pregnancy¹⁷⁻¹⁹.

Vitamin D deficiency during pregnancy has been associated with adverse perinatal outcomes; maternal vitamin D insufficiency may increase the risk of preeclampsia, preterm birth and preterm delivery, small-for-gestational age (SGA) or intrauterine growth restriction (IUGR), and gestational diabetes mellitus²⁰⁻²³.

Few studies have analyzed vitamin D deficiency in Spain. In particular, those conducted in Andalusia (southern Spain) are very scarce. After an extensive and exhaustive bibliographic search, only one study that compared the serum concentration of vitamin D in the Asturian (Northern-Atlantic) and Malaga (Southern-Mediterranean) populations was found. The results

are consistent with previous studies, with higher vitamin D levels in the north of the country²⁴.

The analysis of all these studies reveals the need to know the behaviour of serum vitamin D levels during pregnancy in a specific population, given the heterogeneous distribution of vitamin D. And this, in order to minimize the effects of the consequence of possible hypovitaminosis, both of the mother and the newborn.

The main objective of this study is to determine the serum concentration of 25(OH)D during the first trimester of gestation in relation to solar radiation and the season of the year in women treated at the Virgen de las Nieves University Hospital in Granada.

Hypothesis:

H₀ Vitamin D levels in pregnant women residing in the province of Granada do not vary according to seasonality and solar radiation.

H₁ Vitamin D levels in pregnant women residing in the province of Granada vary according to seasonality and solar radiation.

MATERIALS AND METHODS

Study population

A analytical cross-sectional study was conducted, with a duration of 2 years, in the period between February 2021 and February 2023.

The sample size for the present study was calculated using the finite population formula. $n = \frac{N \cdot Z^2 \cdot p \cdot q}{(N-1) \cdot d^2 + Z^2 \cdot p \cdot q}$

$$(N-1) \cdot d^2 + Z^2 \cdot p \cdot q$$

The total population of pregnant women in the province of Granada in 2020 was N=7,050. The prevalence of vitamin D deficiency was estimated at 51.9% ($p=0.519$) according to the study by Perz Castillo et al.²⁰ A confidence level of 95% ($Z=1.96$), a margin of error of 5% ($d=0.05$), and the complement ($q=1-p$) were applied. Consequently, the calculated sample size was $n=363$ pregnant women.

At the beginning, 500 pregnant women were studied due to the probability of sample loss, selected by simple randomized sampling, using a random number generator. The participants were recruited at the Obstetrics and Gynecology Service of the Virgen de las Nieves University Hospital in Granada in their first trimester of pregnancy. During the study period, 16 were excluded due to incomplete data, so the final population was 474 pregnant women (Figure 1).

The selection of women was carried out in the province of Granada, as the climate in this region has four seasons. The study is also carried out in the first trimester of pregnancy,





Figure 1. Flow diagram of participants

as this is a period in which nutritional intervention could be implemented to reverse vitamin D deficiency. To participate in the study, pregnant women had to meet the inclusion criteria: attend the first trimester obstetric session, be at a gestational age of 10-16 weeks, not be supplemented with vitamin D, and provide consent to participate in the study. The exclusion criteria were patients with mental illness, women who do not regularly attend the clinic and those who participate in another clinical trial.

This is a study nested in the project “Vitamin D supplementation during pregnancy” (D-woman), carried out by the CTS-367 group of the University of Granada. The project received the approval by the Biomedical Research Ethics Committee of the Andalusian Health Service in March 9, 2021 (ref. code 1699-N-20), and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Each pregnant woman was attended in accordance with the protocol established by the Junta de Andalucía and gave their informed consent prior to their inclusion in the study.

Description of the study variables

The variables used in this study are the following:

Primary (independent): Serum concentration of 25 OH D; vitamin D deficiency is considered serum levels below 10 ng/mL of (25 OH D), insufficiency, values less than 20 ng/mL of (25 OH D), serum levels below 30 ng/mL of (25 OH D) as low levels and optimal levels those that exceed 30 ng/mL of (25 OH D), following the recommendations of the Spanish Society of Endocrinology and Nutrition (SEEN)²⁵. This classification was selected for its alignment with the Spanish and Mediterranean context, where local factors like seasonal sun exposure and

Table 1. Classification of 25 OH D.	
Classification	Vitamin D
Deficiency	<10 ng/ml
Insufficiency	<20 ng/ml
Low levels	<30 ng/ml
Optimal levels	>30 ng/ml

Table adapted from García Martín A²⁵.

Classification	UVI Index	Recommended sun protection system based on UVI value
Low exposure	<2	-No sun protection needed
		-Can be outdoors without risk
Moderate exposure	3-5	-Needs sun protection
High exposure	6-7	- Stay in the shade during the middle of the day
		-Put on a shirt, sunscreen and hat
Very high exposure	8-10	-Needs extra protection
		-Avoid going out during the central hours of the day
Extremely high exposure	>11	-Seek the shade
		-A shirt, sunscreen and a hat are essential

Table adapted from the AEMET²⁷.

dietary habits play a bigger role than the more conservative international guidelines. Additionally, this classification aligns with the one used by the laboratory at Virgen de las Nieves University Hospital, where we analyzed the vitamin D samples for this trial (Table 1).

Modifying (dependent) variables: The average daily values of ultraviolet (UV) radiation recorded in Granada were gathered from the public data published by the National Meteorological Agency (AEMET).²⁶ The maximum monthly values of the UVI index were also considered. UVI index measures the intensity of UV radiation on the earth's surface and its value is related to the effects on human skin. UVI <2 was considered as low exposure, between 3 and 5 was moderate exposure, 6 to 7 was high exposure, 8 to 10 was very high exposure and more than 11 was extremely high exposure, according to the criteria from the WHO and the AEMET (Table 2)²⁷.

Season; This refers to the season in which participants were selected to participate in the study. Autumn was considered from September 23 to December 20, winter from December 21 to March 19, spring from March 20 to June 20, and summer from June 21 to September 22.

Descriptive variables

Daily sun exposure: Daily sun exposure was considered to be the time in which the woman is directly exposed to the sun with clothing. It is expressed in minutes a day. It has been

classified as <10 min (zero sun exposure), <20 min (insufficient sun exposure) <30 min (poor sun exposure) and >30 min optimal sun based on the recommendations of the scientific literature^{28,29}.

Daily physical activity: It was considered whether she exercised daily or not. In the case of practicing it daily, it was distinguished if it was outdoors or indoors activity.

Daily sun protection: Here it is a question of knowing whether or not the pregnant women used solar protection on a daily basis.

The body mass index (BMI) was obtained using the equation established by the WHO: kg/m². Participants were classified as follows: low BMI ≤18.5, normal BMI weight >18.5-25, overweight ≥ 25, type 1 obesity ≥30, type 2 obesity ≥35 and type 3 obesity >40 (morbid obesity).

Maternal age, gestational age (GA), place of birth, educational level, and smoking were also registered.

Procedure or collection of information

The study participants were selected at their first visit to the Obstetrics and Gynecology Service of the Virgen de las Nieves University Hospital, during the first trimester of pregnancy.

During this visit, information was obtained from each participant through an interview in which the date of recruitment, place of birth, level of education, smoking, daily sun exposure, application of sunscreen and exercise practiced were required. Other variables were also collected, such as maternal age at the time of selection, GA and BMI of the clinical history using the DIRAYA system of the aforementioned hospital carried out by the obstetricians of the project.

Next, the pregnant woman is scheduled for an appointment with the extraction room for pregnant women, to obtain the sample of Vitamin D in serum.

Serum sample collection

The blood collection was carried out in the extraction room for pregnant women located in the Outpatient Consultation section of the Maternal-Child Hospital of the Virgen de las Nieves Hospital Complex in Granada. A total of 474 fasting maternal blood samples were obtained. Samples were taken by venipuncture; 5 mL of venous blood was deposited in tubes containing anticoagulant (EDTA) and immediately transported to the laboratory for analysis.

Analysis of the blood sample

To determine the concentration of vitamin D in the pregnant woman, serum 25-OH-D levels were assessed. In the laboratory, vitamin D was quantified by microparticle chemiluminescence immunoassay (CMIA), via an Alinity I® analyzer (Abbott, Wiesbaden, Germany). Briefly, the CMIA analysis is based on

Table 3. Sociodemographic variables (n=474)	
Age (years), mean±sd	33.4 ± 5.3
Studies, n (%)	
Primary	16 (3.4%)
Secondary	86 (18.1%)
Bachelor	40 (8.4%)
FP	123 (26.0%)
University	207 (43.7%)
Get a PhD	2 (0.4%)
Smoking, n (%)	
No	422 (89.0%)
Yes	52 (11.0%)
Daily solar exposure without protection, n (%)	
No	199 (42.0%)
Yes	275 (58.0%)
Daily solar exposure, n (%)	
< 10 min	218 (46.0%)
< 20 min	99 (20.9%)
< 30 min	103 (21.7%)
> 30 min	54 (11.4%)
Physical activity, n (%)	
No exercise	307 (64.8%)
Indoor	52 (11.0%)
Outdoor	115 (24.2%)
Country of origin, n (%)	
Spain	431 (90.9%)
Other	43 (9.1%)
Body Mass Index (BMI), mean±sd	25.4 ± 4.7
Body Mass Index (BMI), n (%)	
Underweight	6 (1.3%)
Normal weight	263 (55.5%)
Overweight	131 (27.6%)
Obesity grade I	58 (12.2%)
Obesity grade II	12 (2.5%)
Morbid Obesity	4 (0.8%)
Gestational age (weeks), mean±sd	12.1 ± 0.8
Season, n (%)	
Spring	193 (40.7%)
Summer	97 (20.5%)
Autumn	66 (13.9%)
Winter	118 (24.9%)
Radiation, mean±sd	1923.0 ± 673.8

25(OH)D, mean±sd	21.7 ± 7.9
Vitamin D levels, n (%)	
Deficiency	23 (4.9%)
Insufficiency	167 (35.2%)
Low level	218 (46.0%)
Optimal level	66 (13.9%)

Sociodemographic and clinical characteristics of the study participants. Data are presented as mean ± standard deviation (SD) or as number and percentage (%), as appropriate.

the use of paramagnetic microparticles coated with antibodies. First, it is separated from the vitamin D-binding protein (DBP) to mix with the microparticle coated with anti-vitamin D antibodies. The complex is then marked with acridinium. The reaction conjugate is incubated to be later washed; then the correlation between the emitted chemiluminescence light measured in relative units of light (RLU) and the concentration of 25(OH)D is calculated. According to the manufacturer, the method detection limit for the vitamin D assay is 3.5 ng/mL (8.85 nmol/L) and the coefficient of variation is 3.6% to 99.4 nmol/L (39.8 ng/mL).

Collection of information on average daily values of UV radiation and maximum monthly UVI index values

Public information periodically published by the AEMET, through its website, was collected. With climate monitoring, radiation and ozone, monthly reports on the evolution of the ozone layer and the ultraviolet radiation index are obtained. Data are collected referring to the average daily values of UV radiation and maximum values of the monthly UVI index in the province of Granada, with reference to the Granada air base (northlocation, 37° 07' 38.559", west longitude, 3° 38' 09.687" and altitude 701.1 meters)²⁵

Statistical analysis

A database in an Excel spreadsheet was created for all study variables. Descriptive statistics for the whole population, and between-group comparisons, considering the season and levels of solar radiation were performed. Multivariate analyses including linear and logistic regression were applied to assess the effect of the seasonal period and the radiation levels on serum levels of Vitamin D. Analyses were adjusted for age, use of solar protection, daily solar exposure, and outdoor exercise. Two-sided tests were used for all analyses, and the level of statistical significance was set at 5%. All statistical analysis procedures were performed with Stata 14 (StataCorp LLC, College Station, TX, USA). Statistical tests are detailed in table footnotes.

RESULTS

Characteristics of the study population

Among the participants (n=474) in the study, 218 had low

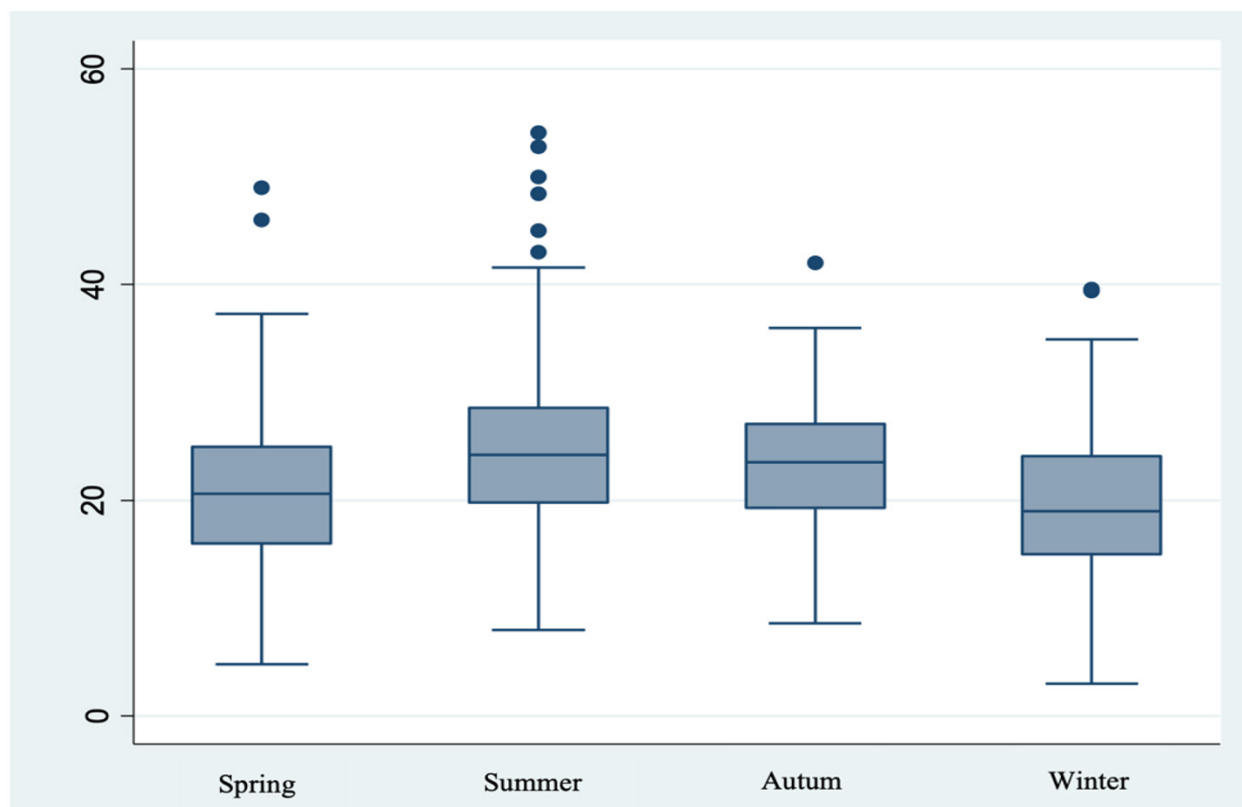


Figure 2. Boxplot diagram. Average levels of 25(OH)D, depending on the season of the year

Table 4. Association of vitamin D-related variables and solar exposure level with the seasonal period in the study participants (n=474).

	Spring	Summer	Autumn	Winter	P-Value
25(OH)D, mean±sd	20.7 ± 7.4	25.7 ± 8.9	22.9 ± 6.6	19.4 ± 7.3	< 0.001a
Vitamin D levels, n (%)					0.001b
Deficiency	9 (4.7%)	1 (1.0%)	2 (3.0%)	11 (9.3%)	
Insufficiency	74 (38.3%)	23 (23.7%)	17 (25.8%)	53 (44.9%)	
Low level	85 (44.0%)	52 (53.6%)	37 (56.1%)	44 (37.3%)	
Optimal level	25 (13.0%)	21 (21.7%)	10 (15.2%)	10 (8.5%)	
Optimal Vitamin D levels, n (%)					0.024b
No	172 (89.1%)	76 (78.3%)	57 (86.4%)	108 (91.5%)	
Yes	21 (10.9%)	21 (21.7%)	9 (13.6%)	10 (8.5%)	
Radiation, mean±sd	2212.1 ± 483.4	2587.4 ± 376.9	1192.4 ± 338.3	1312.6 ± 301.4	< 0.001a

ANOVA^a, ^b Pearson's chi-square

Table 5. Association of vitamin D-related variables with the solar radiation levels in the study participants (n=474).

Radiation, mean±sd		Global p-value ^a	Pairwise comparison among groups	
Deficiency [A]	1634.6 ± 572.4	< 0.001a	Comparison	p-Value ^b
Insufficiency [B]	1831.8 ± 644.4		A vs B	1
Low level [C]	1943.6 ± 684.3		A vs C	0.205
Optimal level [D]	2186.0 ± 668.2		A vs D	0.004
			B vs C	0.612
			B vs D	0.002
			C vs D	0.057

ANOVA ^a, ^b Student's T-test for pairwise comparison of means

Table 6. Multivariate regression analysis on the influence of seasonal period and level of solar exposure on vitamin D levels (n=474).

Model 1 – Linear Regression (mean Vit D levels)		
Season	Coefficient (95% CI)	p-value
Winter	(reference)	(reference)
Spring	1.43 (-0.31, 3.17)	0.108
Summer	6.60 (4.54, 8.66)	<0.001
Autumn	3.76 (1.47, 6.04)	0.001
Radiation	0.002 (0.001, 0.003)	<0.001
Model 2 – Logistic Regression (Vit D optimal levels, yes/no)		
Season	OR (95% CI)	p-value
Winter	(reference)	(reference)
Spring	1.34 (0.61, 2.97)	0.469
Summer	3.01 (1.33, 6.85)	0.008
Autumn	1.60 (0.61, 4.20)	0.342
Radiation	1.000 (1.000, 1.001)	0.003

(*)All models adjusted for age, use of solar protection, daily solar exposure, and outdoor exercise. Winter was chosen as reference category in both models as had lower solar radiation levels.

vitamin D levels (< 30 ng/mL of 25(OH)D), 167 had insufficient levels (< 20 ng/ml of 25(OH)D) and 23 pregnant women had deficient levels (<10 ng/mL of 25(OH)D). Table 3 describes the characteristics of the population and the classification of serum vitamin D levels. It was observed that 89% of the participants did not smoke during pregnancy, 64.8% did not report any physical activity, and 55.5% were at normal weight, according to the BMI classification.

Association between the season of the year and solar radiation at the time of blood collection with serum vitamin D levels of pregnant women

Figure 2 represents the mean serum 25(OH)D in pregnant women, according to the season of the year in which they were enrolled in the study. It is observed that the lowest mean serum 25(OH)D levels, with a mean of 19.4 ng/ml, was reported for the winter period (Figure 2). In the summer, on the other hand, the highest mean serum levels were observed, with a average value of 25.7 ng/ml (Figure 2). Also, a greater number of outliers was observed in this period, presenting widely different mean seru levels than the average 25(OH)D.

Figure 2 shows the evolution of serum vitamin D, depending on the season of the year in which the blood extraction was performed, corresponding to the first trimester of pregnancy. In general, average levels of hypovitaminosis (< 30 ng/mL of 25(OH)D) were seen in all months of the year, including summer.

Table 4 shows the relationship between serum vitamin D levels classified as deficiency (<10 ng/mL of 25(OH)D), insufficiency (< 20 ng/mL of 25(OH)D), low levels (< 30 ng/mL of 25(OH)D), and optimal levels (> 30 ng/mL of 25(OH)D), with the season of the year in which the participants were found.

The results show that in the winter and spring months the percentage of participants with vitamin D deficiency and insufficiency is higher, compared to summer and autumn. On the other hand, the same does not happen with low levels of vitamin D, as the results show 37.3% and 44% in winter and spring compared to 53.6% and 56.1% analyzed in summer and autumn.

The results corresponding to optimal vitamin D levels show, as expected, that the tendency of pregnant women with optimal levels was in summer (21.7%) and in autumn (15.2%), compared to those with lower optimal levels in winter (8.5%) and spring (13%). These ratios have a P value of 0.001, indicating a significant association between serum vitamin D and the season of the year in which the blood is drawn.

Table 4 also analyzes the association between the percentage of participants who have or do not have optimal levels of vitamin D and the season of the year in which they are. These results show that in winter, spring, autumn and summer most of the participants did not reach optimal levels. In summer alone, 21.7% manage to achieve it, maintaining an association with a high level of significance (p=0.001) between the season of the year in which the blood sample is taken and the concentration of vitamin D presented by pregnant women. It also shows the maximum monthly average radiation concentrations in the province of Granada in each season of the year. The results show a significant association, since in autumn there are lower averages of 1192.4 ± 338.3 that rise progressively until the summer, with a higher mean of 2587.4 ± 376.9.

Association between the 4 classifications of serum vitamin D in pregnant women, according to the month of blood collection and the solar radiation present

Table 5 shows the mean solar radiation of the participants who are deficient in vitamin D (1634.6), vitamin D insufficiency (1831.8), low levels of vitamin D (1943.6) and optimal levels (2186). These results show significant differences (p value < 0.001) between solar radiation in the 4 classifications of serum vitamin D. It can be seen that the higher average levels of radiation coincide with the optimal levels of this vitamin. Similarly, they are carried out a comparison in pairs pairs to find significant results in the comparison of A vs. D with a p -value of 0.004, and in B vs. D with a p value of 0.002. Thus, participants with deficiency and insufficiency have significantly lower levels of solar radiation compared to those with optimal levels who have higher levels of solar radiation. In the rest of the comparisons there is no significant comparison regarding the value of solar radiation, despite differences in the values of vitamin D. However, the C vs. D comparison is not significant, but it is close to significance.

Effect of change in vitamin D levels caused by the season of the year and solar radiation and risk of solar radiation

Once the existence of a significant association between the season of the year and the solar radiation in which the blood is drawn and the concentration of serum vitamin D is known, the effect of the season on vitamin D is studied, through a multivariate regression analysis (Table 6). 2 different regression models are used, in which the winter season is taken as a reference, the season with the lowest sun exposure. Both are adjusted for potential confounding variables (*).

The results of model 1 in Table 6 show that the mean levels of vitamin D in spring compared to winter present a coefficient of 1.43, indicating a small change with respect to winter, with a non-significant association (p 0.108). While we found a higher coefficient in both autumn (3.76) and summer (6.60) indicating a significant change in vitamin D levels in these two seasons compared to winter levels, presenting a significant association of 0.001 and < 0.001 respectively. As for the maximum monthly

solar radiation, we found that it also presents a significant association <0.001 ; however, it has a very low coefficient of 0.002 with respect to the serum concentration of vitamin D, indicating a small change in the concentration of vitamin D in the months of higher and lower maximum solar radiation.

In Model 2 (Table 6), the effect of radiation and the season of the year if the pregnant woman has optimal levels of vitamin D has been studied by means of logistic regression. The results show that in summer there is a greater difference compared to winter, participants in summer have a 3.01 higher percentage of having optimal levels of vitamin D than in winter, with a significant association (p 0.008). While pregnant women in spring and autumn have a lower probability of 1.34 and 1.60, respectively, of presenting optimal levels of vitamin D, although the association is not significant. As for solar radiation, the results are an OR of 1.00, with a non-significant association, showing that a higher or lower average maximum monthly radiation does not imply a greater probability of having optimal levels of vitamin D.

The risk of sun exposure measured by the WHO standard UVI index, analyzed in Table 7, shows that pregnant women have higher mean vitamin D levels when the UVI index is extreme, with a mean of 23.0 ng/ml of 25(OH)D and a non-significant association. In the comparison between the categories of vitamin D concentration and the risk classification (UVI index), a non-significant relationship between the two is found; it also happens with the comparison between pregnant women who have optimal levels of vitamin D and the UVI index. The results show that the risk of sun exposure is not associated with the likelihood of achieving optimal vitamin D levels, nor with a specific vitamin D classification, either deficiency, insufficiency, low levels, or optimal levels.

DISCUSSION

With the present study we wanted to address if the

Table 7. Association of vitamin D-related variables with the UVI index in the study participants (n=474).

	Low	Moderate	High	Very High	Extreme	P-Value
Vitamin D, mean $\pm$ sd	20.6 $\pm$ 6.1	19.6 $\pm$ 8.1	20.5 $\pm$ 6.8	22.9 $\pm$ 8.1	23.0 $\pm$ 9.5	0.007a
Vitamin D levels, n (%)						0.014b
Deficiency	1 (2.1%)	9 (12.9%)	5 (5.2%)	6 (2.7%)	1 (2.8%)	
Insufficiency	19 (40.4%)	28 (40.0%)	37 (38.5%)	69 (31.4%)	12 (33.3%)	
Low level	23 (48.9%)	25 (35.7%)	47 (49.0%)	107 (48.6%)	15 (41.7%)	
Optimal level	4 (8.5%)	8 (11.4%)	7 (7.3%)	38 (17.3%)	8 (22.2%)	0.125b
Optimal Vitamin D levels, n (%)						
No	43 (91.5%)	63 (90.0%)	89 (92.7%)	185 (84.1%)	29 (80.6%)	
Yes	4 (8.5%)	7 (10.0%)	7 (7.3%)	35 (15.9%)	7 (19.4%)	

ANOVA^a, ^b Pearson's chi-square



concentration of vitamin D in pregnant women during the first trimester of pregnancy and relate it to solar radiation and the season of the year in the population of Granada.

The results have shown low and insufficient levels (46.0% and 35.2%) of vitamin D in the study population, with only 13.9% with optimal levels of this vitamin. It also showed a prevalence of hypovitaminosis D of 86.07% (<30 ng/mL) according to SEEN thresholds. Alternative classification criteria yield markedly different prevalence estimates: the Endocrine Society threshold (<20 ng/mL for insufficiency) identifies approximately ~40% of cases, while the IOM criterion (<12 ng/mL for deficiency) captures only ~5%. These lower thresholds may underestimate maternal-foetal risks in Mediterranean populations with subclinical deficiency, thereby justifying the contextualised SEEN thresholds for Spain^{13,17}.

They are in line with the study carried out by Pérez Castillo et al., in 2020, also with the pregnant population of Granada, in which very similar data were obtained using the same SEEN classification. 51.9% had what has been classified in this article as low vitamin D levels (<30 ng/ml) and 36.3% suffered from vitamin D insufficiency (<20 ng/mL)²⁰. These results reveal a significant hypovitaminosis, with levels <30 ng/ml of 25 OH D in pregnant women living in Granada.

Vitamin D concentration follows a seasonal pattern. The results show a maximum peak in mean serum concentrations of 25 OH D in summer (25.7 ng/ml) and progressively decrease in autumn (22.9 ng/ml) and winter (19.4 ng/ml, to rise again in spring (20.7 ng/ml). These results coincide with those found in another study that was carried out in Logroño, La Rioja (Spain), in which it was shown that vitamin D hypovitaminosis (levels below 30 ng/ml) follows a seasonal pattern, with the lowest levels of 25(OH)D in winter and the highest in summer. when exceeding 20 ng/ml³⁰. Similar results in cities with a location so far from each other, both in the north and south of Spain, show that hypovitaminosis is widespread, regardless of the geographical location in which they are located, even within the same country.

Regarding location, the results show that there is an association between solar radiation in the geographical place where the pregnant woman lives and the vitamin D levels represented in Table 4 (p value = < 0.001). However, in the linear regression of Table 6, the coefficient of change is low (0.002), which indicates that radiation plays an irrelevant role in vitamin D levels even if a positive association is maintained with respect to this vitamin. This relationship contrasts with what was described by Suarez-Calleja et al., in 2020 in a study carried out in Asturias, in which vitamin D levels were measured in the first trimester of gestation to find that 25.3% had plasma values of 25(OH)D below 20 ng/ml. 36.5% had values between 20 and 30 ng/ml and, paradoxically, the highest frequency was found in pregnant women, with optimal values (>30 ng/ml) with 38.2%³¹.

But if the two frequencies of low levels (< 30 ng/ml) are

grouped together, most pregnant women would be in this situation, with a frequency of 61.8%. These results indicate that, despite the fact that hypovitaminosis is present in this region of Spain, 38.2% have managed to reach optimal levels, although the north of Spain is one of the regions with the lowest sun exposure. This percentage is high, compared to pregnant women in Granada (southern Spain), whose frequency of optimal levels is only 13.9%.

The same situation is observed in one of the regions with the highest sun exposure in Spain, the Canary Islands; there, 65.5% of healthy individuals have 25(OH)D levels below 30 ng/ml, 23.4% below 20 ng/ml and 5% with levels below 14.3 ng/ml³². In the study carried out by García-Dorta et al., it is concluded that, as it is a region with a lot of sun exposure, these data should be considered as optimal levels, since it is not feasible for the population to be exposed to the sun more than in that region of Spain, since these values of this population are already optimal³².

However, our results show that vitamin D concentration does not depend exclusively on sun exposure, since in Table 5 no significant homogeneous results have been found between the association of vitamin D classification and sun exposure; there is only an association between pregnant women with deficiency and insufficiency. that have significantly lower levels of solar radiation, compared to those that have optimal levels of vitamin D and that have higher levels of solar radiation. Thus, according to our results, the theory of Garcia-Dorta et al³². cannot be supported.

The results of vitamin D concentration from the three previously mentioned studies carried out in Spain coincide with the results of our study, which shows that there is a significant deficiency in the pregnant population and that, even in summer, the levels considered optimal are not reached in Table 4^{32,34}.

Similarly, the associations found in the regression analyses (Table 6) indicate a greater relationship of dependence between vitamin D and the seasonality of solar radiation. Therefore, the Mediterranean paradox could be justified that indicates that the countries of the Mediterranean basin have a greater sun exposure, but despite this, they have low levels of this vitamin, compared to countries in northern Europe. A clear example of this is that the level of vitamin D is usually much higher in the Nordic countries than in the Mediterranean. This is explained by the widespread frequent consumption of cod liver oil, fish oil, and vitamin D supplements³². In countries such as Finland, food fortification policies are carried out in their dairy products, which resulted in a significant increase in the serum levels of 25(OH)D in their population in 2011³⁴.

Apart from eating habits, northern European countries are characterized by having developed policies of supplementation during pregnancy. The Scottish government has recommended consuming 400 IU of vitamin D daily from the beginning of pregnancy to all pregnant women, since 2012³⁵. The Swiss

guidelines also recommend a routine supplement of 600 IU of vitamin D to all pregnant women, without prior assessment of their vitamin D levels¹⁹.

All these measures applied in northern European countries confirm the thesis that optimal levels of vitamin D are unlikely to be achieved with sun exposure alone.

It is necessary to take into account how this sun exposure affects people's health; in the results of Table 7, it can be seen that, although the UVI risk may be higher or lower, it is not significantly related to achieving optimal levels of vitamin D. Therefore, recommendations for daily sun exposure should be made taking into account this UVI risk and should be discouraged when the risk is high, especially in a population as vulnerable as pregnant women.

The study carried out in Switzerland by Religi A et al., shows that in winter and autumn, the recommended production of vitamin D without the risk of sunburn is often -unattainable. Up to 6.5 h of sun exposure would be necessary, considering 8-10% of the skin surface uncovered; however, in summer and spring good vitamin D levels can be reached with a daily exposure of 10-15 minutes with a risk of erythema production if they spend between 9 and 45 minutes exposed to the sun³⁶. It is evidenced, once again, that reaching optimal vitamin D values is more related to the season of the year than to sun exposure; it can even be dangerous to try to reach optimal levels of vitamin D only with sun exposure due to the risk of erythema.

It is known that the sun's ultraviolet rays stimulate the synthesis of vitamin D in the skin; however, there are environmental factors and personal characteristics that prevent or impede such dermal synthesis. There are conflicting opinions when it comes to defending sun exposure as a matter of public health to increase the level of vitamin D. It is important to continue researching how to address vitamin D deficiency through dietary supplements rather than sun exposure, given their few side effects and relatively wide margin of safety. The provision of vitamin D 3 could be considered an important, cheap and safe therapy.

We consider that one of the main limitations of this study is that important determinants of vitamin D status were not assessed, including maternal skin phototype, dietary vitamin D intake, socioeconomic status, clothing habits, and cultural sun exposure. These were not collected due to logistical constraints of hospital recruitment and brief questionnaires designed to minimise participant burden. These factors could modulate skin synthesis and absorption (low intake in a non-fortified Mediterranean diet), leading to an underestimation of deficiency in vulnerable subgroups. Future prospective studies should incorporate them via validated questionnaires.

As a strength, however, the number of women participants and

the duration of the study (2 years) are estimated.

CONCLUSIONS

The present results reveal a high prevalence of vitamin D deficiency in the pregnant population of Granada Likewise, and despite the geographical location where the study has been carried out (Granada) characterized by high solar availability, not even in summer does most of the pregnant population reach optimal levels of vitamin D.

It is found that sun exposure does not seem to be the main variable when it comes to achieving optimal levels of vitamin D, but, rather, the time of year in which they are found. Likewise, it is considered that the solution to obtain optimal levels of vitamin D does not seem to be sun exposure, as there is a high risk for the skin, if such exposure is excessive.

These results reveal the need for a public health intervention in the Spanish female population as a whole, Specific interventions are proposed: universal minimum supplementation with vitamin D (400-1000 IU/day from preconception); regional policies for systematic prenatal screening of 25(OH)D in the first trimester in Andalusia; and national food fortification (milk, cereals) similar to Finland, raising population levels to ~20 nmol/L. These cost-effective measures would mitigate the observed seasonal deficiency and its consequent complications in pregnancy.

AUTHOR CONTRIBUTIONS

Author 1, AMRC: helped conceive and design the study, coordinated participant recruitment, oversaw data collection, collaborated in the interpretation of results and wrote the initial and final version of the manuscript. Author 2 , MJAC conceive and design the study, assisted in the interpretation of results, critically revised the manuscript for important intellectual content and provided feedback at all stages. Author 3 AMF contributed to data extraction, dataset management, performed statistical analyses and contributed to the formal English language and grammatical editing of the manuscript. Author 4, JLGV helped conceive and design the study, helped with participant recruitment and provided feedback at all stages. Author 5 MPSA contributed to conceive and design the study and collaborated in the interpretation of results. Author 6 RBMJ7 contributed to dataset management and performed statistical analyses. Author 7 NNAM contributed to critically revised the manuscript. Author 8 NSJ contributed to critically revised the manuscript and provided feedback at all

All authors contributed to the review and approval of the final version of the manuscript for submission.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.



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