

## Seasonal Variations of Vitamin D in Vegetarians

Gabriella Lavalle\*

Clinical Pathology Laboratory, Bracciano Hospital, Bracciano, Rome, Italy

\*Corresponding author: Gabriella Lavalle, Clinical Pathology Laboratory, Bracciano Hospital, Bracciano, Rome, Italy, Tel: 00390699890299; E-mail: gabriella.lavalle@aslroma4.it

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### Abstract

**Aim:** Adequate levels of vitamin D are vital for bone homeostasis throughout the life cycle, especially at older ages. We aimed to assess 25-hydroxyvitamin status and seasonality variations in a sample of vegetarians stratified for age and gender.

**Methods:** The study included 347 vegetarians, whose serums were collected between March and May (before summertime-PreS) and then between August and September (after summertime-PostS). Serum 25-hydroxyvitamin (Vitamin D) was measured by an automated immunoassay (ARCHITECT, Abbott).

**Results:** PreS samples showed a Vitamin D median of 18.3 ng/mL and 57% resulted as deficient (<20 ng/mL) while only 13% resulted as sufficient (>30 ng/mL). The deficient fraction was the mode class at all ages. No statistically significant differences among age and sex classes were detected. PostS samples showed a Vitamin D median of 36.0 ng/mL with a significant increase of 32% as compared to PreS; 26% resulted as deficient while only 36% resulted as sufficient. In subjects aged >69 years this increase was statistically smaller than in other age groups. Mode was the sufficient class up to 49, the insufficient class (20-30 ng/mL) between 50 and 69 and the deficient class over 69 years.

**Conclusions:** These results confirm that exposure to the sun increases the levels of vitamin D in young and middle-aged vegetarians but not in the elderly and highlight the need for dietary strategies to increase vitamin D intake especially in the older age classes.

**Keywords:** Vitamin D; Vegetarian diet; Seasonal variations; Elderly; Prevention

### Introduction

Vitamin D is a lipophilic pro-hormone essential for calcium homeostasis and bone health especially in old age [1]. However, many studies have shown that this hormone, deceptively simple, has pleiotropic action and affects many aspects of the human physiology. This metabolite influences the optimal function of cardiac and skeletal muscle [2], is involved in the modulation of inflammatory and immune system [3] and also regulates the proliferation, cell differentiation and apoptosis [4]. A recent meta-analysis study [5] has also shown that the proper calcium metabolism and therefore proper intake of vitamin D, contributed substantially to the increase in longevity that occurred in western countries.

Vitamin D can be obtained through the diet, in the form of vitamin D<sub>2</sub> (from plants) and vitamin D<sub>3</sub> (from animals), but it is mostly synthesized in the skin after sun exposure [6]. The ultraviolet light in the range of 280-320 nm (UVB) [7] penetrate through the skin and convert 7-dehydrocholesterol present in the body into Vitamin D<sub>3</sub>. Both molecular forms, D<sub>2</sub> and D<sub>3</sub>, are transported to the liver, where they are subjected to a hydroxylation reaction and turned into 25 (OH) D (25-hydroxyvitamin D), that represents the prevalent circulating form of vitamin D. In kidneys, the enzyme 1-Alpha hydroxylase converts 25-hydroxyvitamin D into 1.25-dihydroxyvitamin D, which is the active form and plays the main role, as it promotes the absorption

of calcium in kidneys and the absorption of phosphorus and calcium in the intestine.

Many factors (food intake, body mass index, skin melanin, intestinal absorption) influence vitamin D status in addition to sun exposure [8,9]. Of these factors, diet is of fundamental importance: milk, dairy products and fish such as salmon, herring, cod and mackerel contain a high amount of vitamin D thus allowing a right supplementation. Vegetarian diet has gained popularity in Italy. Vegetarian diets exclude meat, seafood, and products containing these foods. With the adoption of vegetarian eating styles, there is a growing interest to study vitamin and nutrients adequacy in diets excluding animal foods.

The aim of this work was to analyze the influence of vegetarian diet on vitamin D mean before and after the summer season according to different age groups and in both genders.

### Materials and Methods

The study was carried out during the period from March to September 2016 in the laboratory of the Bracciano Hospital, which is located in the north area of the province of Rome (Italy). Blood samples were collected from 347 vegetarian outpatients. All patients gave their written consent and testing was approved by the Ethics Committee of the institutions involved.

Demographic and anthropometric data (age, gender, weight, height) and data on vitamin D and dietary supplement use and pregnancy

state were collected based on self-reports. Body mass index (BMI) was calculated as the weight (in kg) divided by the height (in m) squared.

Exclusion criteria included treatment with vitamin D or calcium supplement, use of dietary supplement, obesity (BMI<28) and pregnancy.

The samples, collected in patients that has been fasting for at least 12 hours, were centrifuged at 3000 rpm for 10 minutes and analyzed within two hours for Vitamin D serum concentration using the fully-automated chemiluminescent method in routine use at the laboratory (Architect i2000SR, Abbott Diagnostics).

In the period before summer (PreS: from March to May), 347 serums from white skin vegetarians (178 females and 170 males) were analyzed. After summer (PostS), in August-September, samples from the same population were collected.

The patients were divided according to gender and grouped into four age groups mentioned as A (<30 years), B (30-49 years), C (50-69 years) and D (>69 years). The results obtained were divided into three classes: deficient results (<20 ng/mL), inadequate results (20-30 ng/mL) and sufficient results (>30 ng/mL) (>75 nmol/L) [10,11].

### Statistical Analysis

Data were processed with a commonly-used spreadsheet (MS Excel) powered by a statistical analysis plug-in (Analyse-it Software Ltd., UK, Method Validation Edition ver. 2.30). Differences between groups were analyzed using ANOVA with Bonferroni correction (p<0.0020 for significance). The distribution was expressed through the median and interquartile range (IQR). As the reference interval was considered the only upper limit to 95%, with a non-parametric confidence interval of 95% (CI95%). Grading percentages for the various classes (sex, age and time of year) were compared using Chi-square test.

Vitamin D ng/MI	n	Min	1st Quartile	Median	95% CI	3rd Quartile	Max	IQR
Pre	347	5	13.6	18.3	17.10 to 19.50	24.95	76.3	11.35
Post	346	6.5	19.48	26.7	25.70 to 28.10	34.43	105.1	14.94

**Table 1:** Distribution of Vitamin D (ng/mL) before (Pre) and after (Post) summer.

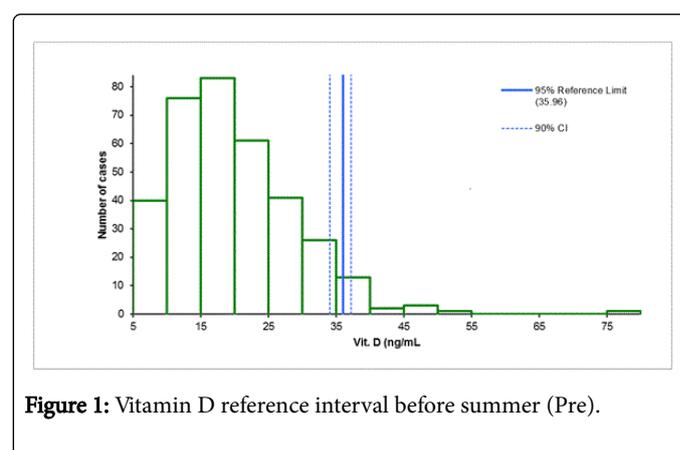
	Pre-A	Pre-B	Pre-C	Pre-D	Post A	Post B	Post C	Post D
Deficient	59%	39%	57%	65%	15%	10%	22%	41%
Insufficient	32%	48%	27%	24%	26%	38%	42%	39%
Sufficient	9%	13%	17%	12%	59%	51%	36%	20%
Total	34	54	115	144	39	68	110	129

**Table 2:** Age-dependent Vitamin D levels.

In the PostS period, the median vitamin D levels were of 26.7 ng/mL (IQR=14.9 ng/mL) and a significant overall increase of 32% as compared with the pre-summer period (Table 1). The 95th percentile of the post-summer population resulted to be 48.5 ng/mL (CI95%: 45.5 ng/mL to 51.7 ng/mL) with a non-gaussian distribution (kurtosis=8.5, symmetry=1.4, significant) (Figure 2).

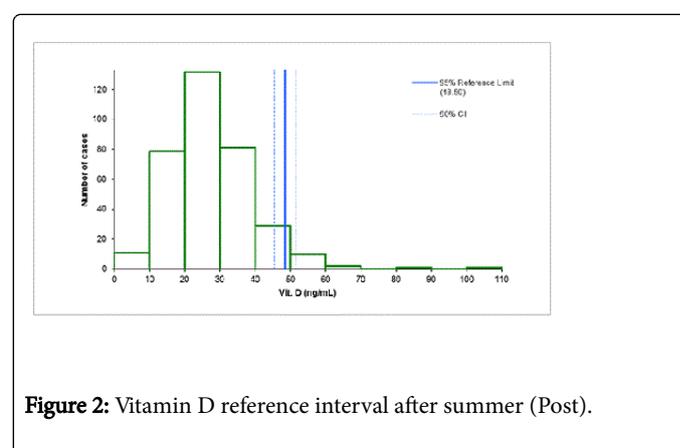
### Results

As summarized in Table 1, in the PreS period the levels of Vitamin D had a median value of 18.3 ng/mL (IQR=11.3 ng/mL) with no significant differences between genders. The concentration of vitamin D was almost always not sufficient (95th percentile=36.0 ng/mL, CI95%: 34.0 ng/mL to 37.2 ng/mL) with non-gaussian distribution (kurtosis=6.9, symmetry=1.4, significant) (Figure 1).



**Figure 1:** Vitamin D reference interval before summer (Pre).

When considering the result categories in detail, 57% (199 subjects) of the samples proved to be deficient in vitamin D, 29% (102 subjects) was found insufficient and only 13% (46 patients) had a sufficient value (Table 2).



**Figure 2:** Vitamin D reference interval after summer (Post).

In all age classes the percentage of insufficient patients were reduced after summer (Table 2). No statistically-significant differences were detected in this study between males and females neither before

summer nor after summer. In the PreS population no significant difference was detected among age classes while in the PostS population group D (>69 years old) showed lower levels (median=23.0 ng/mL) and a reduced, statistically-significant increase compared to all other groups (respectively: A median=31.7 ng/mL, B median=30.6 ng/mL, C median=27.4 ng/mL).

Younger age classes (A and B) after the summer showed a significant reduction of the percentage of patients classified as deficient

and insufficient (respectively from 91% to 41% and from 87% to 49%). In contrast, in older patients (C and D) a still statistically significant but quantitatively less noticeable percentage reduction of patients with vitamin D deficiency (respectively from 83% to 64% and from 88% to 80%) was observed, with an ensuing increase in the insufficient class (20-30 ng/mL) which indicates an incomplete transition to the upper class of values (Table 3).

	Class Composition								Class variation (Post-Pre)			
	Pre-A	Pre-B	Pre-C	Pre-D	Pos A	Pos B	Pos C	Pos D	A	B	C	D
Deficient	58.80%	38.90%	56.50%	64.60%	15.40%	10.30%	21.80%	41.10%	74%	74%	61%	36%
Insufficient	32.40%	48.10%	27%	23.60%	25.60%	38.20%	41.80%	38.80%	21%	21%	-55%	-64%
Sufficient	8.80%	13%	16.50%	11.80%	59%	51.50%	36.40%	20.20%	-568%	-297%	-120%	-71%

**Table 3:** Age class composition and frequency variation.

### Discussion

Low serum levels of vitamin D, which is frequent among the elderly and during aging, may affect brain oxidative status, while adequate serum levels of Vitamin D protect it from free radicals, preventing damage and harmful consequences [12]. In addition, vitamin D deficiency can also cause an up-regulation of the renin-angiotensin system causing a hypertrophy of the left ventricle and vascular smooth muscle cells, increasing these cardiovascular risk factors for early mortality [13]. UVB sunlight exposure, rather than diet, has been reported as the main source of vitamin D of the population.

Some research [14-16] suggests that vegetarian diets are associated with lower bone mineral density and s25(OH) mostly in elderly people.

In the current study, the prevalence of vitamin D deficiency among vegetarians analyzed before the summer months, was 57%, with high percentage also in young donors, in agreement with literature data [17].

Several papers show a significant increase of vitamin concentration after summer [18,19], and many other studies as the EPIC-Oxford study [20] have shown that vegetarian people show generally low level of Vitamin D but, as far as we know, these two factors were never considered together in according to age and gender as in this work.

Our study points out that the beneficial effect of the summer (Vitamin D increase) is somehow lowered by the aging. After summer, sufficient class turns to be the moda only for younger people while elder ones, despites improved median levels, keep mostly deficient or insufficient.

However, our study shows that the elderly vegetarians would not benefit enough of solar exposure as the other classes of age and as elderly non-vegetarians [19]. Dietary factors may be not sufficient to increase the levels of Vitamin D. This indicates the need for a stronger dietetic prevention in the elderly vegetarians.

It is known that observed phenomenon could have several causes to investigate, e.g., a lower solar exposure in older subjects (this study not investigated the time of sun exposure and clothing material) or a metabolic inhibition in the synthesis of 25OHD [21].

Whatever the causes, it is conceivable that the effect observed may be corrected by implementing preventive interventions on nutrition and lifestyle habits (clothing, physical activity).

IOM [22] and Australian recommendations [23] indicate that, in order to improve the status of vitamin D circulating solar exposures are mandatory. Arm discoveries, of 6-7 minutes per day during the summer and from 7 to 40 minutes during winter depending on latitude might be enough. Where sun exposure is reduced as in elderly people (mostly vegetarians), dietary intakes of vitamin D are recommended, (at least 600 IU per day in people 70 years and at least 800 UI for those above 70 years, in association with dietary intake of calcium [24].

### Conflict of Interest

The author declares to have no conflicts of interest.

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