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Article

# Determinants of Vitamin D Status: An Analysis in a Primary Care Setting in Lithuania of Age, Gender and Seasonality

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## Abstract

**Background and Objectives:** Vitamin D deficiency is a pervasive public health issue in high-latitude regions, yet large-scale population data for the Baltic states remain sparse. This study aimed to determine the prevalence of vitamin D status and identify its primary determinants within a primary care setting in Lithuania. **Materials and Methods:** We conducted a retrospective cross-sectional analysis of serum 25-hydroxyvitamin D [25(OH)D] concentrations from 14,330 unique patients (aged 1–101 years) collected during 2025 at a major clinic in Vilnius. Vitamin D status was categorized according to the Central and Eastern European Expert Consensus thresholds. **Results:** The overall median 25(OH)D concentration was 68.3 nmol/L (Mean: 74.7 nmol/L; SD: 35.1), placing the population average in the "insufficiency" range (50–75 nmol/L). Seasonality emerged as the most significant predictor of deficiency; multivariable logistic regression showed a maximal risk reduction in September (OR 0.33; 95% CI: 0.27–0.41) and August (OR 0.34) compared to January, while June and November provided no significant protection. Age-specific analysis revealed a non-linear "U-shaped" distribution: children aged 0–6 years had the highest levels (mean ~100 nmol/L), likely due to rickets prophylaxis, whereas adolescents (12–18 years) exhibited the highest vulnerability, with approximately 80% suffering from deficiency or insufficiency. Males faced a 13.9% higher likelihood of deficiency than females (OR 1.14;  $p = 0.0036$ ), potentially due to lower rates of elective supplementation. **Conclusions:** These findings suggest that current supplementation strategies successfully protect infants but fail to sustain adequacy through adolescence and adulthood, particularly during the "vitamin D winter." Targeted public health interventions for adolescents and year-round monitoring are recommended to mitigate the high prevalence of suboptimal vitamin D status in Lithuania.

**Keywords:** vitamin D; vitamin D deficiency; seasons; age factors; sex factors; Lithuania; cross-sectional studies; public health surveillance

## 1. Introduction

Vitamin D deficiency represents a major global public health concern, affecting an estimated one billion individuals worldwide across all age groups, geographic regions, and socioeconomic backgrounds [1,2]. Notably, this burden is not limited to regions with low sun exposure. Systematic reviews have demonstrated that deficiency remains prevalent even in countries with year-round sunlight [2]. Vitamin D is primarily linked to calcium and phosphorus metabolism and bone health. Moreover, the clinical significance of vitamin D extends beyond skeletal health. Studies have demonstrated associations between low vitamin D status and increased risk of several chronic conditions, including certain cancers, type 2 diabetes, cardiovascular disease, and autoimmune disorders [3–5]. There remains debate in recent literature regarding which 25(OH)D concentrations should define deficiency and sufficiency. According to the latest Endocrine Society guidelines,

published in 2024, healthy adults younger than 75 years require no routine screening for 25(OH)D in the general population and empiric vitamin D supplementation [6]. In contrast, The Institute of Medicine considers the minimal 25(OH)D concentration of 50 nmol/L as physiologically adequate for at least 97.5% of the population [7]. A Central and Eastern European Expert Consensus Statement published in 2022 confirmed vitamin D deficiency as <50 nmol/L, suboptimal status 50 - 75 nmol/L, and optimal concentration 75 - 125 nmol/L [8,9]. In our study, we adopted the Central and Eastern European consensus thresholds, as these were specifically developed for populations sharing Lithuania's geographic, climatic, and nutritional characteristics, have been widely implemented across the neighboring countries and are consistent with the classification used in prior Lithuanian studies, allowing further comparisons [9,10].

Within Europe, the prevalence of vitamin D deficiency remains high and exhibits marked geographical heterogeneity [11,12]. Paradoxically, Northern European countries such as Finland, Sweden, and Norway often report higher population 25(OH)D concentrations compared to Central and Eastern European countries, where vitamin D deficiency is considerably more prevalent [12–14]. Lithuania, situated at approximately 54° - 56°N latitude, falls into a zone where cutaneous synthesis of vitamin D via ultraviolet B (UVB) radiation is physiologically limited from October through March, restricting endogenous production to a narrow seasonal window [11]. Combined with limited dietary vitamin D sources, low habitual consumption of oily fish, and the absence of a mandatory vitamin D food fortification policy, endogenous and exogenous vitamin D supply in Lithuania remains insufficient for most of the year [15,16]. Furthermore, lifestyle factors common across European populations at similar latitudes, including predominantly indoor occupational activities, reduced time spent outdoors during the cold season, and limited deliberate sun exposure even in summer, may further contribute to the risk of vitamin D deficiency in the Lithuanian population [17].

Despite the recognized public health significance of vitamin D deficiency, population-level data on 25(OH)D concentrations in Lithuania remain limited. The largest population-based study to date was conducted by Bleizgys and Kurovskij in 2018, including 9581 subjects, and demonstrated a high overall prevalence of vitamin D deficiency (67%). In contrast, a high prevalence of vitamin D hypervitaminosis was observed among young children [10]. The largest study investigating a pediatric population was performed by Butkute et al., who analysed 2008 samples from the Children's Hospital, and reported that 51.3% of the study population were vitamin D deficient [18]. Other existing studies have focused on specific subgroups. Strazdiene et al. examined elderly individuals and found that 72.2% of women and 65.6% of men were vitamin D deficient [19]. Punceviciene et al. confirmed that vitamin D deficiency is highly prevalent among patients with rheumatoid arthritis, with 25(OH)D levels significantly lower compared to healthy controls [20]. Several smaller studies have further characterized vitamin D status in Lithuanian subpopulations: Gailyte et al. examined men of the Lithuanian Armed Forces aged 19 - 25 years and found that nearly all participants were vitamin D deficient; Zabuliene et al. assessed young female school graduates and reported that only 3.4% had sufficient vitamin D levels and Bleizgys studied young healthy males and observed clear seasonality, with mean 25(OH)D levels during the warm season being approximately two-fold higher than during the cold season, while more than one-third of participants exhibited low vitamin D levels in both seasons [21–23].

To our knowledge, our study represents the largest population-based assessment of vitamin D status conducted in Lithuania. Specifically, we aimed to, firstly, determine the prevalence of vitamin D status in a large sample of 14,330 patients, secondly, evaluate the independent and combined effects of age, sex, and season on serum 25(OH)D concentrations using multivariable regression and, thirdly, compare our findings with previously published Lithuanian data over the past decade.

## 2. Materials and Methods

### 2.1. Study Design and Setting

We conducted a retrospective cross-sectional study analyzing serum 25-hydroxyvitamin D [25(OH)D] concentrations in a primary care setting in Lithuania. Anonymized data were retrieved from the laboratory information system of Karoliniskiu Clinic in Vilnius, Lithuania. The study aimed to evaluate the prevalence of vitamin D deficiency across different age, seasonal and gender groups. To ensure the representation of the baseline population health status, we analyzed historical laboratory data collected over a period from January 2<sup>nd</sup> 2025 to December 31<sup>st</sup> 2025. The study adhered to the principles of the Declaration of Helsinki and followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

The serum concentration of total 25-hydroxyvitamin D [25(OH) vitamin D] was determined using a two-step competitive binding immunoenzymatic assay (paramagnetic particle, chemiluminescent immunoassay). All analyses were performed on the UniCel DxI Immunoassay System (Beckman Coulter, Inc.), utilizing sheep monoclonal anti-25(OH) vitamin D antibodies and a vitamin D analog-alkaline phosphatase conjugate to achieve high analytical sensitivity and specificity.

### 2.2. Study Participants and Sampling

The initial dataset contained 15,421 laboratory entries. To avoid selection bias introduced by repeated measurements (e.g., follow-up tests after prescribed supplementation), we applied a strict data deduplication protocol. Unique patients were identified using a combination of birth date, gender, and the referring physician ID. Only the first chronologically recorded test (baseline measurement) for each unique individual was included in the final analysis. Subsequent tests for the same patient were excluded to reflect the natural prevalence of vitamin D status before intervention.

The final study sample consisted of 14,330 unique subjects, ranging in age from infancy to centenarians. No exclusions were made based on comorbidities or clinical department.

### 2.3. Variables and Measurements

In the absence of specific national guidelines for vitamin D reference ranges in Lithuania, we adopted the classification criteria proposed by the Central and Eastern European Expert Consensus Statement (2022). This consensus, developed by a panel of experts from the region (including Poland, Latvia, and Estonia), aligns with major international epidemiological studies [9]. Specifically, we utilized the consensus-endorsed threshold of <30 nmol/L for deficiency and >75 nmol/L for sufficiency to ensure our findings are comparable. Based on this clinical data, vitamin D status was classified into the following categories:

- Severe Deficiency: <30 nmol/L
- Deficiency: 30–50 nmol/L
- Insufficiency: 50–75 nmol/L
- Sufficiency (Optimal): 75–125 nmol/L
- Elevated Level: >125 nmol/L
- Absolute Hypervitaminosis: > 374 nmol/L

### 2.4. Data Analysis

Statistical analysis and data visualization were performed using R Statistical Software (v4.x, R Foundation for Statistical Computing) within the RStudio environment. The tidyverse and ggplot2 packages were utilized for data manipulation and graphical representation.

Descriptive Statistics: Normality of the data distribution was assessed visually using histograms and kernel density estimation (KDE) plots. Since the distribution of 25(OH)D levels showed a positive skew (right-tailed), continuous variables are reported as means with standard deviations (SD) and

medians with interquartile ranges (IQR). Categorical variables are presented as absolute numbers (n) and percentages (%).

**Comparative Analysis:** To compare vitamin D levels between two independent groups (e.g., males vs. females), the non-parametric Mann-Whitney U test was applied. For comparisons across multiple age groups, the Kruskal-Wallis H test was used, followed by pairwise post-hoc analysis. The Chi-square ( $\chi^2$ ) test was employed to compare the frequency of clinical deficiency categories between groups.

**Regression Analysis:** To identify predictors of vitamin D deficiency (<50 nmol/L), we performed binary logistic regression. A multivariate logistic regression model was fitted to calculate adjusted Odds Ratios (aOR) with 95% Confidence Intervals (CI), estimating the independent risk associated with male gender and specific age groups.

A p-value of <0.05 was considered statistically significant for all tests.

### 2.5. Ethical Considerations

This study is a retrospective, descriptive cross-sectional analysis focused on public health surveillance rather than the testing of a specific clinical hypothesis. According to the Law on Ethics of Biomedical Research of the Republic of Lithuania, a study is only classified as "biomedical research" if it meets four cumulative criteria. Our study does not satisfy the first criterion, as it does not aim to test a new scientific hypothesis or create novel theoretical knowledge; instead, it provides a statistical overview of vitamin D prevalence in a specific population.

Furthermore, the study utilized fully anonymized retrospective data. In accordance with the guidelines of the Lithuanian Bioethics Committee, studies that do not meet all four legal criteria for biomedical research are exempt from mandatory Institutional Review Board (IRB) approval. This research was conducted following the "Ethical Principles of Non-Biomedical Research Involving Human Health," ensuring high standards of data protection and de-identification.

## 3. Results

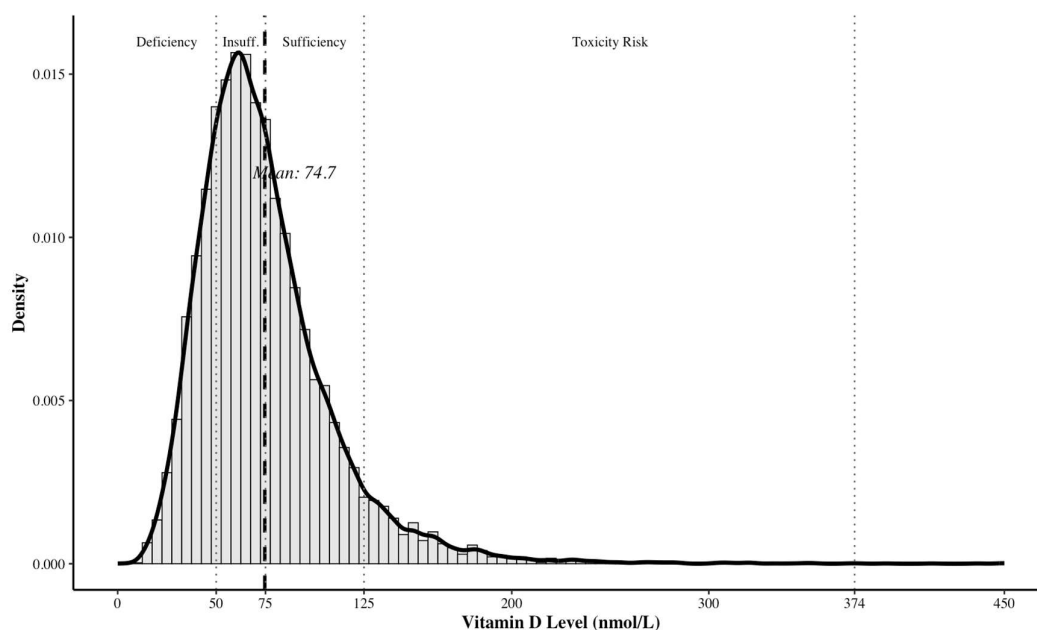
### 3.1. Descriptive Statistics

A total of 14330 unique subjects were retained in the final analysis following the exclusion of duplicates. The study population spanned a broad demographic spectrum (Table 1), ranging from 1 to 101 years of age. The mean age was 38.8 years (Median: 37.0; IQR: 30.0), and the distribution was normal (Figure 1).

The analysis of serum 25(OH)D concentrations revealed a non-normal distribution with a positive skew. The overall mean 25(OH)D concentration was 74.7 (95% CI: 74.1–75.2). However, the median value was notably lower at 68.3 nmol/L

**Table 1.** Descriptive Statistics of Age and Serum 25(OH)D Concentrations.

	Age (Years)	Vitamin D (nmol/L)
Sample Size (N)	14330	14330
Mean	38.8	74.65
Median	37.0	68.32
Standard Deviation (SD)	22.3	35.11
Standard Error (SEM)	0.2	0.29
95% CI (Lower – Upper)	38.4 - 39.1	74.0 - 75.2
Range (Min – Max)	1.0 - 101.0	2.3 - 500.7
1st Quartile (Q1)	24.0	52.0
3rd Quartile (Q3)	54.0	89.1
Interquartile Range (IQR)	30.0	37.0

**Figure 1.** Distribution of serum 25(OH)D levels in the study population.

### 3.2. Vitamin D Distribution by Age

Regarding gender composition (Table 2), the study population was predominantly female (72.3%, n=10359), while males accounted for 27.7% (n=3971) of the participants.

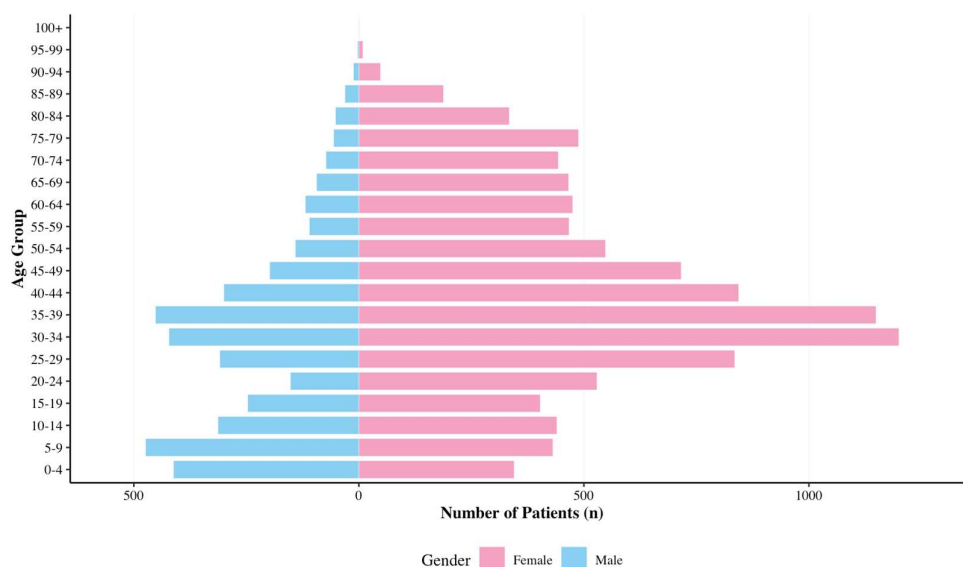
The age distribution differed significantly by gender (Table 3), with females generally being older. The median age for females was 39.1 years (IQR: 28.0–57.9), compared to 31.0 years (IQR: 11.3–43.0) for males. The female cohort spanned from 1.0 to 102.0 years, showing a broader distribution in older age brackets. In contrast, the male cohort (range: 1.0 to 97.2 years) was more heavily concentrated in younger demographics (Figure 2).

**Table 2.** Demographic Characteristics of the Study Population by Gender.

Gender	Count (n)	Percentage (%)
Female	10359	72.3%
Male	3971	27.7%
Total	14330	100.0%

**Table 3.** Distribution of Serum 25(OH)D Concentrations with Median and Quartile Ranges.

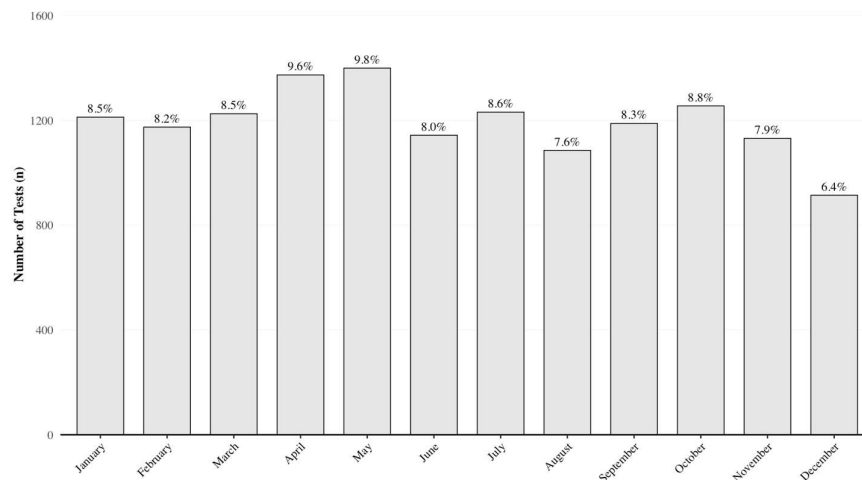
Gender	Minimum	Q1	Median	Q3	Maximum
Female	1.0	28.0	39.1	57.9	102.0
Male	1.0	11.3	31.0	43.0	97.2

**Figure 2.** Population pyramid illustrating the demographic structure of the study cohort by age and gender.

Data collection was distributed relatively evenly (Table 4; Figure 3) throughout the year, with peak activity observed in May (9.8%) and April (9.6%), while the lowest volume of tests was recorded in December (6.4%).

**Table 4.** Monthly Distribution of Vitamin D Testing.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Count (n)	1212	1174	1225	1373	1399	1143	1231	1085	1188	1255	1131	914	14330
Percentage (%)	8.5%	8.2%	8.5%	9.6%	9.8%	8.0%	8.6%	7.6%	8.3%	8.8%	7.9%	6.4%	100.0%

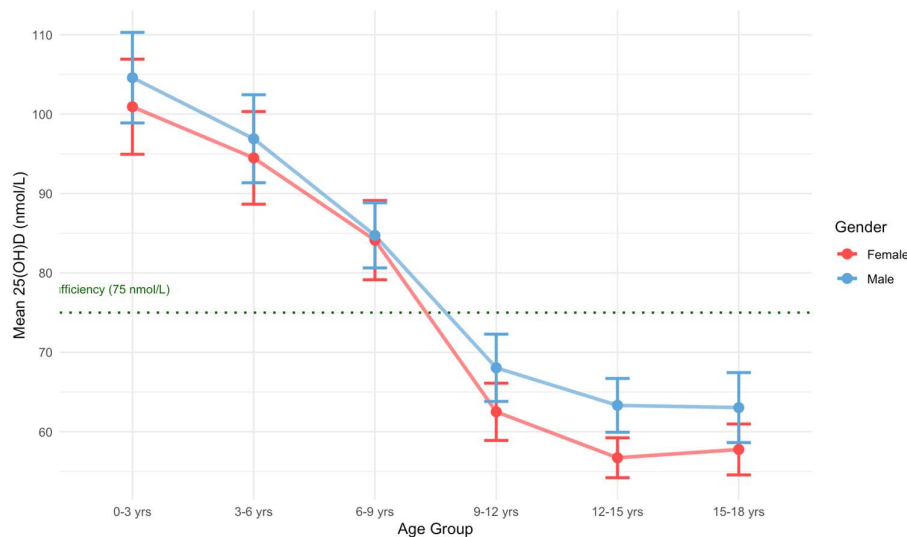


**Figure 3.** Monthly and Seasonal Distribution of Vitamin D Testing.

The biggest changes in vitamin D levels were recorded in patients under the age of 18. This is why a more precise analysis of this group was conducted. The pediatric analysis (Table 5) demonstrated a strong inverse relationship between age and Vitamin D serum levels. The highest concentrations were recorded in the 0–3 years group, where both females (99.8 nmol/L) and males (100.3 nmol/L) exhibited mean levels reaching the 100 nmol/L threshold. Conversely, the lowest concentrations were found in the 12–15 and 15–18 years cohorts, with female mean levels dropping to 55.1 nmol/L and 58.2 nmol/L, respectively. While Vitamin D levels remained comparable between genders in early childhood, a divergence became apparent in the 9–12 years group, where males maintained a higher mean (81.1 nmol/L) compared to females (73.6 nmol/L). Overall, median values showed a consistent decline from over 90 nmol/L in toddlers to approximately 53–57 nmol/L in adolescents (Figure 4).

**Table 5.** Vitamin D Serum Levels (nmol/L) Across Pediatric Age Groups and Gender.

Age Group	Gender	n	Median	Mean [95% CI]
0–3 yrs	Female	165	90.6	99.82 [92.1 – 107.4]
	Male	194	90.7	100.37 [94.3 – 106.4]
3–6 yrs	Female	264	87.6	99.08 [92.6 – 105.5]
	Male	312	86.6	102.27 [95.8 – 108.7]
6–9 yrs	Female	233	71.6	81.00 [76.1 – 85.8]
	Male	274	73.2	81.86 [77.2 – 86.5]
9–12 yrs	Female	302	63.3	73.62 [68.8 – 78.4]
	Male	232	72.4	81.19 [76.1 – 86.2]
12–15 yrs	Female	252	53.0	55.17 [52.2 – 58.0]
	Male	187	57.2	62.39 [58.8 – 65.9]
15–18 yrs	Female	264	53.8	58.29 [55.3 – 61.2]
	Male	174	57.0	62.49 [58.7 – 66.2]



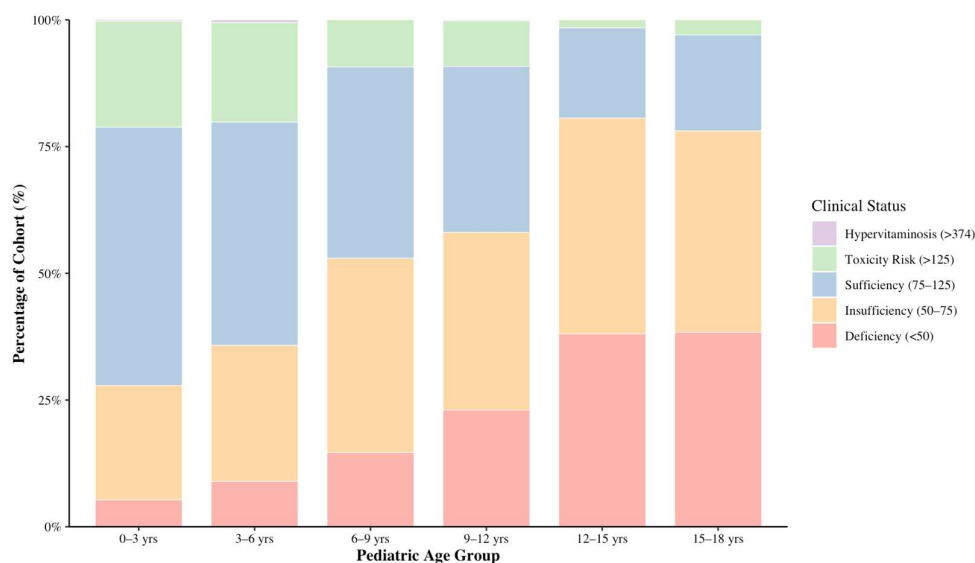
**Figure 4.** Vitamin D Serum Levels (nmol/L) Across Pediatric Age Groups and Gender.

The categorical analysis (Table 6; Figure 5) reveals a significant shift in Vitamin D status as children age, transitioning from a high prevalence of sufficiency and elevated levels in early childhood to widespread deficiency in adolescence. In the 0–3 years group, 51.0% of children achieved sufficiency, while a notable 20.9% were at elevated level. Similarly, the 3–6 years group maintained high levels, with 20.5% at elevated level.

However, as age increased, the proportion of children with adequate levels decreased sharply. By the 12–15 and 15–18 years age groups, deficiency (less than 50 nmol/L) and insufficiency (50–75 nmol/L) became the dominant clinical states, together affecting approximately 80% of those cohorts. Specifically, deficiency reached its peak in the 15–18 years group at 38.4%, while elevated level group fell to just 3.0% in the same age bracket. Hypervitaminosis remained extremely rare across all pediatric groups, occurring in 0.5% or less of the population.

**Table 6.** Vitamin D Status Prevalence and Clinical Categories by Pediatric Age Group.

Age Group	Deficiency	Insufficiency	Sufficiency	Elevated Level	Hypervitaminosis
0–3 yrs	19 (5.3%)	81 (22.6%)	183 (51.0%)	75 (20.9%)	1 (0.3%)
3–6 yrs	51 (8.9%)	153 (26.6%)	251 (43.6%)	118 (20.5%)	3 (0.5%)
6–9 yrs	74 (14.6%)	194 (38.3%)	191 (37.7%)	48 (9.5%)	0 (0.0%)
9–12 yrs	123 (23.0%)	187 (35.0%)	175 (32.8%)	48 (9.0%)	1 (0.2%)
12–15 yrs	167 (38.0%)	187 (42.6%)	78 (17.8%)	7 (1.6%)	0 (0.0%)
15–18 yrs	168 (38.4%)	174 (39.7%)	83 (18.9%)	13 (3.0%)	0 (0.0%)



**Figure 5.** Distribution of Vitamin D Status From Deficiency to Elevated Levels Across Pediatric Age Groups.

### 3.3. Vitamin D Distribution by Gender

The analysis of vitamin D status revealed a high level of consistency between genders, although female participants exhibited slightly higher central values (Table 7). The median 25(OH)D concentration for females was 68.9 nmol/L, which is 2.0 nmol/L higher than the median of 66.9 nmol/L observed in males. Both medians fall within the "Insufficiency" range (50–75 nmol/L) as defined by the Central and Eastern European Expert Consensus.

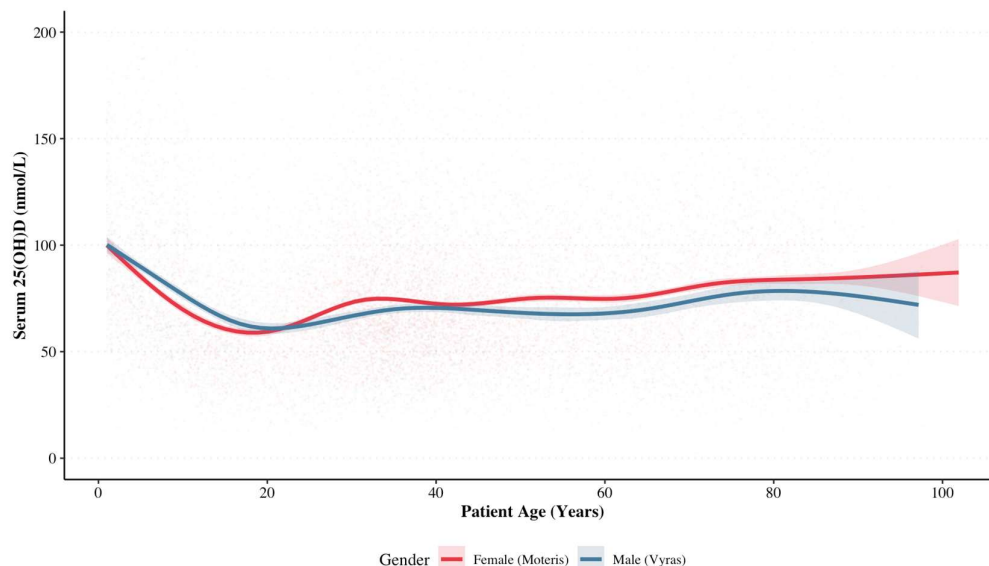
The mean values for females (74.9 nmol/L) and males (74.1 nmol/L) are numerically close, with a narrow 95% Confidence Interval for both groups. Males exhibited a higher Standard Deviation (SD = 37.8) compared to females (SD = 34.0), suggesting a broader spread of results and a higher frequency of extreme values within the male cohort.

In both groups, the mean is significantly higher than the median (a difference of 6.0 nmol/L for females and 7.2 nmol/L for males), confirming a right-skewed distribution driven by outliers with high vitamin D concentrations.

**Table 7.** Serum 25(OH)D Concentrations Stratified by Gender.

Gender	n	Mean	Median	SD	95% CI
Female	10359	74.9	68.9	34.0	74.2 – 75.5
Male	3970	74.1	66.9	37.8	72.9 – 75.3

The oldest cohort (Q4) exhibited the highest levels, with a mean of 79.06 nmol/L (95% CI [77.9 – 80.2]) and a median of 73.6 nmol/L. Conversely, the lowest concentrations were recorded in the young adult cohort (Q2), which presented a mean of 70.8 nmol/L (95% CI [69.86 – 71.8]) and a median of 65.9 nmol/L. These results indicate a non-linear distribution where serum Vitamin D levels are highest in the youngest and oldest population segments and reach their nadir in the young adult group (Figure 6).



**Figure 4.** Vitamin D Mean Level Distribution by Age and Gender.

The study population of 14,330 participants was stratified into eight distinct cohorts (Table 8) based on gender and age quartiles (F1–F4 and M1–M4). Age divisions were established using the population's interquartile range: Q1 included individuals up to 24 years, Q2 and Q3 spanned from 24 to 54 years, and Q4 represented those above 54 years.

### 3.4. Vitamin D Distribution by Seasonality

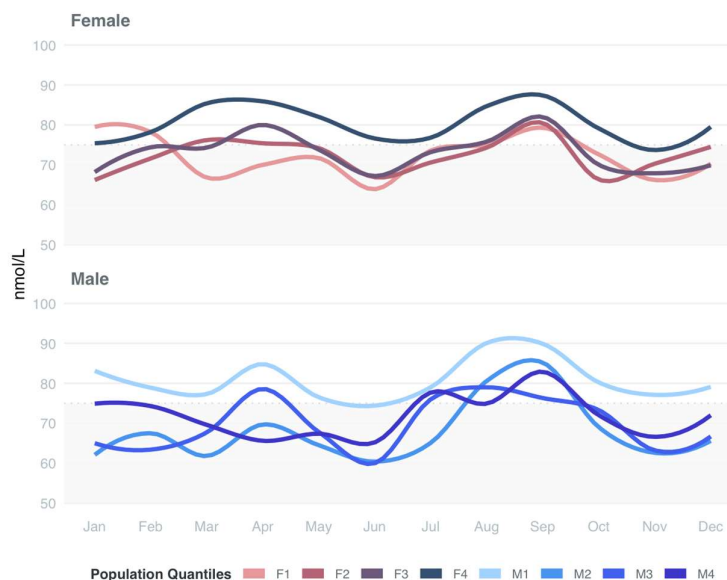
To make a more precise analysis of the vitamin D distribution, the evaluation of seasonality was performed. Seasonal Vitamin D dynamics follow a distinct biphasic pattern (Figure 7). The primary peak occurs across all groups during August and September, reaching maximum concentrations in the M1 (90.1 nmol/L) and F4 (87.4 nmol/L) cohorts. A secondary, less pronounced elevation is observed in mid-spring (April), followed by a transient decline in May and June.

Troughs are most evident during the winter months and the start of summer. Middle-age groups (M2, M3) show the most significant fluctuations, dropping to 60.0–60.4 nmol/L in June. While older cohorts and youngest males (F4, M1) maintain higher baselines, they follow the same trend: an April rise, a June dip, and a definitive annual peak in late summer.

**Table 8.** Monthly Vitamin D Distribution (nmol/L).

Group	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
F1	79.5	78.2	66.8	70.0	71.7	63.9	73.6	75.1	79.3	72.6	66.2	70.3
F2	66.2	71.6	76.2	75.4	74.2	66.9	70.6	74.3	80.5	66.3	70.3	74.5
F3	68.2	74.4	74.3	80.0	73.8	67.2	73.2	75.8	82.0	70.0	67.9	69.9
F4	75.4	78.2	85.4	85.9	82.0	76.6	76.8	84.7	87.4	79.1	73.7	79.5
M1	83.1	78.9	77.3	84.8	76.5	74.4	79.0	90.1	89.9	80.2	77.1	79.1
M2	62.1	67.5	61.8	69.7	64.5	60.4	65.1	80.6	85.2	69.0	62.6	65.4
M3	65.0	63.4	67.7	78.6	67.8	60.0	76.0	79.0	76.3	73.3	63.2	66.7
M4	74.9	74.3	69.6	65.6	67.4	65.2	77.7	74.9	82.9	72.0	66.6	72.0

\*The prefix F identifies Female groups, M identifies Male groups, and indices 1–4 indicate the respective population age quantiles from lowest to highest.



**Figure 7.** Comparative Analysis of Vitamin D Distribution by Gender and Quantile.

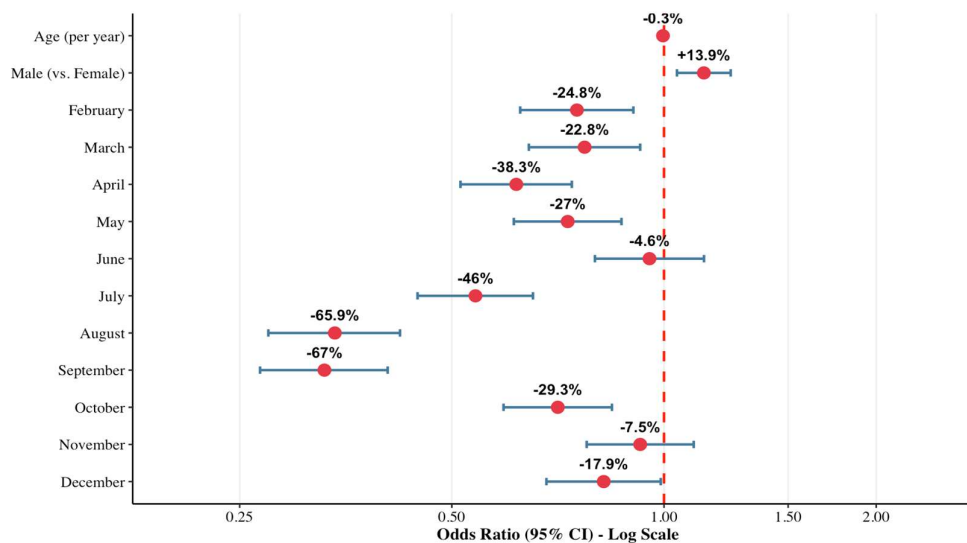
### 3.5. Multivariate Logistic Regression

To validate the primary analysis, a multivariate logistic regression models were created. The models (Table 9) identified age, gender, and seasonality as significant predictors of Vitamin D deficiency. Gender emerged as a notable risk factor, with males exhibiting a 13.9% higher likelihood (OR 1.140;  $p=0.0036$ ) of falling into the deficiency range compared to females. Age showed a statistically significant but negligible protective effect (OR 0.997;  $p<0.001$ ).

Seasonality exerted the most substantial impact on risk levels. September and August provided the highest protective effect (Figure 8), reducing risk by 67% and 65.9% respectively. A secondary period of significant risk reduction occurred in July (-46%) and April (-38.3%). In contrast, months such as June and November showed no statistically significant change in risk. The data confirms that while biological factors like gender are influential, the magnitude of risk is predominantly driven by seasonal variation.

**Table 9.** Multivariate Logistic Regression Analysis of Factors Associated with Vitamin D Deficiency.

Factor	Odds Ratio (OR)	95% CI	Risk Change (%)	p-value
Age (per year)	0.997	0.995 – 0.998	-0.3%	< 0.001
Male	1.140	1.040 – 1.240	+13.9%	0.0036
February	0.752	0.625 – 0.905	-24.8%	0.0025
March	0.772	0.643 – 0.925	-22.8%	0.0052
April	0.617	0.514 – 0.740	-38.3%	< 0.001
May	0.730	0.612 – 0.870	-27.0%	< 0.001
June	0.954	0.798 – 1.140	-4.6%	0.6013
July	0.540	0.447 – 0.652	-46.0%	< 0.001
August	0.341	0.275 – 0.422	-65.9%	< 0.001
September	0.330	0.267 – 0.405	-67.0%	< 0.001
October	0.707	0.592 – 0.844	-29.3%	< 0.001
November	0.925	0.777 – 1.100	-7.5%	0.3832
December	0.821	0.681 – 0.989	-17.9%	0.0387



**Figure 8.** Odds Ratios for Factors Influencing Vitamin D Status Based on Multivariate Logistic Regression Analysis.

## 4. Discussion

### 4.1. Vitamin D Status Across Age groups

In our study, 25(OH)D concentrations varied substantially across age groups, with the highest mean levels observed in the youngest (0 - 3 years: ~100 nmol/L) and oldest (Q4, ≥54 years: 79.1 nmol/L) groups, and the lowest in adolescents aged 15 - 18 years. Age is a well-established determinant of vitamin D status, and the European Calcified Tissue Society has identified young children, adolescents, and older adults as key risk groups [14]. In a pooled analysis of standardized data from over 55,000 Europeans spanning all age groups, Cashman et al. reported an overall prevalence of vitamin D deficiency (<30 nmol/L) of 13%, with considerable variation by age group and latitude [12]. However, direct comparison of age-stratified data across studies is complicated by substantial heterogeneity in age group classification. For instance, in Lithuanian studies Bleizgys and Kurovskij employed broad decade-wide groupings, Butkute et al. divided children only into those under and over 2 years, while European studies used varying pediatric categories, for example, 0 - 1, 2 - 5, 6 - 10, 11 - 15, 16 - 20, 21 - 30, 31 - 40 and etc. years in the Czech cohort, 0 - 1, 1 - 6, 7 - 12, 13 - 18 years in the Belgian cohort [10,18,24,25]. Similarly, adult and elderly cohorts range from ≥50 to ≥70 years in different studies [10,26–29]. Despite these methodological differences, the literature consistently points to two vulnerable age periods: young children, who tend to have the highest concentrations largely driven by supplementation practices, and adolescents, who show the steepest rise in deficiency prevalence. Among older adults, findings are more heterogeneous, with some studies reporting increasing concentrations with age in supplement-using populations (26), while others document high deficiency rates in the institutionalized elderly [19,26,29]. We examine these patterns below in two subgroups: children and older adults, where the clinical implications are most pronounced.

#### 4.1.1. Vitamin D Status in Pediatric Age Groups

Our study revealed that the highest 25(OH)D concentrations were recorded in the 0 - 3 years age group, where both females (99.8 nmol/L) and males (100.3 nmol/L) exhibited mean levels approaching the 100 nmol/L threshold. A similar pattern was observed in the 3 - 6 years age group, with mean concentrations of 99.08 nmol/L for females and 102.27 nmol/L for males. These findings are consistent with those of Bleizgys and Kurovskij, who reported that children aged nine years and

younger had the highest mean 25(OH)D levels (113.3 nmol/L) [10]. In contrast, in the largest Lithuanian pediatric cohort to date, Butkute et al. found that only 29.0% of children under 2 years of age and 19.7% of children over 2 years had optimal vitamin D levels [18].

Consistent with the broader literature, our findings indicate that young children are at greater risk of elevated 25(OH)D concentrations. In our cohort, 20.9% of children in the 0 - 3 years age group had elevated 25(OH)D concentrations. Similarly, the 3 - 6 years age group maintained a comparable prevalence of elevated levels (20.5%), although hypervitaminosis D remained extremely rare across all pediatric age groups, occurring in only 0.5% of cases. Other Lithuanian pediatric cohorts have reported similar results. Butkute et al. found that 52.1% of children under 2 years of age had increased vitamin D concentrations. Overall, increased concentrations (125 - 250 nmol/L) were found in 20.9% of cases, extremely high concentrations (250 - 500 nmol/L) in 4.0%, and toxic concentrations (>500 nmol/L) in 0.3% of all children [18]. In the cohort analyzed by Bleizgys and Kurovskij, 72 cases had 25(OH)D levels greater than 250 nmol/L, of whom 71 were younger than 1 year of age [10].

Severe vitamin D deficiency manifests as rickets in children. In most European countries, including Lithuania, infant vitamin D supplementation of 400 IU/day is now well established for rickets prophylaxis, which may partly account for the high 25(OH)D concentrations observed in the 0 - 3 years age group in our study [30,31].

Regarding 25(OH)D concentrations in children across other European countries, studies from Ukraine, the Czech Republic, Belgium, and Romania demonstrate similar patterns: infants and children up to 5 - 6 years of age are at the lowest risk of vitamin D deficiency, although elevated 25(OH)D concentrations are relatively common in these age groups, while hypervitaminosis remains rare [24,25,32,33]. A recent Ukrainian pediatric study reported no cases of vitamin D deficiency in children aged 1 - 2 years, however, toxic levels were recorded in 4.2% of children in this age group (95% CI: 1.4 - 9.6) [32]. A large Czech cohort study revealed that 65.6% of infants had sufficient 25(OH)D levels, with a slightly higher prevalence of sufficiency among females (65.8%). Hypervitaminosis was very rare, with 25(OH)D concentrations exceeding 250 nmol/L occurring in only 0.1% of infants aged 0 - 1 year [34]. In a large Belgian cohort, only 23.2% of children in the youngest age group had insufficient vitamin D levels, while 22.3% had severely deficient levels [25]. A Romanian cohort reported a vitamin D deficiency prevalence of 11% among toddlers and preschoolers aged 2 - 5 years [33].

The majority of available evidence indicates that vitamin D deficiency rates among children increase with age, beginning in those older than 6 - 7 years, with the highest deficiency rates observed in adolescent cohorts [10,25,32-34]. Our study confirmed this pattern: by the 12 - 15 and 15 - 18 years age groups, deficiency (<50 nmol/L) and insufficiency (50-75 nmol/L) together constituted the dominant clinical categories, affecting approximately 80% of individuals in those cohorts. A similar pattern was observed in another Lithuanian cohort, where Bleizgys and Kurovskij found that participants aged 10 - 19 years had significantly lower mean 25(OH)D levels than other age groups ( $p < 0.05$ ) [10]. In the Belgian cohort, the combined prevalence of vitamin D insufficiency and deficiency was highest among children aged 7 - 12 years (24.9%) and 13 - 18 years (20.7%) [25]. In the Romanian cohort, vitamin D deficiency was reported in 33% of children aged 6 - 11 years and 39% of those aged 12 - 18 years [33]. In the Czech cohort, the lowest prevalence of sufficiency was observed in the 6 - 15 years age group (19.2%) [34]. The progressive decline in 25(OH)D concentrations with increasing age likely reflects, at least in part, the discontinuation of routine vitamin D supplementation after infancy, combined with reduced parental oversight of health behaviors during adolescence.

Beyond supplementation, other modifiable factors influence pediatric vitamin D status. A large European multi-country study combining samples from eight countries determined that the principal determinants of childhood 25(OH)D concentrations were UVB, time spent outdoors, and dietary vitamin D intake, while concentrations decreased modestly with increasing BMI and age. Notably, children who spent one additional hour per day outdoors had a 21% higher probability of non-deficient status compared with their peers [17].

#### 4.1.2. Vitamin D Status in Older Adults

In our cohort, the oldest age group (Q4,  $\geq 54$  years) exhibited the highest 25(OH)D levels, with a mean of 79.06 nmol/L (95% CI: 77.9 - 80.2) and a median of 73.6 nmol/L. This finding stands in contrast to other Lithuanian studies that included elderly populations. In the study by Strazdiene et al., the lowest vitamin D levels were found in persons aged 80 years and older, particularly among women. 72.2% of women and 65.6% of men were classified as vitamin D deficient [19]. In the cohort analyzed by Bleizgys and Kurovskij, individuals older than 50 years did not differ significantly from other age groups, although there was a modest trend toward lower 25(OH)D concentrations with advancing age: median concentrations were 54.4 nmol/L in the 50 - 59 years group, 52.9 nmol/L in the 60 - 69 years group, and 46.7 nmol/L in the  $\geq 70$  years group [10].

Findings from other European studies present a varied picture, although direct comparison of prevalence rates is limited by differences in the cutoff values used to define deficiency and insufficiency across studies. In the Dutch study by Verbakel et al., results were consistent with our findings, as vitamin D concentrations increased with age. Women had higher median 25(OH)D concentrations than men in both the 50 - 69 years group (76.5 vs. 50.6 nmol/L) and the  $\geq 70$  years group (85.9 vs. 73.0 nmol/L). In the oldest group, only 13% of women and 26% of men were classified as deficient [26]. In a cohort of older adults aged  $\geq 65$  years in Great Britain, 54.8% were classified as having insufficient 25(OH)D levels [27]. In a large Irish cohort, the prevalence of deficiency (25(OH)D  $< 30$  nmol/L) was considerably lower at 13.1% (95% CI: 12.1 - 14.2) [28]. In an Austrian cohort of nursing home residents, 22.2% had vitamin D deficiency and 17.4% had insufficient levels, with a combined prevalence of inadequate vitamin D status of 39.5% [29].

Several European studies have examined risk factors for vitamin D deficiency in older adults. Laird et al. found that deficiency was more prevalent among non-supplement users, during winter months, in smokers, in obese individuals, in the physically inactive, in those living alone, and in the oldest old ( $> 80$  years). The main predictors ( $p < 0.05$ ) of 25(OH)D concentration were supplement use (coefficient: 27.2 nmol/L; 95% CI: 15.3 - 39.2), smoking (- 8.9; 95% CI: - 12.6 to - 5.2), summer season (5.9; 95% CI: 2.7 - 9.1), and obesity (- 4.0; 95% CI: - 6.3 to - 1.7) [28]. Verbakel et al. reported that older age, female sex, and lower BMI were significantly associated with higher vitamin D status [26]. In the EUREYE study, a large multi-country cohort spanning seven countries from northern to southern Europe, multivariable analysis revealed that 25(OH)D concentrations were significantly lower ( $p < 0.05$ ) in smokers and participants with self-reported diabetes, and significantly higher with increasing dietary vitamin D intake and supplement use, including fish liver oil, omega-3 fatty acids, and vitamin D supplements. Additionally, 25(OH)D concentrations were positively associated with higher intakes of oily fish and greater UVB exposure [35].

It should be noted that our Q4 group encompasses a broader age range than many of the comparison cohorts discussed below, which focused on populations aged  $\geq 65$  or  $\geq 70$  years. This difference may partly contribute to the higher mean concentrations observed in our study, as the inclusion of younger, more active individuals aged 54 - 65 years likely shifts the group mean upward.

#### 4.2. Sex Differences in Vitamin D Status

In our cohort, females exhibited a modest, but statistically significant higher median 25(OH)D concentration compared to males (median difference was +2 nmol/L). In multivariate logistic regression analysis, male sex was associated with an increased likelihood of vitamin D deficiency with 13.9% higher odds of falling into the deficiency range (OR 1.140;  $p = 0.0036$ ). Notably, our results contrast with those reported in previous Lithuanian population-based cohort in which men demonstrated significantly higher mean 25(OH)D concentrations than woman ( $81.0 \pm 55.7$  nmol/L vs.  $64.8 \pm 41.6$  nmol/L, respectively,  $p < 0.001$ ) [10].

Our findings contrast with several European population-based cohorts. In the German national survey analyzed by Rabenberg et al., no statistically significant sex differences in serum 25(OH)D concentrations were observed [36]. Similarly, Palaniswamy et al. reported no significant gender differences in a Finnish population cohort [37]. In contrast, data from the Dutch cohort analyzed by

Verbakel et al. demonstrated higher mean 25(OH)D concentrations among males [26]. Ukrainian population data likewise showed no statistically significant sex differences in adults. However, among adolescents, females exhibited significantly higher serum 25(OH)D levels. Notably, in the Ukrainian cohort comprising 11,462 participants, the prevalence of severe vitamin D deficiency was higher in males (4.5%; 95% CI: 3.7 - 5.3) compared with females (2.4%; 95% CI: 2.1 - 2.7 [32]. Consistent with our results, a large Czech study by Holmannova et al. including 119,925 individuals reported a significant negative association between male sex and 25(OH)D concentrations ( $p < 0.001$ ) [24].

Several factors may explain this discrepancy. First, differential supplementation behavior is likely influential. Women are more engaged in preventive health practices, including vitamin D use. Holmannova et al. reported that females had 2.47 - fold higher odds of regular supplementation and 1.82 - fold higher odds of irregular supplementation compared with males [34]. Such patterns may result in systematically higher circulating 25(OH)D concentrations among women in ambulatory cohorts. Second, the marked sex imbalance in our sample (72.3% female vs. 27.7% male) may introduce selection and indication bias, as women are more likely to undergo routine biochemical testing. Third, residual confounding cannot be excluded. Sex-specific differences in BMI, adiposity distribution, alcohol consumption, smoking prevalence, occupational exposure, and habitual sun exposure-all established determinants of vitamin D status-may influence circulating 25(OH)D concentrations. In addition, hormonal factors may contribute. In the cohort analyzed by Palaniswami et al., female oral contraceptive users exhibited approximately 10% higher mean 25(OH)D concentrations compared with non-users, suggesting that exogenous estrogen exposure may modulate vitamin D metabolism [37]. Collectively, these factors indicate that the observed sex differences are likely multifactorial and context-dependent rather than purely biological.

#### 4.3. Seasonal Variation

Lithuania is located at approximately 54 - 56°N latitude, within the "vitamin D winter" zone where UVB radiation is insufficient for cutaneous vitamin D synthesis from roughly October through March [11]. Pan-European pooled data confirm a clear seasonal divide, with 17.7% of Europeans having 25(OH)D below 30 nmol/L in winter versus 8.3% in summer [12]. Lithuanian data are consistent: Bleizgys and Kurovskij reported the nadir in January - April and peak in August - September [10]. In the paediatric population, Butkute et al. while younger children showed no significant seasonal variation - likely reflecting routine supplementation - children over 2 years had significantly lower vitamin D concentrations in spring, indicating that once supplementation ceases, the same seasonal dependency seen in adults emerges rapidly [18].

Our results are broadly consistent with these prior Lithuanian findings. The primary 25(OH)D peak occurred in August - September across all cohorts, reaching 90.1 nmol/L (M1) and 87.4 nmol/L (F4), with corresponding maximum deficiency risk reduction of 67.0% and 65.9%, respectively. A secondary April elevation was observed, followed by a transient May - June decline most pronounced in middle-aged groups M2 and M3 (60.0 - 60.4 nmol/L). The spring dip in older children reported by Butkute et al. mirrors this pattern - a vulnerable transitional period when winter stores are depleted but sustained UVB-driven synthesis has not yet commenced [18].

Meteorological conditions in 2025 could modulate these patterns. According to the Lithuanian Hydrometeorological Service, April was the sunniest month of the year, while overall winter and summer recorded 9% and 16% fewer sunshine hours than normal, respectively. January had only 19 hours of sunshine (53% below the climatological norm) [38]. The unusually sunny April likely enabled earlier cutaneous synthesis - the UVB production threshold at Lithuanian latitudes is crossed in late March - April - while below-average summer sunshine may explain the May - June dip, particularly in employed middle-aged cohorts with limited midday outdoor exposure [11]. The absence of statistically significant risk reduction in both June and November is consistent with this interpretation - the former reflecting attenuated early-summer UVB, the latter the cessation of vitamin D-effective radiation at these latitudes.

Seasonal variation in vitamin D status has been consistently demonstrated across Europe. In the Netherlands, Lifelines cohort data showed marked monthly fluctuation, with near-zero deficiency (0 - 5%) between May and October, but 12–17% deficiency in late winter. Median 25(OH)D concentrations in March rose significantly between 2011 and 2023 (from 45 - 49 to 54 - 75 nmol/L), likely reflecting increased supplement use [26]. In Slovenia, 63.4% of non-supplementing adults were insufficient (<50 nmol/L) in winter versus only 5.5% in summer, though half still had suboptimal concentrations (<75 nmol/L) even in the warm season [39]. Central European longitudinal data showed wintertime mean 25(OH)D of 20 - 23 ng/mL across all ages, with the summer increase strongly age-dependent - 20 ng/mL in children but only 5 - 6 ng/mL in octogenarians [40]. A Czech study confirmed the same pattern, with levels decreasing during winter and early spring [24]. Among Belgian children (n = 14,887), only 28.8% had sufficient levels (>30 ng/mL), with the lowest status at end of winter when up to 77% fell below the sufficiency threshold [25]. Pan-European pooled estimates place the overall picture in context: 17.7% of Europeans have 25(OH)D below 30 nmol/L in winter versus 8.3% in summer [12].

## 5. Conclusions

In this retrospective cross-sectional study of 14,330 individuals - the largest clinic-based assessment of vitamin D status in Lithuania to date - the overall median serum 25(OH)D concentration was 68.3 nmol/L, falling within the insufficiency range by Central and Eastern European consensus criteria.

Seasonality was the strongest determinant of deficiency risk, with August - September associated with up to 67% reduced odds compared to January, while June and November showed no significant protective effect - underscoring that even nominally warm months do not reliably support adequate vitamin D synthesis at Lithuanian latitudes. Male sex was independently associated with modestly higher odds of deficiency (OR 1.14; p = 0.004), potentially reflecting differences in supplementation behavior rather than biology alone. Age effects were non-linear: children aged 0 - 6 years exhibited the highest concentrations, likely attributable to infant supplementation policies, while adolescents aged 12 - 18 years had the poorest status, with deficiency and insufficiency affecting approximately 80% of this group.

These findings highlight adolescents as a priority population for targeted supplementation interventions beyond early childhood and support the rationale for routine October - March supplementation for the general Lithuanian population. Future prospective, multi-center studies incorporating data on supplementation use, BMI, dietary intake, and sun exposure are warranted to further clarify the contributions of these modifiable factors.

## 6. Limitations of the Study

Several limitations of this study should be noted. First, the retrospective cross-sectional design means we can only show associations, not prove cause and effect. Second, our data did not include information on lifestyle factors that influence Vitamin D, such as diet, Body Mass Index (BMI), skin type, or the specific amount of time spent in the sun. Third, because we used laboratory records, we could not track whether participants were taking over-the-counter Vitamin D supplements before their blood test. While we removed duplicate tests to focus on baseline levels, some patients might have already started supplementing, which could skew the results. Fourth, since the data comes from a single clinic in Vilnius, the findings may not represent the entire country or people living in rural areas. Finally, because the study covers only one year, we cannot account for weather changes between different years that might affect sun exposure.

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**Informed Consent Statement:** Informed consent was not required as the data provided was anonymized.

**Data Availability Statement:** The dataset supporting the conclusions of this publication is proprietary and will not be publicly shared. However, additional information regarding the study methodology and analysis can be provided upon reasonable request to the corresponding author.

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