*Article*

# Vitamin D Status in a Rural Italian Population

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**Abstract:** Vitamin D is known as an antirachitic factor, although it also plays a critical role in several nonskeletal diseases. In our study, we evaluated vitamin D status and sex, age and seasonal association in a general population cohort living in central Italy. Data from 1174 men and 2274 women aged 20–81 were analyzed, and stored serum samples were assayed for 25-hydroxyvitamin D (25(OH)D). Vitamin D was low in both sexes with values significantly lower in women than in men; furthermore, its deficiency was highly correlated with age. The younger men had just sufficient 25(OH)D levels (32.3 ng/mL ± 13.2), which decreased with increasing age. The younger women showed insufficient 25(OH)D levels (24.8 ng/mL ± 11.9) that, as with men, further decreased with increasing age. This study demonstrated that hypovitaminosis D may be a very frequent condition also in a rural central Italian area with remarkable solar irradiation throughout the year. Our data clearly indicated an evident seasonal trend: at the end of the winter, serum 25(OH)D levels of the examined cohort were below the official sufficient value for both adult sexes. Sufficient levels were just reached in summer for men and only at the end of summer for young women.

**Keywords:** vitamin D; 25-hydroxyvitamin D; aging; seasonal variation

## 1. Introduction

The main biochemical forms of vitamin D are: vitamin D2 (ergocalciferol), which is found naturally in vegetal foods, and vitamin D3 (cholecalciferol), which is synthesized in the skin by exposure to ultraviolet B light and is also found in some foods of animal origin.

The major sources of vitamin D in humans are cutaneous UVB photosynthesis of vitamin D3, followed by dietary intake from foods that naturally contain animal vitamin D3 or vegetal vitamin D2. In addition, the use of fortified foods and vitamin D supplements could represent other possible sources of vitamin D in some populations.

Vitamin D (D2, D3) has no biological activity without a two-step hydroxylation process. The first step, in the liver, leads to the major circulating form—25-hydroxyvitamin D (25(OH)D)—while the second step, in the kidneys, forms the active metabolite 1,25(OH)D (calcitriol) [1]. However, recent studies provide new insight into vitamin D metabolism and its mechanism of action [2]. Specifically, vitamin D can also be activated in the skin [3]; therefore, alternative pathways of its activation by CYP11A1 have been described, producing hydroxyderivatives that are biologically active in the body [4–10].

Vitamin D, directly or indirectly, controls the expression of more than 200 genes, such as the Epidermal Growth Factor receptor, Phospholipase C, Gamma 1, Insulin Growth Factor binding protein 3 and many more that are responsible for the regulation of cellular proliferation, differentiation, apoptosis, and angiogenesis [11].

In humans, vitamin D is well known for its role in calcium and phosphate homeostasis and its clinical properties have been most extensively studied as an antirachitic factor, including its effects on intestinal and renal handling of mineral ions and regulation of osteoblast activity [12]. However, in recent years, more and more of the literature suggests that vitamin D may also be a critical factor for several nonskeletal system and related diseases, such as cardiovascular dysfunction, diabetes, cancer, immune function and some chronic diseases associated with aging [13,14].

Clinical evidence indicates that low circulating levels of vitamin D may be associated with increased risk of cardiovascular diseases, including coronary heart disease, stroke and mortality for major cardiovascular events [15,16]. Some data suggest that vitamin D deficiency is associated with the progression of heart failure (HF) and may be an independent predictor of mortality in HF patients [17]. However, despite experimental evidence suggesting a role of vitamin D in cardioprotection, a cause–effect relationship has not yet been established; mechanisms have been hypothesized in the suppression of parathyroid hormone modulation, the renin–angiotensin axis, vascular smooth-muscle function, nitric oxide or immune function [18]. Statin therapies have been shown to reduce major cardiovascular events but, despite being well-tolerated, their use has been associated with myopathy [19] and some studies supported a possible interaction between statin use and vitamin D status, although it is unclear how statins interact with vitamin D [20].

Hypovitaminosis D has also been identified as a common feature between diseases widely diffused in senescence such as osteoporosis, sarcopenia (a condition characterized by both reduced muscle mass and muscle strength "dynapenia"), and cognitive impairment. It has been suggested that hypovitaminosis D is one of the causative factors [21]. In elderly, hypovitaminosis D is mainly due to the reduced ability of the skin to synthetize cholecalciferol from its precursor, 7-dehydrocholesterol. Together with a reduced synthesis of vitamin D, older subjects show a reduced expression of vitamin D receptors (VDRs). These two phenomena cooperate in the amplification of the effect of hypovitaminosis D during aging [22]. Although a relationship between low vitamin D levels, sarcopenia and dementia is well known, interventional studies disagree in recommending vitamin D supplementation to efficiently treat these two diseases [10,23].

Furthermore, more recently, it has been suggested that deficient vitamin D status is associated with increased coronavirus disease 2019 (COVID-19) infection risk [24], and more severe systemic inflammatory response with higher pulmonary involvement and respiratory failure in COVID-19-affected patients [25,26].

The most representative index of vitamin D status is the serum 25(OH)D concentration. In fact, serum 25(OH)D level reflects the total stored quantity derived from both endogenous and exogenous sources and has a long half-life. The most current guidelines classified vitamin D status into: deficient (25(OH)D < 20 ng/mL), insufficient (25(OH)D < 30 ng/mL), sufficient (25(OH)D ≥ 30 ng/mL) or toxic (25(OH)D ≥ 150 ng/mL) [27].

Therefore, 25(OH)D levels < 20 ng/mL are considered inadequate for maintaining bone health; however, a consensus is still to be reached regarding an optimal level for maintaining nonskeletal health and achieving possible cardiovascular benefits [12].

Vitamin D supplementation can be used to prevent a decrease in circulating 25(OH)D concentrations below 30 ng/mL if skin synthesis of vitamin D is low or absent. However, several studies suggested health benefits for some diseases and only if vitamin D supplementation was administered to vitamin-D-deficient populations; on the contrary, few benefits were found when supplementation was given to vitamin-D-sufficient/insufficient populations [28]. Although the main source of vitamin D is sun exposure, other factors, such as age, gender, skin color, fat mass, and lifestyle, are relevant in vitamin D intake [29].

In this study, we evaluated the vitamin D status of an Italian population cohort living in four adjacent rural towns of central Italy in order to investigate the serum 25(OH)D content by sex, age classes and seasonality at this latitude and to assess possible critical deficiencies.

It is noteworthy that we analyzed a cohort of men and women enrolled between 1993 and 1996, a period when, in a rural Italian population food supplemented with vitamin D was generally not consumed and the consumption of vitamin D supplements was unusual.

## 2. Materials and Methods

### 2.1. Study Population

Baseline data of the Malattie Cardiovascolari Aterosclerotiche, Istituto Superiore di Sanità (MATISS) Project were collected from 1993 to 1996 in men and women, aged 20–81 years, randomly selected from the Italian general population stratified by age and sex [30,31]. The cohort was extracted from the electoral rolls of a geographical area located about 100 km southeast of Rome (central Italy) and comprising four adjacent rural towns (Sezze, Roccagorga, Bassiano, and Priverno). The MATISS study was approved as part of the CUORE Project by Ethical Committee of the Institute Superiore di Sanità (ISS, the Italian National Institute of Health) on 15 March 2006; the Ethical Committee approved the use of pooled samples for research activity in epidemiological and genetic/genomic studies. People were invited by letter at the screening center.

Participation rate was 60%. Personal data are stored and managed according to the Italian legislative Decree 196/03 and the Code of Conduct to manage personal data for statistic and scientific purposes (2004). Personal data are excluded from the database used for statistical analyses that, instead, includes a unique code for each person. The same codes were used to label the serum samples collected at the study baseline that were preserved in nitrogen vapors and stored in the biological bank of the Cardiovascular, and Endocrine-Metabolic Diseases, and Aging Department of ISS.

### 2.2. Measurement of 25-Hydroxyvitamin D Concentrations and Risk Factors

The MATISS cohort is part of the MORGAM (MOnica Risk, Genetics, Archiving and Monograph) and BiomarCaRE (Biomarker for Cardiovascular Risk Assessment in Europe) international projects. The BiomarCaRE consortium is an EU-funded consortium including over 30 partners from academia and industry [32]. BiomarCaRE aims to determine the value of established and emerging biomarkers to improve risk estimation of cardiovascular disease in Europe. Within the BiomarCaRE consortium, a large panel of biomarkers was assessed in the MATISS Study to improve disease prediction among different European populations. Among the panel, stored serum samples of 1174 men and 2274 women were thawed and assayed for 25(OH)D concentration at the BiomarCaRE laboratory in Germany between February and May 2016.

25(OH)D was measured using the Abbott ARCHITECT i2000 system (Abbott Diagnostics, Abbott Park, IL, USA) by a chemiluminescent microparticle immunoassay with an assay range of 8–160 ng/mL [33]. The intra-assay coefficient of variation (CoV) was 4.51% and inter-assay CoV 2.87%. The assay is highly sensitive to natural 25(OH)D3.

Risk factors were collected using standardized procedures published elsewhere [34,35].

### 2.3. Statistical Analysis

Mean, standard deviation (SD), and 95% confidence interval of 25(OH)D serum levels were assessed by sex, by sex and age group (20–29, 30–39, 40–49, 50–59, 60–69 and 70–81 years), and by sex and month of the year when serum sample was collected. Mean, SD and 95% confidence interval of 25(OH)D serum levels were also assessed by sex, age group and month of the year in combination; in this case, in order not to incur an excessive fragmentation of the sample size in each stratum, age groups were aggregated into 20–39, 40–59 and 60–81 years. In consideration of the central limit theorem, confidence intervals for continuous variables were reported only for strata with more than 30 persons, as well as the assessment of t-test to compare sex and age groups. Values of 25(OH)D serum levels

are reported as both crude and age-standardized values using the direct method, referring to the age- and sex-specific distributions of the 1994 Latina 20–81 years old population (Italian National Institute of Statistics—ISTAT). The values of 25(OH)D serum levels were stratified into four subgroups: deficiency (<20 ng/mL), insufficiency (<30 ng/mL), sufficiency (≥30 ng/mL) and toxic (≥150 ng/mL); prevalence and 95% confidence interval were assessed by sex. Age-standardized cardiovascular risk factor levels and risk conditions were assessed by 25(OH)D classes (<20 ng/mL, <30 ng/mL, ≥30 ng/mL) and sex showing means, SD, prevalence and 95% confidence intervals. Statistical analyses were performed using IBM SPSS Statistics (IBM, New York, USA) and SAS software, release 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

## 3. Results

Blood serums from 1174 men and 2274 women 20–81 years aged (mean age was 53 years both in men and women) were analyzed for their 25(OH)D levels. The mean serum concentrations of 25(OH)D in men and women were $24.1 \pm 12.6$ and $17.2 \pm 10.0$, respectively; despite low levels in both sexes, vitamin D levels were significantly lower in women than in men ($p < 0.0001$) (Figure 1 and Tables 1 and 2). Overall, 68.6% of women and 40.6% of men showed a deficient vitamin D status; the insufficient status was observed in 22% of women and 30.2% of men, while only 9.4% of women and 29.2% of men had sufficient 25(OH)D levels. Furthermore, one woman showed a toxic status.
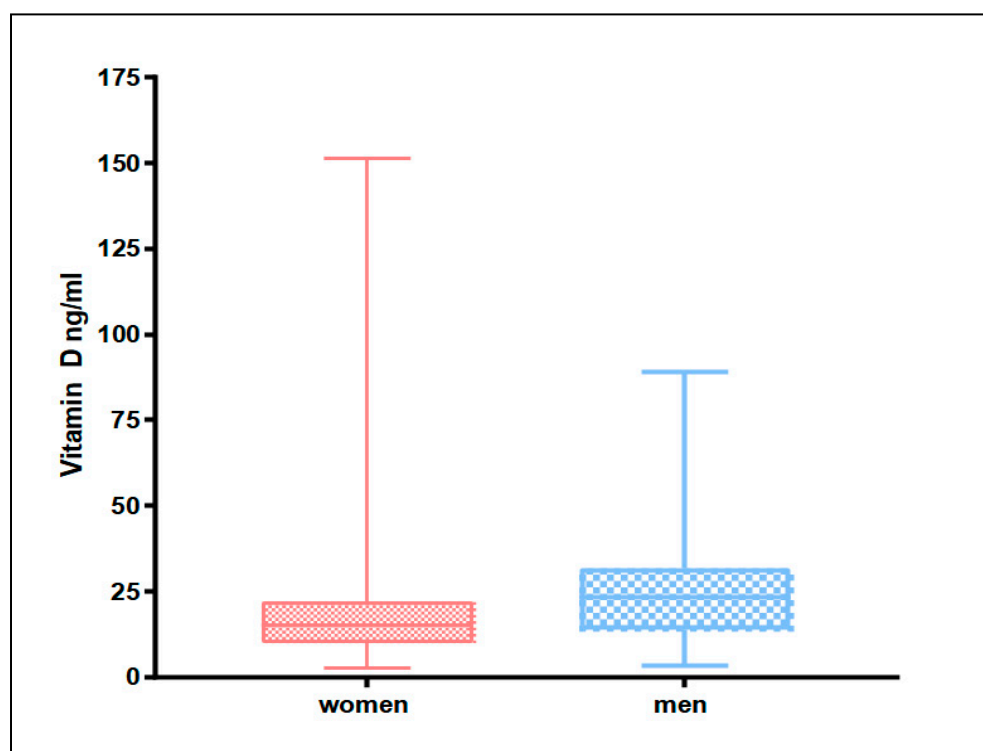


**Figure 1.** Vitamin D (25(OH)D) serum level distribution by sex. Women and men 20–81 years old, MATISS study 1993–1996. The bars in the figure indicate the minimum and maximum value of vitamin D (25(OH)D).

**Table 1.** Vitamin D (25(OH)D) serum mean level distribution by sex. Women and men 20–81 years old, MATISS study 1993–1996 [30,31].

| | WOMEN | | | | | MEN | | | |
|---|---|---|---|---|---|---|---|---|---|
| *n* | Mean | SD | 95% CI | | *n* | Mean | SD | 95% CI | |
| 2274 | 17.2 | 10.0 | 16.8 | 17.6 | 1174 | 24.1 | 12.6 | 23.3 | 24.8 |

**Table 2.** Prevalence of 25(OH)D serum levels by sex and stratified into four subgroups: deficiency (<20 ng/mL), insufficiency (<30 ng/mL), sufficiency (≥30 ng/mL) and toxic (≥150 ng/mL). Women and men 20–81 years old, MATISS study 1993–1996 [30,31]. SD: standard deviation. CI: confidence interval. Red values were for vitamin D sufficiency.

| Vitamin D Status ng/mL | WOMEN | | | | MEN | | | |
|---|---|---|---|---|---|---|---|---|
| | *n* | % | 95% CI | | *n* | % | 95% CI | |
| <20 Deficiency | 1560 | 68.6 | 66.7 | 70.5 | 477 | 40.6 | 37.8 | 43.4 |
| 20–30 Insufficiency | 500 | 22.0 | 20.3 | 23.7 | 354 | 30.2 | 27.5 | 32.8 |
| ≥30 Sufficiency | 213 | 9.4 | 8.2 | 10.6 | 343 | 29.2 | 26.6 | 31.8 |
| ≥150 Toxic | 1 | 0.04 | - | - | 0 | 0 | - | - |

Vitamin D deficiency was highly correlated with age in both men and women (Figure 2 and Table 3). The younger men (20–29 years) had just sufficient 25(OH)D levels (32.3 ng/mL ± 13.2), which decreased with increasing age, becoming deficient (14.4 ng/mL ± 8.7) in men aged between 70 and 81. In women, vitamin D deficiency was more evident. In fact, the younger women already showed insufficient 25(OH)D levels (24.8 ng/mL ± 11.9) that, as with men, decreased with increasing age; women aged between 70 and 81 had 10.2 ng/mL ± 4.8 of 25(OH)D. It is worth noting that the women of the first five age classes showed about 7 ng/mL less than men; instead, in the last class (70–81), the difference was slightly decreased (4 ng/mL). In fact, although the deficiency was always more evident in women than in men, the men of this class exhibited a relatively steep 25(OH)D level decrease (Figure 2 and Table 3).
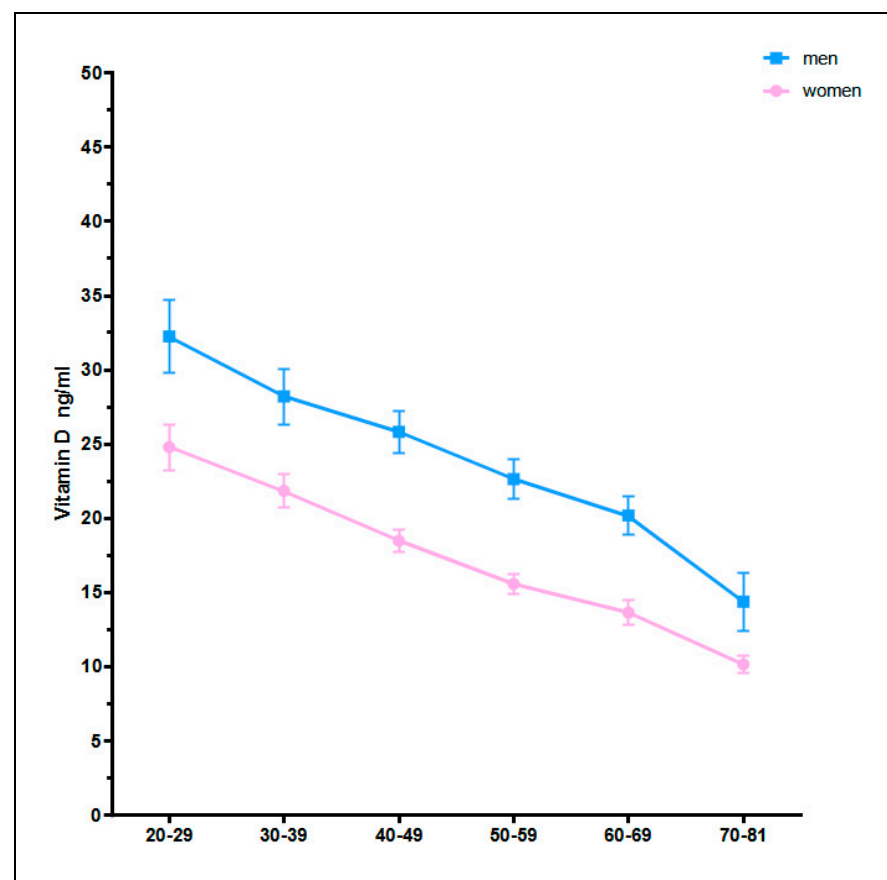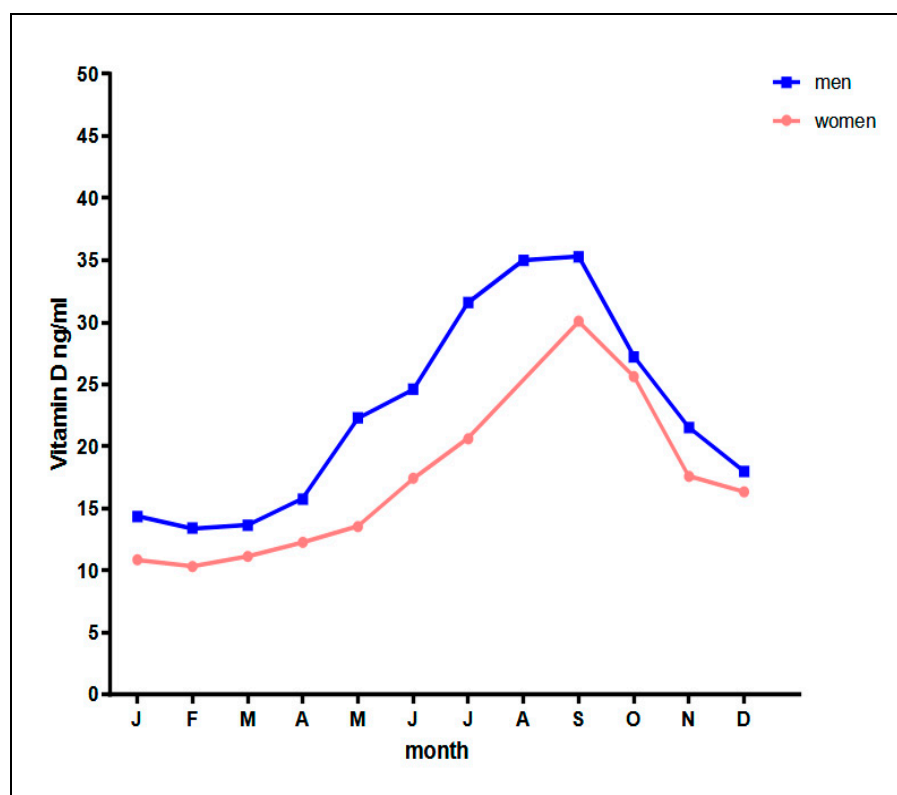


**Figure 2.** Vitamin D (25(OH)D) mean values by sex and age-classes. Women and men 20–81 years old, MATISS study 1993–1996. Bars refer to 95% confidence intervals. Number of persons, means, standard deviations and 95% confidence intervals were reported in Table 3.

**Table 3.** Vitamin D (25(OH)D) mean values, standard deviations, 95% confidence intervals by sex and age-classes. Women and men 20–81 years old, MATISS study 1993–1996 [30,31].

| Age Classes (Years) | WOMEN | | | | | MEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Mean | SD | 95% CI | | *n* | Mean | SD | 95% CI | |
| 20–29 | 233 | **24.8** | 11.9 | 23.2 | 26.3 | 116 | **32.3** | 13.2 | 29.8 | 34.6 |
| 30–39 | 373 | **21.9** | 11.3 | 20.7 | 23.0 | 230 | **28.2** | 14.4 | 26.3 | 30.0 |
| 40–49 | 471 | **18.5** | 8.5 | 17.7 | 19.2 | 264 | **25.8** | 11.6 | 23.0 | 25.8 |
| 50–59 | 478 | **15.6** | 7.7 | 14.8 | 16.2 | 255 | **22.6** | 10.8 | 21.3 | 23.9 |
| 60–69 | 487 | **13.6** | 9.2 | 12.8 | 14.4 | 231 | **20.2** | 10.1 | 18.8 | 21.5 |
| 70–81 | 232 | **10.2** | 4.8 | 9.5 | 10.7 | 78 | **14.4** | 8.7 | 12.4 | 16.3 |

Vitamin D concentrations were significantly lower in women than in men for each age group (t-test $p < 0.0001$). The comparisons between six age groups are all significant (t-test $p < 0.05$) for both women and men, except the comparison in men in the classes between 40–49 and 50–59, and 50–59 and 60–69 years ($p = 0.0710$ and $p = 0.0105$, respectively). SD: standard deviation. CI: confidence interval. Means values are in bold.

Considering that a seasonal variability in 25(OH)D levels has previously been reported [36], in order to explore this effect in our cohort, we stratified data for the month in which the serum samples were collected; unfortunately, at the time of sample collection, the stratification was not foreseen for season and, therefore, not all months are represented with the same number of subjects. Nevertheless, in both men and women, we observed a high variability during the year, indicating an evident seasonal trend (Figure 3 and Table 4). The curve had a minimum value in February and March and a maximum value in August and September but, unfortunately, in August, we did not have enough women to analyze. The results show that sufficient levels of serum 25(OH)D were just reached in July, August and September for men and only in September for women.



**Figure 3.** Vitamin D (25(OH)D) mean values by sex and month of blood collection. Women and men 20–81 years old, MATISS study 1993–1996. Serum samples from female were not available in August. Number of persons, means, standard deviations and 95% confidence intervals were reported in Table 4.
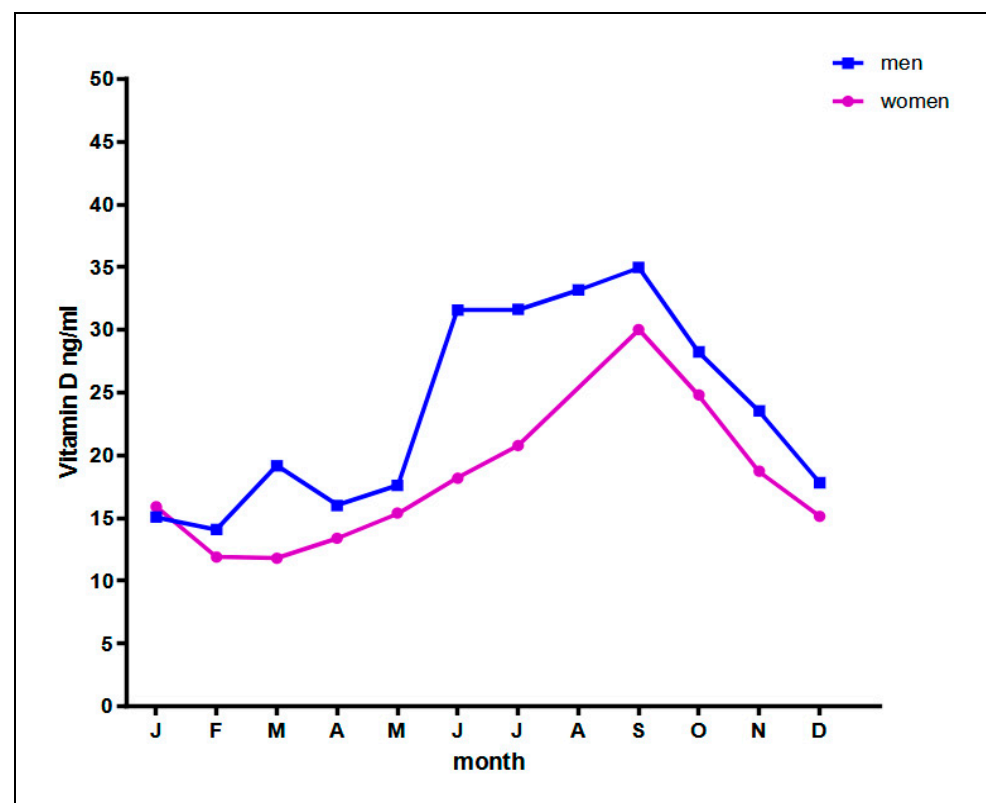
**Table 4.** Vitamin D (25(OH)D) mean values, standard deviations, 95% confidence intervals by sex and month of blood collection. Women and men 20–81 years old, MATISS study 1993–1996 [30,31]. Means values are in bold.

| Month of the Year | WOMEN | | | | | MEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Mean | SD | 95% CI | | *n* | Mean | SD | 95% CI | |
| J | 58 | **10.9** | 5.9 | 9.3 | 12.4 | 131 | **14.4** | 6.4 | 13.2 | 15.4 |
| F | 174 | **10.3** | 7.4 | 9.2 | 11.4 | 218 | **13.4** | 6.9 | 12.4 | 14.3 |
| M | 296 | **11.1** | 5.0 | 10.5 | 11.7 | 41 | **13.7** | 7.6 | 11.2 | 16.0 |
| A | 235 | **12.3** | 5.3 | 11.5 | 12.9 | 60 | **15.7** | 6.8 | 13.9 | 17.4 |
| M | 313 | **13.5** | 9.4 | 12.4 | 14.5 | 4 | **22.3** | 6.7 | - | - |
| J | 112 | **17.4** | 6.8 | 16.1 | 18.7 | 4 | **24.6** | 14.9 | - | - |
| J | 122 | **20.6** | 6.5 | 19.4 | 21.9 | 48 | **31.6** | 9.5 | 28.8 | 34.3 |
| A | serum sample not available | | | | | 161 | **35.0** | 12.4 | 33.0 | 36.9 |
| S | 254 | **30.1** | 11.1 | 28.7 | 31.4 | 141 | **35.3** | 11.9 | 33.2 | 37.2 |
| O | 223 | **25.6** | 9.4 | 24.3 | 26.8 | 311 | **27.2** | 8.6 | 26.2 | 28.1 |
| N | 385 | **17.6** | 7.2 | 16.8 | 18.3 | 48 | **21.5** | 8.2 | 19.1 | 23.9 |
| D | 102 | **16.3** | 7.5 | 14.8 | 17.8 | 7 | **18.0** | 5.7 | - | - |

Serum samples from female were not available in August. SD: standard deviation. CI: confidence interval. Means values are in bold.

Similar trends were observed when mean values of serum 25(OH)D were age-standardized (Figure 4 and Table 5).



**Figure 4.** Vitamin D (25(OH)D) age-standardized mean values by sex and month of blood collection. Women and men 20–81 years old, MATISS study 1993–1996. Means were age-standardized by Italian National Institute of Statistics—ISTAT Latina population 1994. Age-standardized values referred to 20–81 years old population resident in Latina in 1994 (ISTAT data). Serum samples from female were not available in August. Number of persons, means, standard deviations and 95% confidence intervals were reported in Table 5.
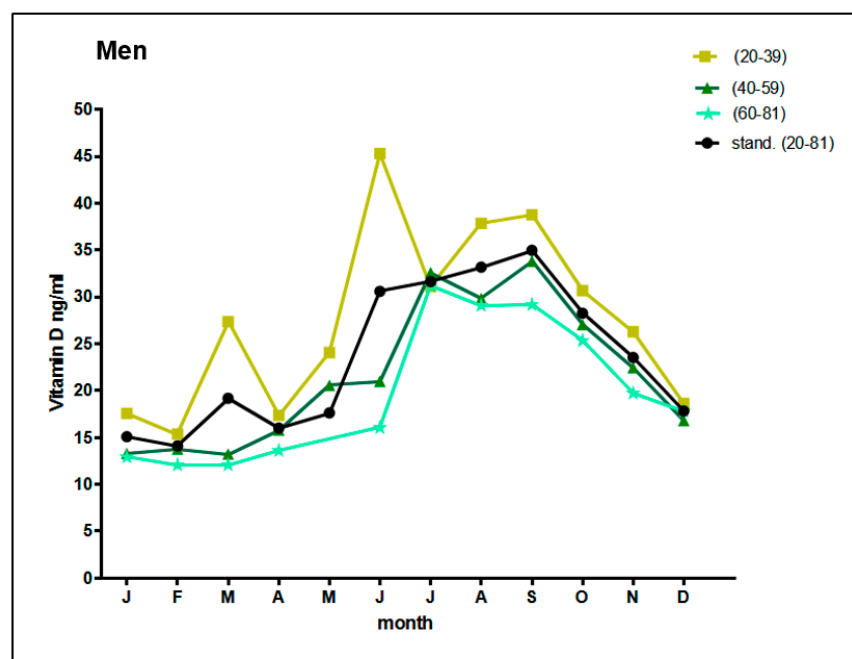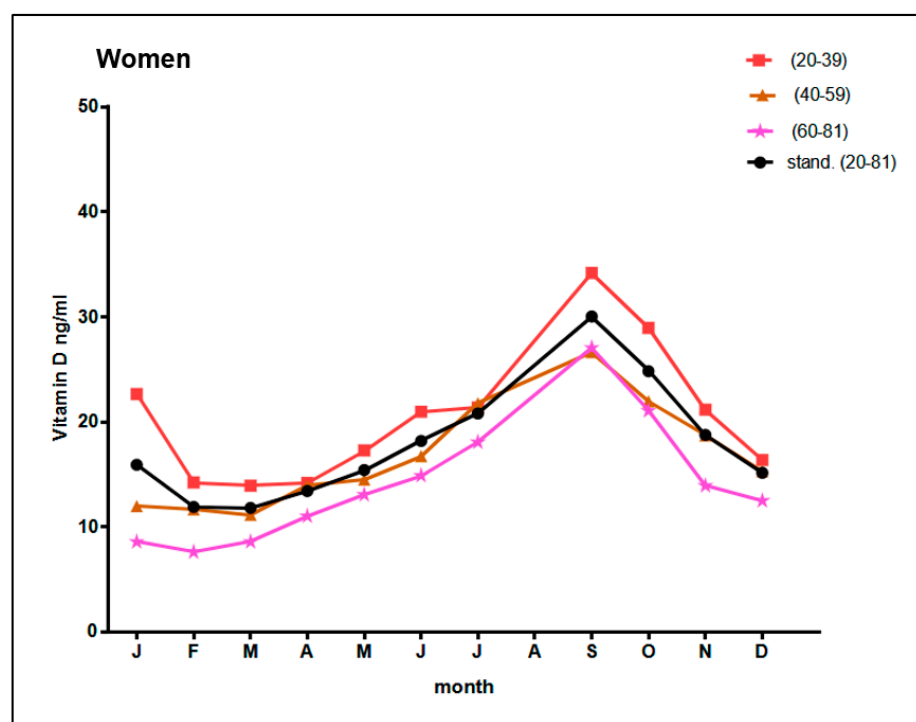
**Table 5.** Vitamin D (25(OH)D) age-standardized mean values and standard deviations, 95% confidence intervals by sex and month of blood collection. Women and men 20–81 years old, MATISS study 1993–1996 [30,31]. Means and standard deviations were age-standardized by Italian National Institute of Statistics—ISTAT Latina population 1994.

| Month of the Year | WOMEN | | | | | MEN | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Mean | SD | 95% CI | | *n* | Mean | SD | 95% CI | |
| J | 58 | **15.9** | 5.3 | 14.5 | 17.3 | 131 | **15.1** | 6.2 | 14.0 | 16.1 |
| F | 174 | **11.9** | 7.4 | 10.8 | 13.0 | 218 | **14.1** | 6.9 | 13.2 | 15.0 |
| M | 296 | **11.8** | 4.6 | 11.3 | 12.3 | 41 | **19.2** | 7.0 | 17.0 | 21.3 |
| A | 235 | **13.4** | 5.3 | 12.7 | 14.1 | 60 | **16.0** | 6.6 | 14.3 | 17.7 |
| M | 313 | **15.4** | 10.1 | 14.3 | 16.5 | 4 | **17.6** | 8.1 | - | - |
| J | 112 | **18.2** | 6.3 | 17.0 | 19.3 | 4 | **31.6** | 27.6 | - | - |
| J | 122 | **20.8** | 6.3 | 19.7 | 21.9 | 48 | **31.6** | 8.8 | 29.1 | 34.1 |
| A | serum sample not available | | | | | 161 | **33.2** | 12.0 | 31.3 | 35.0 |
| S | 254 | **30.0** | 10.7 | 28.7 | 31.3 | 141 | **35.0** | 11.9 | 33.0 | 36.9 |
| O | 223 | **24.8** | 8.9 | 23.6 | 26.0 | 311 | **28.2** | 8.3 | 27.3 | 29.2 |
| N | 385 | **18.7** | 6.8 | 18.0 | 19.4 | 48 | **23.5** | 7.9 | 21.3 | 25.8 |
| D | 102 | **15.1** | 7.1 | 13.8 | 16.5 | 7 | **17.8** | 4.9 | - | - |

Age-standardized values referred to 20–81 years old population resident in Latina in 1994 (ISTAT data). Serum samples from female were not available in August. SD: standard deviation. CI: confidence interval. Means values are in bold.

The mean serum 25(OH)D levels of men and women, aged 20–39, 40–59 and 60–81, were stratified for month and compared with the age-standardized curve (Figures 5 and 6, Supplementary Tables S1 and S2). Looking at the three age subgroups, although the number of cases in some months was lower with respect to those from the others, we observed that the mean curves of all age subgroups had the same seasonality trend: low levels in winter that increase in spring and have the highest level in summer to decrease again in the fall. Compared with the age-standardized curve, participants aged 20–39 had higher values, subjects aged 40–59 had similar values, while the last group, 60–81, were lower. In particular, we observed that young men showed sufficient 25(OH)D levels from June to October with a maximum level (45.3 ng/mL) in June (Figure 5 and Supplementary Table S1), while the young women showed sufficient 25(OH)D levels only in September (34.1 ng/mL) (Figure 6 and Supplementary Table S2), having no data for the month of August. In the second class (40–59 years), the men showed sufficient levels from July to September, while the women in none of these months. The men of the last class (60–81) showed sufficient levels only in July (31.1 ng/mL) but the women of this class were insufficient in all the months observed. Finally, we assessed the level and prevalence of some cardiovascular risk factors and conditions by vitamin D classes (Supplementary Tables S3 and S4). In particular, both in women and in men, systolic blood pressure, total cholesterol and diabetes were inversely associated with vitamin D levels, while a direct association was found with the HDL values.

**Figure 5.** Vitamin D (25(OH)D) serum levels by age classes and month of blood collection. Men 20–81 years old, MATISS study 1993–1996. Number of persons, mean, standard deviation and 95% confidence intervals were reported in Supplementary Table S1. For the age-standardized curve, means were age-standardized by Italian National Institute of Statistics—ISTAT Latina population 1994.



**Figure 6.** Vitamin D (25(OH)D) serum levels by age classes and month of blood collection. Women 20–81 years old, MATISS study 1993–1996. Number of person, mean, standard deviation and 95% confidence intervals were reported in Supplementary Table S2. For the age-standardized curve, means were age-standardized by Italian National Institute of Statistics—ISTAT Latina population 1994.

## 4. Discussion

Clinical evidence indicates that low circulating levels of vitamin D may be associated with increased risk of several skeletal and nonskeletal systems and related diseases.

Hypovitaminosis D status includes traditional risk categories such as young children, elderly, pregnant women, postmenopausal women, and obese persons. However, hypovitaminosis D status has a high prevalence over the world, not only in these risk groups; it was reported that other individual factors such as age, gender, skin pigmentation, lifestyle, a diet lacking in Vitamin D content and some drugs have contributed to make vitamin D deficiency one of the most prevalent health problems. Furthermore, the change in modern environment, air pollution and excessive sunscreen use to avoid the risks of skin cancer must also be considered.

The optimal serum concentration of 25(OH)D continues to be a controversial issue; most current guidelines agree that levels > 30 ng/mL are considered adequate for bone health, defined as the concentration that maximally suppresses serum parathyroid hormone [37], but it is not certain if this level is also optimal for other nonskeletal-related diseases. In this study, we analyzed the serum content of vitamin D in the MATISS cohort to assess its distribution status at the time of sampling.

Our results in the MATISS cohort revealed that, in the geographical area located in central Italy, the incidence of hypovitaminosis D status is high; in fact, only 9.4% of women and 29.2% of men had sufficient 25(OH)D levels. In order to better evaluate widespread vitamin D insufficiency, we divided our cohort into six age classes to reduce potential confounding age-related factors. We observed that vitamin D deficiency was highly correlated with age in both men and women and only younger men (20–29 years) had sufficient 25(OH)D levels. Sunlight is an essential determinant of vitamin D status and the variation in seasonal irradiance plays an important role everywhere; the direct effect of sunlight on vitamin D metabolism is, hence, dependent upon direct sunlight irradiation, expected to be higher in summer and lower in winter. Hence, to better understand the effect of the seasonal change on our cohort, we stratified data for the month in which the blood sampling was performed. The results clearly indicated an evident seasonal trend: the resulting fitting curves reported in Figure 3 show that at the end of the winter, the mean serum 25(OH)D level is below the official sufficient value reported for adult men and women. In this cohort, sufficient levels of serum 25(OH)D were just reached in summer for men and only at the end of summer for women. These data therefore indicate that low levels of 25(OH)D can also be found in rural populations of the central Mediterranean area with appreciable sun irradiation across the year; our results are in agreement with those obtained in studies from other countries with a similar type of sun exposure [38–40]. Moreover, a further stratification of data, according to the three age classes, generated more consistent results: in fact, the seasonal trend is observed in each class and the differences in serum 25(OH)D levels are confirmed throughout the year. It is of particular interest that the class of elderly women studied, although insufficient and predisposed to vitamin D deficiency (reduced ability of the skin to synthetize cholecalciferol, reduced expression of VDRs), also achieved its highest serum 25(OH)D value at the end of summer. Different studies suggest that vitamin D may have a relationship with senescence-linked diseases; however, data obtained by intervention studies are not sufficient to indicate that the administration of vitamin D, even at high doses, may be useful for patients with these diseases [21].

An adequate amount of sunlight exposure remains the most important source of vitamin D for the elderly population also; therefore, the most appropriate medical advice should be to improve vitamin D status in this population through proper sun exposure throughout the year, without compromising skin health and the risk of skin cancer. Sun exposure may also be useful in other vitamin-D-linked diseases for which there is no evidence that supplementation reduces both risk of incidence and perhaps death. In terms of mechanisms, the production of vitamin D3 in the skin makes it accessible to the CYP11A1, while orally delivered vitamin D is hydroxylated in the liver to 25(OH)D, which is not recognized by the CYP11A1 [41]. However, vitamin D administration can be considered

in order to prevent the negative effect of hypovitaminosis D in skeletal integrity and in many other nonskeletal diseases with convincing evidence of efficacy. The strength of this study is that our data refer to the time of the sampling (1993–1996) and some confounding factors were absent or not relevant. For instance, although unfortunately no information on additional vitamin D intake was reported in the questionnaire, at that time, fortified foods in Italy were absent or very few, as well as the use of vitamin D supplements, especially in rural areas. In the same way, we could exclude, in our cohort, a possible effect of statin on vitamin D levels because until 1997 in Italy, this therapy was not yet widely recommended [42]. Moreover, it is important to note that serological analyses for vitamin D content were performed in 2016 using highly precise and standardized methods and the serum samples were in perfect storage conditions. In conclusion, although vitamin D deficiency has already been described in various Italian regions, our study also confirms its relevance in a Mediterranean rural population analyzed for several health risk factors and clearly indicates that this hypovitaminosis was already seriously present in the 1990s when lifestyles were different. In particular, this epidemiological research underlines the importance of serum 25(OH)D measurement in at-risk Italian populations. In attempt to avoid vitamin D insufficiency, results from this study are of particular relevance in establishing future recommendation in some critical population. In fact, for the important biological link with health status, the screening of patients at risk of developing vitamin D deficiency should be recommended, not only for preventing osteoporosis, but also for restoring an appropriate concentration of vitamin D that would be effective to counteract the development of other and more severe pathological conditions.

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