

# Vitamin D Status in Osteoporotic and Diabetic Patients and Athletic Healthy Individuals from Northern Greece

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## Abstract

**Background:** Vitamin D deficiency is recognised as a pandemic in the developed world. However, the importance of prudent sun exposure tends to be overlooked, which is responsible for this pandemic.

**Methods:** We investigated the vitamin D status in 326 adults, 165 females and 161 males: 99 Osteoporosis patients, 53 Type 1 Diabetes patients, 51 Type 2 Diabetes patients, and 123 Athletic Healthy individuals, from Northern Greece, through the measurement of total calcidiol in winter and summer by immunoenzymatic assay.

**Results:** In the Whole Sample 23.31% had severe deficiency, 13.50% mild deficiency, 17.48% insufficiency, and 45.71% adequacy at the end of winter. Mean concentrations differed significantly ( $p < 0.001$ ) between males and females. The prevalence of deficiency in the young was significantly lower than in the middle-aged ( $p = 0.004$ ) and in the elderly ( $p < 0.001$ ), while it was significantly lower ( $p = 0.014$ ) in the middle-aged than in the elderly. The best vitamin D status was found in the Athletic Healthy individuals, followed by the Type 1 and Type 2 Diabetic patients, while Osteoporotic patients had the poorest status. The difference in mean concentrations between winter and summer was significant ( $p < 0.001$ ).

**Conclusions:** Vitamin D status deteriorated with increasing age and it was better in males than in females. Our findings suggest that outdoor physical activity in a Mediterranean country can cover the vitamin D needs of the young and the middle-aged, but not of the elderly, without the need for dietary supplements.

**Keywords:** Total calcidiol, osteoporosis, Type 1 diabetes, Type 2 diabetes, Vitamin D status.

## Introduction

Vitamin D has been produced under the influence of solar radiation for more than 500 million years (1). Vitamin D deficiency has been recognised since the late 20th century as a pandemic affecting all age groups in the developed world (1,2). However, the importance of prudent sun exposure tends to be overlooked, which is responsible for the global pandemic of vitamin D deficiency (1). On average, 40%–60% of children and adults worldwide are deficient or insufficient (3). Total calcidiol or 25-hydroxyvitamin D is considered to

be the most relevant indicator of vitamin D status (4). Many diseases and other negative health outcomes have been linked to vitamin D deficiency, including osteoporosis and increased risk of fractures (5), type 1 diabetes (6), and type 2 diabetes (7).

In Greece, despite the high sunshine levels and the strength of solar radiation at lower latitudes, many studies show poor vitamin D status in more or less all age groups (8–17). In fact, in some European multicentre studies, mainly in the

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Received: 16 Jun, 2022; Accepted: 12 Oct, 2022

elderly, a decrease of vitamin D concentrations from North to South was observed, contrary to what was expected (18,19). Research on vitamin D status in Greece, and especially in Northern Greece, to date, are few and usually partial, only in one age group or only in one gender, only in healthy individuals or only in patients, usually examining the association with a single health outcome.

The aim of the present study, which is part of bigger research, is the comprehensive assessment of vitamin D status in adults from Northern Greece, among genders and age subgroups, firstly through the measurement of total calcidiol in winter and summer, in groups of patients with osteoporosis, type 1 and type 2 diabetes, also in a group of athletic healthy individuals with intense outdoor activity, to investigate whether natural outdoor exercise and exposure to solar radiation can cover the vitamin D needs of a Mediterranean Caucasian throughout the year.

## Materials and Methods

### *Subjects*

A total of 326 individuals, 165 females and 161 males, aged 20–77 years, with a mean age  $49.8 \pm 13.5$  years, participated in this study. More specifically, 99 subjects, 75 females and 24 males, with a mean age  $56.3 \pm 11$  years, were patients with diagnosed Osteoporosis, as revealed by the T-scores of bone density, which ranged between -2.50 and -3.40. All were receiving anti-absorptive treatment. 53 subjects, 26 females and 27 males, with a mean age  $36.7 \pm 8.8$  years, were diagnosed with Type 1 Diabetes (T1D) and another 51 subjects, 11 females and 40 males, with a mean age  $58.9 \pm 7$  years, with Type 2 Diabetes (T2D). All diabetics were treated with insulin. The above participants formed the patient's groups. Another 123 individuals, 53 females and 70 males, with a mean age  $46.4 \pm 13.3$  years, were perfectly healthy. They all exercised regularly in outdoor activities. They were recruited from swimming, cycling, naturalist, hiking, etc. groups, through personal acquaintances or social media advertisements. These participants formed the Athletic Healthy group.

### *Study design*

Serum samples were taken from all participants between 15–31 March 2018, at the end of winter. At the end of summer, between 20–31 September 2018, a second blood draw was performed and total calcidiol concentration was measured in 35 subjects with osteoporosis, 16 subjects with T1D, four subjects with T2D, and 51 Athletic Healthy, a total of 106 subjects, 61 females and 45 males. T2D patients with who were examined for the second time in the summer were very few, so we included them together with T1D patients in a single Diabetes group, especially for the analyses of variation between winter and summer.

In addition, Professor Stella Mitka, co-author of the present study, kindly provided us with unpublished data of the total calcidiol concentrations from one of her own studies, concerning 49 healthy individuals, 22 female and 27 male, with a mean age of  $53.7 \pm 15.9$  years, who had no diagnosed medical conditions and were not engaged in outdoor physical activity, measured at the end of March 2016, in the same laboratory and with the same method as all the other specimens. These participants formed the Regular Healthy group.

None of the participants had vitamin D supplementation and all elderly individuals were independent, living in the community. Written informed consent was obtained from each.

Participants were excluded from the study if during the last six months had used a medication known to affect calcium and vitamin D metabolism, except for antiresorptive used by osteoporotic participants. Other exclusion criteria were the presence of diseases that affected calcium homeostasis, such as metabolic bone diseases other than uncomplicated osteoporosis, osteoporotic fractures, and liver and kidney disease.

The protocol was reviewed and approved by the Committee of Ethics and Deontology in Research, of the Democritus University of Thrace, under number Θ24/Δ.Σ7/28.2.2081.

From all patients and healthy subjects, fasting blood samples were collected between 8 and 10 am and stored at  $-70^{\circ}\text{C}$  for subsequent

analysis, and their height and weight were measured.

### **Biochemical analysis**

The measurements of biochemical and hormonal markers in all samples were performed in the diagnostic laboratory Bioanalysis, certified with ISO 9001:2008 by the Hellenic Accreditation System (ESYD), using standard automated laboratory methods. Urea, Creatinine, AST, and ALT measurements were performed on a Hitachi 902 biochemical analyser (Roche Diagnostics, Mannheim, Germany) to ensure that participants did not suffer from liver or kidney disease.

Of the 75 osteoporotic women, 16 were premenopausal and 59 were postmenopausal, as confirmed by FSH, LH, and Total Estrogen concentrations measured on an Elecsys immunoanalyser (Roche Diagnostics, Mannheim, Germany), using the chemiluminescence method.

A questionnaire was completed for each participant, including age, gender, type of diabetes, bone mass in osteoporotic patients, underlying diseases, medical history, medications.

The measurements of total calcidiol concentrations were carried out at the Clinical Chemistry Laboratory, Faculty of Biomedical Sciences, International University of Greece, by the immunoenzymatic method, using commercial kits: Vitamin D Total ELISA (DIAsource ImmunoAssays, Ottignies-Louvain-la-Neuve, Belgium), according to the manufacturer's instructions.

For the prevalence analysis of vitamin D deficiency and insufficiency, we categorised the serum total calcidiol levels as in previous research (14): Severe Deficiency ( $\leq 10$  ng/ml), Mild Deficiency (10–20 ng/ml), two categories aggregated as Deficiency ( $< 20$  ng/ml), Insufficiency (20–30 ng/ml) and Adequacy ( $\geq 30$  ng/ml). In some cases, the categories of Deficiency and Insufficiency are reported together as Suboptimal Levels ( $< 30$  ng/ml).

Participants from each healthy and patient group were divided into three age subgroups: young, with ages 20–39 years, middle-aged,

with ages 40–59 years, and elderly, with ages  $\geq 60$  years.

### **Statistical analysis**

All statistical analyses were performed using IBM SPSS Statistics, version 23 (IBM, Armonk, NY). Before each statistical analysis, except chi-square, normality tests were performed: a Shapiro-Wilk test, also a visual inspection of the histograms, Q-Q normal plots, and box and whiskers plots.

In addition, all the other assumptions of the various statistical tests were examined by conducting the appropriate tests as required (15). When this examination showed that the assumptions were met, accurately or approximately, the appropriate parametric tests were carried out, otherwise, the corresponding non-parametric tests were performed. In all cases of positive correlations, the corresponding effect size test was conducted and was interpreted according to the APA instructions (16).

Prevalence frequencies and mean concentrations are accompanied by 95% Confidence Intervals. The CIs of the frequencies were calculated using the Clopper-Pearson Exact binomial test. In all cases, a probability level of  $\alpha = 0.05$  was considered statistically significant.

## **Results**

### **End of winter**

The prevalence of total calcidiol levels categories for the Whole Sample and the Study Groups and subgroups as well as the correlations between them are presented in Figures 1 and 2.

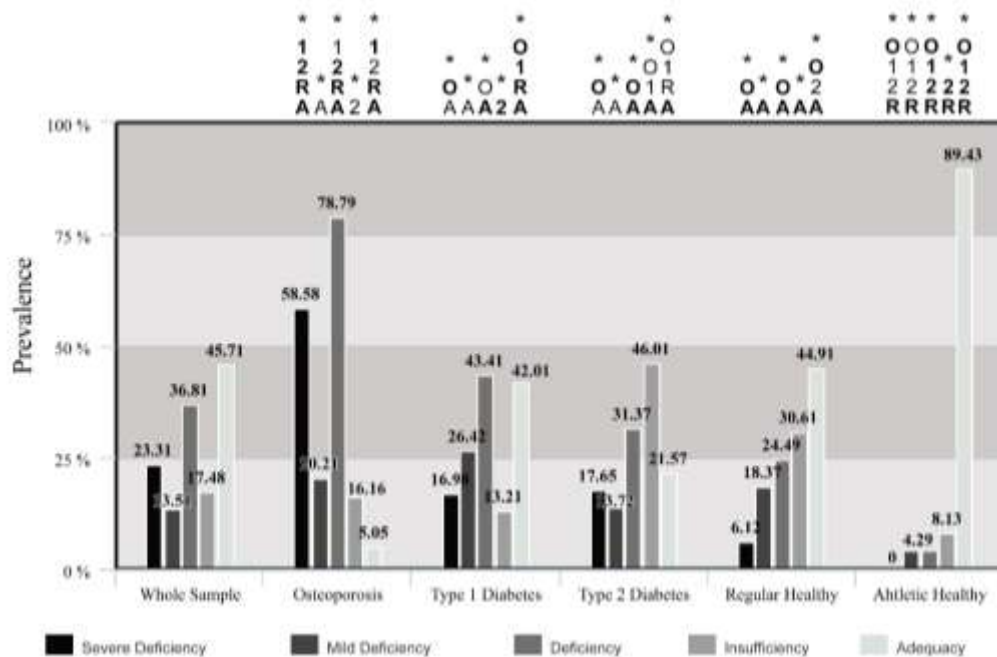
Prevalence between genders, in the Whole Sample, showed a mixed picture, regarding the suboptimal levels categories, while it did not differ significantly in the category of total calcidiol adequacy (data not shown). The vast majority of Athletic Healthy had a total calcidiol adequacy. Of note, all 13 individuals showing suboptimal values in this group belonged to the elderly age subgroup. Athletic Healthy had a significantly higher prevalence of total calcidiol adequacy than all other Study Groups, with strong to very strong correlations.

Athletic Healthy outperformed Regular Healthy in all categories of calcidiol levels with strong to very strong correlations.

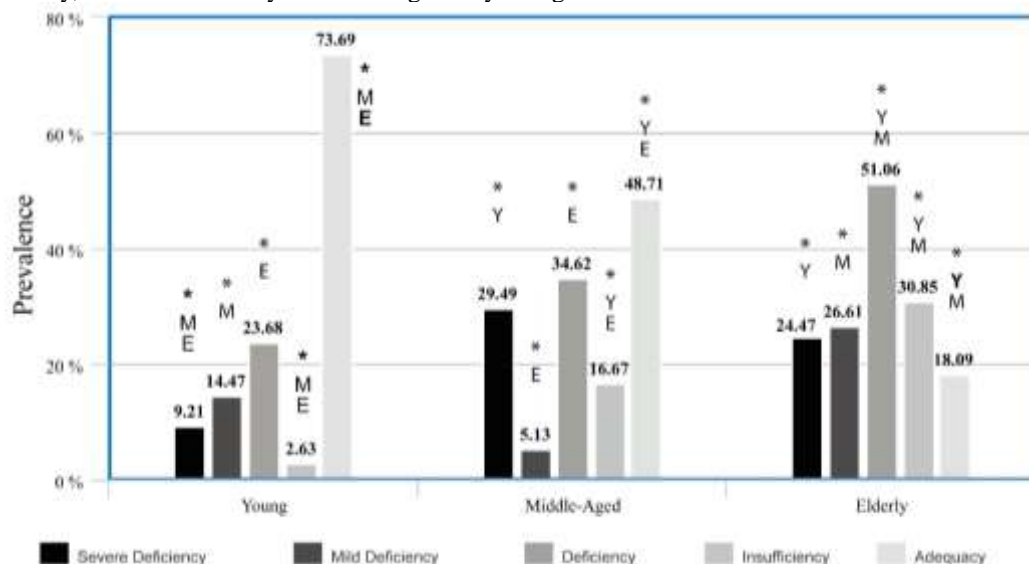
The prevalence of adequacy in T1D and T2D compared to Osteoporosis was significantly higher with a strong correlation for the former and a mild correlation for the latter. T2D showed a significantly lower

prevalence of total calcidiol adequacy than T1D, with a weak correlation.

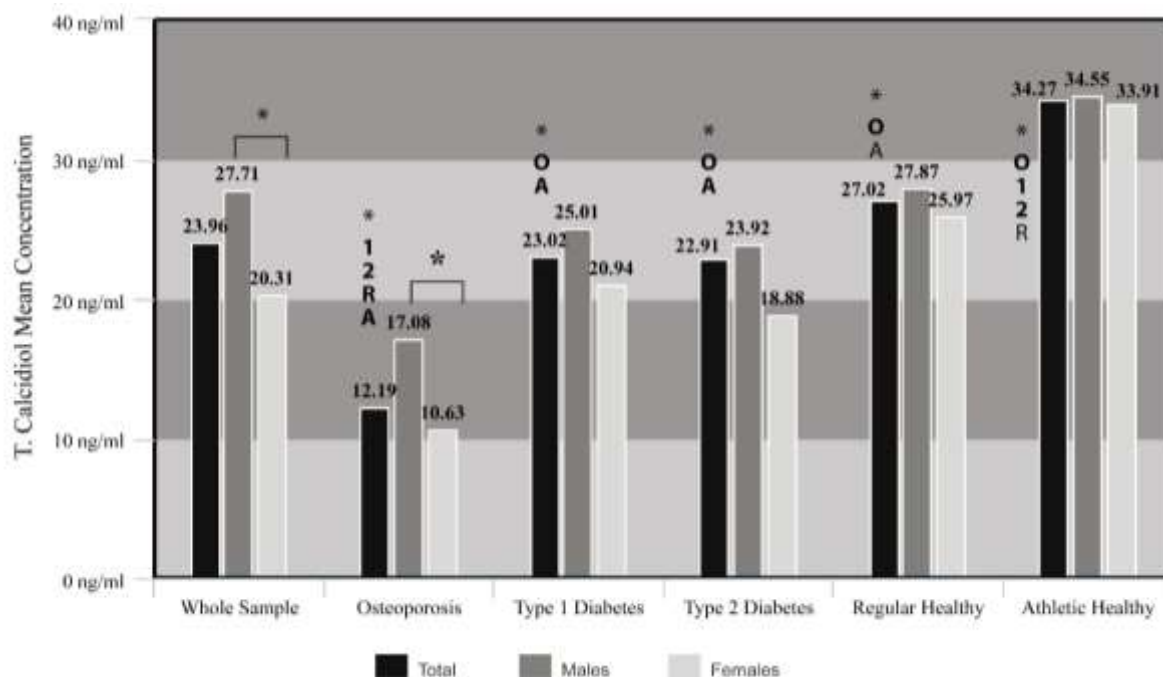
Figures 3 and 4 show the mean total calcidiol concentrations in total and by gender in the Whole Sample and the Study Groups as well as the corresponding correlations the mean concentrations in the Study Groups by age subgroup as well as the corresponding correlations.



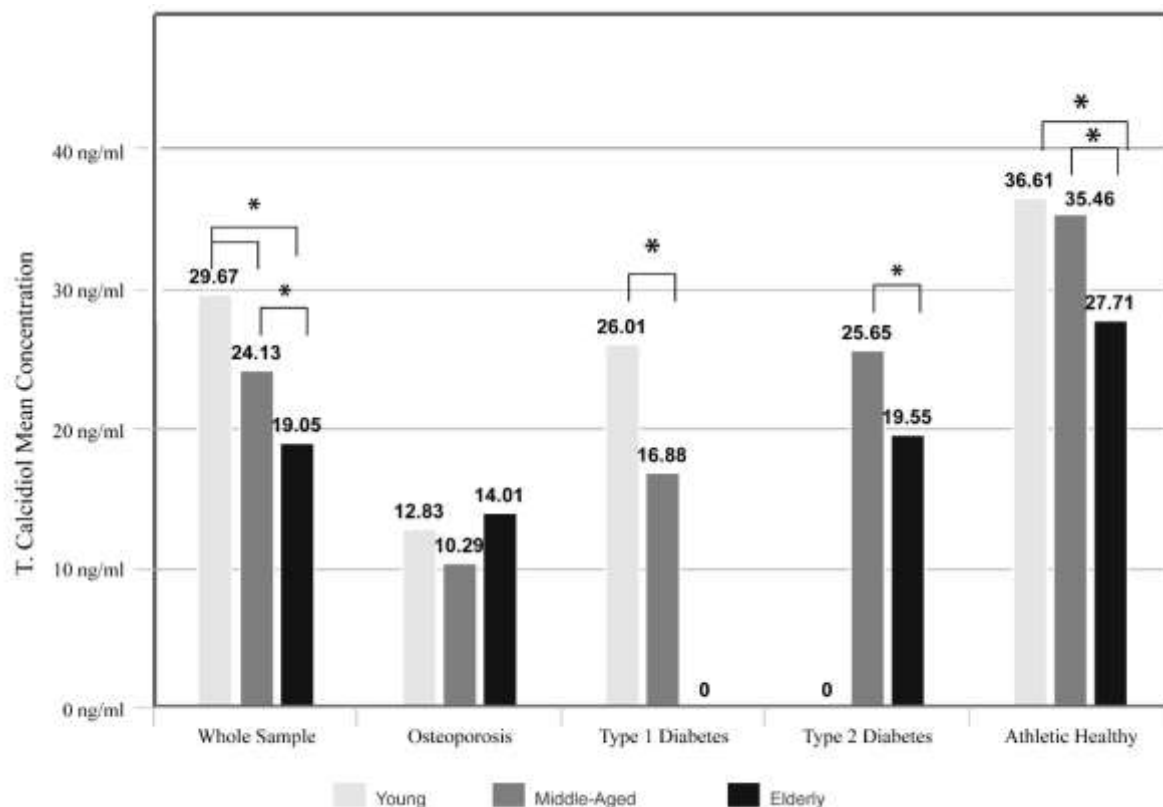
**Fig. 1.** Prevalence of total calcidiol levels categories for the Whole Sample and the Study Groups at the end of winter. \*: Statistically significant difference with the corresponding category of another group. O: Osteoporosis, 1: Type 1 Diabetes, 2: Type 2 Diabetes, R: Regular Healthy, A: Athletic Healthy. **Bold:** Strong or very strong correlation. Normal: Weak or mild correlation.



**Fig. 2.** Prevalence of total calcidiol levels categories in the Whole Sample by age subgroup at the end of winter. \*: Statistically significant difference with the corresponding category of another age subgroup. Y: Young, M: Middle-aged, E: Elderly. **Bold:** Strong or very strong correlation. Normal: Weak or mild correlation.



**Fig. 3.** Mean total calcidiol concentrations in the Whole Sample and the Study Groups at the end of winter, in total and by gender. \*: Statistically significant difference. O: Osteoporosis, 1: Type 1 Diabetes, 2: Type 2 Diabetes, R: Regular Healthy, A: Athletic Healthy. **Bold:** Strong or very strong correlation. Normal: Weak or mild correlation.



**Fig. 4.** Mean total calcidiol concentrations in the Whole Sample and the Study Groups by age subgroup at the end of winter. \*: Statistically significant difference. **Bold:** Strong or very strong correlation. Normal: Weak or mild correlation.

In the Whole Sample, the mean concentration was significantly greater in males than in females, with a mild correlation. Mean concentrations decreased significantly from the young to the middle-aged and then to the elderly, with mild correlations.

In Athletic Healthy, mean concentrations did not differ significantly between genders, neither between the young and the middle-aged, however, both of these age subgroups had significantly higher mean concentrations than the elderly, with strong correlations. Athletic Healthy showed significantly higher mean concentration than the two diabetes groups, with strong correlations, while the mean concentrations in the latter two groups did not differ significantly from each other.

The mean concentration in Osteoporosis was significantly lower than in all other Study Groups, with strong correlations, of which the strongest one was with Athletic Healthy. The mean concentration was significantly higher in osteoporotic males than in osteoporotic females with a very strong correlation. There was no significant difference in the mean concentrations among the three age subgroups. Mean concentrations did not differ significantly between premenopausal and postmenopausal females.

In T1D, mean concentrations were not significantly different between genders, similar to T2D. In T1D, mean concentration was significantly higher in the young than in the middle-aged, with a very strong correlation. Only 1 elderly individual was included in this group, so the statistical analysis was not feasible.

In T2D, mean concentration was significantly higher in the middle-aged than in the elderly with a mild correlation. T2D did not include any young adults.

The mean total calcidiol concentration was significantly lower ( $p < 0.001$ ) in Regular Healthy than in Athletic Healthy, with a mild correlation. Of course, Regular Healthy had a significantly higher mean age than Athletic Healthy ( $p = 0.005$ ), but the strength of the association was negligible ( $r = 0.046$ ), i.e., a

minimal part of the variance between the two groups was due to this difference.

The mean total calcidiol concentration in Regular Healthy was significantly higher than in Osteoporosis, with a very strong correlation, whereas it was not significantly different from that of the two diabetes groups.

### ***End of summer***

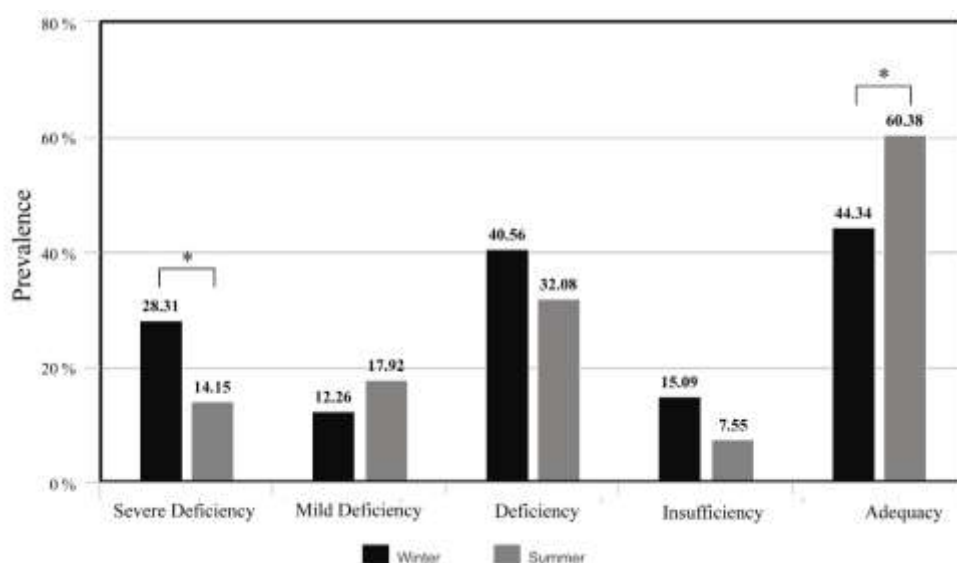
The prevalence of total calcidiol levels categories at the end of winter and at the end of summer together with the corresponding correlations, for the 106 participants from which a second sample was taken, is presented in Figure 5.

27 of the 106 subjects with a second measurement (25.47%, 17.50–34.90) moved up at least one category of total calcidiol concentrations, from severe deficiency to mild deficiency, etc., at summer measurement, whereas no subjects dropped a category. If, in fact, it is taken into account that 47 out of these 106 participants already had adequate levels in winter and therefore could not move up a category, the rate of moving up a category becomes even higher, since it is actually 27 out of 59 (45.76%, 95% CI = 32.70–59.20).

The number of people with severe deficiency in winter halved in summer. The prevalence of severe total calcidiol deficiency in winter was significantly higher than in summer, with a very weak correlation. In the categories of mild deficiency, deficiency, and insufficiency the differences between winter and summer were not significant.

The prevalence of suboptimal levels in winter (55.66%, 45.70–65.30) was significantly higher ( $p = 0.028$ ) than in summer (39.62%, 30.30–49.60), again with a very weak correlation.

Total calcidiol concentration in summer (30.85 ng/ml, 95% CI = 27.93–33.78) increased significantly ( $p < 0.001$ ) in comparison to winter (23.06 ng/ml, 95% CI = 20.65–25.47) with a strong correlation ( $r = -0.576$ ). Notably, of the 106 subjects with a second measurement 95 had a higher concentration in summer, only five had a higher concentration in winter, while six had the same concentrations.



**Fig. 5.** Prevalence of total calcidiol levels categories at the end of winter and at the end of summer, for the 106 participants from which a second sample was taken. \*: Statistically significant difference. The two significant correlations were very weak.

## Discussion

The prevalence analysis and the mean concentrations study in the Whole Sample showed a much better picture of the vitamin D status in Northern Greece, compared to previous epidemiological studies, although they were not completely comparable to the present study, either because they included only children and adolescents (7), or only one gender (9) or only one age subgroup (11), or because patients were affected by another disease (8), or because different levels were used to determine vitamin D deficiency (10). The results of these Greek studies are rather comparable to those of Osteoporosis and T2D in the present study.

From the gender prevalence analysis in the Whole Sample, we could conclude that, in general, the overall vitamin D status of females was somewhat poorer than that of males, although not in all categories. This mixed picture in the prevalence of total calcidiol levels categories between genders is largely observed in the literature, where other studies found overall poorer vitamin D status in females than in males (17), while in other cases no significant differences were observed (18).

The correlation of mean total calcidiol concentrations between genders shows that in the Whole Sample males had significantly higher levels than females. The same is

suggested by the study of Xyda et al. in a bigger sample of the Northern Greek population, whereas in a Greek Cypriot population no such difference was found (12).

The significant difference in mean concentrations between genders, with males showing a better vitamin D status, is widely reported in the literature from almost all regions of the world, although in all cases the strength of the association was not reported (2,17). However, there are studies where the mean total calcidiol concentration did not differ significantly between genders (19).

Prevalence analysis suggests that total calcidiol status in the Whole Sample deteriorates with increasing age, since in general the young had a lower prevalence of suboptimal values, the middle-aged intermediate and the elderly higher. The correlations of the mean total calcidiol concentrations in the age subgroups support this notion. Similar observations have been published in the previous literature, from various parts of the world (20).

In the study by Papadakis et al., in 596 Greek female patients, with a mean age 65.3 years, with osteoporosis, the prevalence of suboptimal values in winter was found to be 92.2% (21). These data would probably be more appropriately compared to our subgroup of the elderly. Indeed, the elderly osteoporotic



subgroup in the present study had a mean age of 66 years and suboptimal values of 97.83% (95% CI = 88.50–99.90). Therefore, our findings are consistent with those of Papadakis *et al*.

In Osteoporosis, mean concentrations did not differ significantly between premenopausal and postmenopausal females, which is consistent to the findings of Abdulmahdi Mokif *et al* (22).

Based on the prevalence analysis in T1D, the vitamin D status was similar to that of the Whole Sample and appeared relatively better than in the other two patient groups. This is probably because T1D is mostly a disease of the young (23), therefore this group has the highest proportion of young people. As aforementioned, the young in general had better vitamin D status than middle-aged and elderly people.

In a 2009 US study of 128 children and adolescents with T1D, only 26% of the sample had wintertime total calcidiol adequacy (24). However, the most important difference to that study is that our T1D patients were all adults, over the age of 20. Previous research has shown that adolescents are likely to be at higher risk on average, in Greece (7), and in the UK (25).

Nevertheless, even if the total calcidiol status in T1D was better than expected, the disease was not prevented. This is possibly because very high intracellular concentrations of calcitriol are necessary to stimulate or silence genes in the extra-renal tissues, which are probably less sensitive to this regulation than the genes in the tissues of the classical vitamin D hormonal system, and therefore higher concentrations of total calcidiol in the circulation are required to produce the necessary calcitriol (26). On the other hand, studies in both animals and humans have shown that high levels of calcidiol or calcitriol are not effective when achieved after the onset of insulinitis and when beta-cell depletion is already underway (27,28). However, Derakhshanian *et al*. found that vitamin D treatment resulted in a significant increase in insulin concentration, which could improve hyperglycaemia in diabetic rats, with diabetes being artificially induced after STZ administration, which provides an alternative model for the study of T1D (29).

In a 2011 study from South Korea, among 276 males and females with T2D, only two percent had adequate levels of total calcidiol (30). Our T2D had a much better status, as the prevalence of adequacy was shown to be almost tenfold. Of course, the number of T2D patients in the present study was much smaller, which leaves room for more random variation, but still, the difference is very large.

In another 2015 study, again from South Korea, in 257 males and females with T2D, 16% had total calcidiol adequacy, a proportion close to ours (31).

In another 2016 survey from China, of 117 males and females with T2D, the results were consistent with ours, with 21.1% showing adequacy, but roughly with a reversal of the rates in the categories of deficiency, which was 52.6% in the Chinese population sample, and insufficiency, which was 26.3% in the Chinese population sample (32).

We should, of course, bear in mind that the above are studies of very different ethnic provenance. Several examples are showing that what applies to Asians does not necessarily apply to Caucasians (33).

In a 2019 large Australian study, in 468 male and female participants with T2D, where 25% of the participants were immigrants from Southern Europe, 19.2% had calcidiol adequacy, a rate consistent with ours (34).

The idea of recruiting a group of people of all ages who practise sports regularly but not professionally and engage in outdoor physical activities aimed at investigating the contribution of this type of activity to vitamin D status without the use of dietary supplements, since it is now considered almost incompatible with Western culture to photosynthesise the required amounts of cholecalciferol by exposure to the sun (35) without the risk of skin cancer (36). However, 90%–95% of the body's vitamin D needs are naturally met by sun exposure (1), since most natural foods, except fish and seafood, contain very small amounts of vitamin D (37). WHO recommends that most of the required vitamin D should be obtained from sun exposure and vitamin D supplements should be taken during winter and at latitudes greater than



42° N or S, i.e., above the northernmost tip of Greece, where skin photosynthesis is practically eliminated in autumn or even earlier (38).

Surprisingly, southern European countries, especially Greece, with very high levels of sunshine and suitable latitude to photosynthesise vitamin D<sub>3</sub> in the skin for more than six months per year (39), have a poorer vitamin D status than countries located at more northern latitudes. Avoidance of sunlight exposure is attributed to the high temperatures in southern latitudes and because of the fear of skin cancer (40).

To assess whether the excellent vitamin D status observed in the Athletic Healthy is due to outdoor activities, we borrowed the results of a group of Regular Healthy persons from another, unpublished, study. Both the prevalence analysis and the study of mean concentrations between the two groups showed that the vitamin D status of the Athletic Healthy was significantly better than that of the Regular Healthy, while the latter had essentially no significant differences from the two diabetes groups and showed a clearly better vitamin D status only from Osteoporosis.

The recruitment of Athletic Healthy individuals made this study fairly original in the literature, except perhaps for a study of children in a rural area of São Paulo, Brazil, where 27.9% of the sample engaged in outdoor physical activity (41), whereas in the present study only adults were included. In other studies, similar results were seen among urban and rural residents in India, Africa, and Fiji but not in the Western countries, due mainly to work rather than sport, both in terms of mean total calcidiol concentration and prevalence of deficiency (42).

The findings of the present study, concerning Athletic Healthy, suggest that it is feasible to improve the vitamin D status of the general population through physical outdoor exercise, and the comparison with Regular Healthy supports this notion. This would significantly improve the general health of the population and reduce costs to the insurance funds, pressure on the National Health System during the winter months at very low cost. It could also help reduce morbidity and mortality from COVID-19 (43).

The elderly Athletic Healthy had a relatively high prevalence of total calcidiol deficiency in

winter, with no significant differences between genders. Older people have a lower concentration of 7-dehydro-cholesterol, or pre-vitamin D, resulting in only 25% of the photosynthetic capacity compared to people in their 20s (44). This finding suggests that older people cannot meet their vitamin D needs with outdoor activities alone and that they should also receive dietary supplements.

In the group of Athletic Healthy as a whole and in all its age subgroups, no difference in vitamin D status between males and females was found. Based on the results of the other groups in the present study, where gender differences did not show a distinct pattern, it could be assumed that any differences observed are due to social factors and lifestyle differences rather than to any physiological or genetic differences between genders. Similarly, in a rural area of São Paulo, where a significant proportion of the participating children engaged in outdoor physical activity, no significant difference in vitamin D status between genders was found (41).

The significant increase in total calcidiol levels in the summer is a commonplace finding in the international literature from many parts of the world (45). This suggests that the change in lifestyle during summertime, the lighter clothing, the increase in outdoor activities, and the subsequent increase in the time of exposure of a larger skin surface to the sun can improve the vitamin D status of the population, despite the widespread use of sunscreens, which are known to drastically inhibit the photosynthesis of vitamin D in the skin (1,39).

In the study of Bolland et al., from New Zealand (45), it was estimated that one should have a concentration of at least 24–30 ng/ml at the end of summer to ensure that even in winter they will not be deficient. In the present study, the mean serum total calcidiol concentration of the 106 subjects with a second sample was at the upper end of the aforementioned range at the end of summer and, indeed, the mean total calcidiol concentration, even the lower limit of its confidence interval, remained above the threshold of vitamin D deficiency during winter.

## Acknowledgements

All participants in this study are acknowledged.

## Funding

No financial support was received.

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