Vitamin D status in Belgian young adults: a retrospective study

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To our dear reader(s):
The paper directly below, marked with a ***bordeaux border****, is the preliminary research conducted for masterproef II, it is a literature review that provides the context for the actual thesis. The main body of text (Masterproef III) can be found in the pages following thereafter.*

Literature Review:

Vitamin D deficiency in young adults

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Introduction

Vitamin D, also known as calciferol, is a fat-soluble vitamin that may be sourced either exogenously via diet or endogenously through synthesis via cutaneous exposure to sunlight. It plays a critical role in calcium homeostasis and is essential in bone (re)modelling [1]. To determine vitamin D status, we use serum 25-hydroxyvitamin D (25(OH)D) since it is the optimal biochemical marker primarily due to its relatively long half-life [2-3]. Unsurprisingly, being severely vitamin D deficient has clinical drawbacks for both children and adults as it causes nutritional rickets and osteomalacia for these populations respectively. The consensus on the titer of 25(OH)D (the optimal biochemical marker of vitamin D status) needed to prevent rickets in children has been established quite unanimously.[4-5] In contrast, the serum 25(OH)D concentrations or vitamin D intake required for adequate extra skeletal health are still the subject of intense debate. However, this cannot be attributed to lack of academic effort, many RCT's have been performed over the last 10 years but no real consensus has been reached [6]. This is unfortunate, considering vitamin D deficiency has been associated with cancer, diabetes mellitus, cardiovascular disease [7], dementia [8-9], frailty [10] and even immune disorders [11-12].The latter being based primarily on substantial preclinical and in vitro models where suboptimal serum 25(OH)D is associated with both higher risk of infections and development of auto-immune diseases [13-14].

Vitamin D deficiency poses a significant problem both in modern day Europe and globally. This endocrinological pandemic is estimated to cost the EU 187.000 million Euro per year, rendering it a problem worth combatting [15]. At present there are no official guidelines for vitamin D supplementation in Belgium, forcing most clinicians to rely on Dutch guidelines for daily practice. Although not ideal, the similarity in populations and culture in combination with geographical proximity makes these the most relevant guidelines available. Notably, young adult patients are not systematically advised to supplement vitamin D regardless of seasonality in these guidelines. The arguments for not supplementing this age group are relatively poorly defined and do not seem based on high quality regional data. Furthermore, we are still uncertain of the clinical impact that vitamin D deficiency carries with respect to young adults [16].

Present systematic review was performed to assess the prevalence of vitamin D deficiency in the age group 18-25 and a classical review was subsequently performed to review its clinical impact.

2. Methods

2.1 Search and study collection

We consulted the following databases for this review: PubMed, Science Direct and Thomson Reuters Web of Science. The following search terms were used: ((((vitamin D[Title]) OR (vit d[Title])) AND (deficien*[Title])) OR (insufficien*[Title])) AND ((young[Title]) OR (adolescen*[Title])) AND (y_10[Filter]). All articles published before the 1st of October 2021 were included in this search, starting from the 1st of October 2011. No additional search filters were applied, with the exception of 'Medicine and Dentistry', 'review articles', 'research articles' and 'practice guidelines' in the Science Direct search. The latter also did not support wildcards for search queries. This problem was solved by readjusting the search terms to "insufficiency" and "adolescents". The results were directly imported into EndNote© after which duplicates were removed using the 'find duplicates' function.

2.2 Study selection

Two independent reviewers (C.H. and S.W.) filtered the search results by manual exclusion based on title. If there were differing opinions on title relevancy, this was settled by debate until a mutual agreement was reached. Studies deemed to have irrelevant titles to the research question were subsequently discarded. A second round of exclusion was performed using a customized set of criteria for abstract relevancy. These criteria may be found below:

- The article must be fully written in English and/or Dutch.
- The article must be a study, review (systematic or otherwise) or meta-analysis.
- The article must feature a study (sub)population between the ages of 18-25.
- The article must feature a study (sub)population above or below the 40th parallel*

Articles that met each criterium were withheld for further analysis. After this step, each article was assessed via full text read-through by the aforementioned independent reviewers. Articles lacking in validity, failed to reasonably describe potential bias or conflict of interest were discarded. If the article measured vitamin D levels in patients where supplementation status was unknown it was also discarded. The remaining articles were estimated to be up to rigorous scientific standard and relevant to our research question and thus were included in this review.

*This criterion is essential. The distribution of solar ultraviolet radiation in the world is unreservedly unequal as it can only reach the skin when the sun is adequately high in the sky for the UVB to penetrate all layers of the atmosphere. This means regions beyond the 40th parallel in mid and high latitude regions only get sufficient UVB exposure in a very limited amount of time, broadly speaking this comprises the middle of spring to the end of the summer. [17]. This can be observed in Figure 2: A world map of UVB radiation distribution.

2.3 Study quality

Study quality in this review was assessed with the Oxford Centre for Evidence Based Medicine (OCEBM), version 2.1, 2011 [18-19]. In the case of conflicting opinions regarding study quality, it was debated until a mutual agreement was reached between independent reviewers.

2.3 Data Selection

Data extracted from the articles included author, publication date, seasonality, region, number of patients included between the ages 18-25, the vitamin D titers of these patients including units used, sex, specific patient traits and 25(OH)D cut-off values. This data is shown in Table 1.

3. Results

3.1 Literature search

The search was performed using the terms and filters described above in the methods section, it yielded 1246 results. PubMed and Science Direct yielded 330 and 916 articles respectively. No records were identified through alternative resources or by any other means available. After deduplicating these articles, 143 were removed and 1103 remained. The remaining records were screened for title, 1024 articles were found to have irrelevant titles and were discarded, narrowing the search down to 79 articles.

Based on abstract only 19 of the 79 articles were withheld for further analysis. After full text assessment only 5 articles were deemed eligible for inclusion, based on the criteria mentioned above. The process of data collection and selection is shown in Figure 1: PRISMA flow diagram showing the process of data collection, selection, and organization.

3.2 Vitamin D prevalence in young adults

Out of 5 selected articles, only one study was based in Russia [20] and all others were conducted in the US [21-24]. There were no European studies that fit the criteria necessary for inclusion. Total study population was 10962 patients, 10831 (98.8%) of whom were from US studies.

Definitions for the cut-off values pertaining to 25(OH)D insufficiency and deficiency were set at 20-30 ng/mL and <20 ng/mL respectively for 3 of the 5 included studies [20-23-24]. The other studies used a slightly higher cut-off for insufficient titers at 20-32 ng/mL [21] or pooled insufficient with sufficient titers and denominated them as 'not deficient' [22]. The pooled data was not used for the analysis of this study, the slightly elevated cut-off for VDI was accepted without correction as the absolute difference was deemed insignificant.

None of the included study populations met the exact age criteria, so the closest approximating age-group was used. Total patient age range was 2-40y, however more than 94% of patients were in the 18-39 age bracket. Seasonality is an important factor in interpreting 25(OH)D titers, all the included studies had samples from both seasonal extremes (winter and summer) except for Bezuglov et al, in which blood was only sampled during the winter.

Out of the 5 included studies, two featured a population with no known underlying conditions. Ammerman et al studied only female patients with lower extremity injuries. Sadhir et al examined and compared patients with PCOS and healthy subjects, both groups were included since there is no proven effect of PCOS on 25(OH)D titers. Kalkwarf et al compared healthy patients to similarly aged patients with various stages of chronic kidney disease. Only the

healthy group of patients were withheld for this study because of the proven effect of CKD on 25(OH)D titers.

Across all included studies the reported 25(OH)D titers were sufficient in 2483 (22.7%), insufficient in 3886 (35.4%) and deficient in 4403 (40.2%) patients. 190 (1.7%) patients were denoted 'non-deficient' and could not be readily categorized as either sufficient or insufficient.

	Country	Patients N = 10962	Age	Sex	Season	Sufficient 25(OH)D	Insufficient 25(OH)D	Deficient 25(OH)D	Traits	Titer cut-off (N, I, D)
Bezuglov et al. (2019)[20]	Russia	131	12-23	M	Wi	75 (57.3%)	26 (18.4%)	30 (22,9%)	Soccer players	(>30, 20-30, <20)
Ammerman et al. (2021) [21]	USA	105	16-40	F	Wi, Sp, Su, Fa	36 (34.3%)	41 (39.0%)	28 (26.7%)	Lower extremity injury	(>32, 20-32, <20)
Liu et al. (2018) [23]	USA	10343	18-39	M, F	Wi, Su	2358 (22.8%)	3785 (36.6%)	4200 (40.6%)	N/A	(>30, 20-30, <20)
Sadhir et al. (2015) [24]	USA	107	12-21	F	Wi, Sp, Su, Fa	14 (13.1%)	34 (31.8%)	59 (55.1%)	PCOS & control	(>30, 20-30, <20)
Kalkwarf et al. (2012) [22]	USA	276	5-21	M, F	Wi, Su	0-190	0-190	86 (31.0%)	N/A	(N/A, N/A, <20)

Table 1 Overview of included studies and data results

4. Discussion

Clinical significance of Vitamin D in- and deficiency in young adults

The negative effects of vitamin D deficiency specifically in the young adult population (18-25) has not been studied extensively. However, this age group has been commonly featured in major trials studying the effects of vitamin D on both the skeletal and extraskeletal systems. Impact of vitamin D titers on skeletal health is both extensively described and well recognized [25-26], however the same cannot be stated for its effects on the extraskeletal systems. The latter have been subject of conflicting data and consequently, intense debate.

Skeletal Health

The best-known effect of a deficiency in vitamin D in the non-geriatric skeletally mature adult is the development of osteomalacia. This disease is marked by the decreased mineralization of newly formed osteoid at the sites of bone turnover. The underlying mechanism is relatively simple and has been known for a few decades. Deficiencies in vitamin D and calcium prevent the deposition of calcium in the skeletal structures. As this deficiency progresses and dips below <20 ng/mL it maximally stimulates the parathyroid glands which causes secondary hyperparathyroidism [27-29]. This extra secretion of PTH increases the metabolism of 25-hydroxyvitamin to 1,25-dihydroxivitamin D which makes the deficiency worse. In addition, supraphysiological PTH causes hypophosphatemia through increased phosphaturia. Both these processes result in an inadequate calcium-phosphorus product which diminishes the mineralization of collagen matrix [27-30]. The symptoms traditionally associated with osteomalacia are diffuse joint and bone pain, muscle weakness and difficulty walking [31-32].

Extraskeletal Health

Type 1 Diabetes (T1DM)

Studies researching the role of vitamin D status and the development of type 1 diabetes are plentiful due to pancreatic beta cells expressing 1-alpha hydroxylase through vitamin D receptors [33]. While both interventional [34-35] and observational studies [36-39] have found a beneficial effect of vitamin D supplementation causing a protective effect in preventing the development of T1D and improving outcomes. There are many other studies that have not been able to demonstrate such a beneficial relation, mainly through finding no benefit to maternal vitamin D supplementation on the risk of developing T1D in the offspring [40] and not being able to demonstrate a positive impact on beta-cell function [41]. It is still unclear what the reason is for these conflicting findings, more qualitative RCT's investigating the relationship between vitamin D and preventing and/or prolonging endogenous insulin secretion should be conducted.

Multiple sclerosis (MS)

Multiple sclerosis is a heterogeneous disorder mainly in the young adult with variable clinical and pathologic features reflecting different pathways to tissue injury [42]. It was determined to have higher prevalence in persons living above 35 or below -35 degrees latitude [43], leading to the theory that exposure to sunlight might be protective [44-46]. A few studies have demonstrated an inverse relationship between vitamin D titers, exposure to sunlight or ultraviolet radiation and prevalence of MS [46-53] or even disease activity in previously

diagnosed patients [54-56]. Some data suggests that 25(OH)D titers above 20 ng/mL in the mother and infant are protective against MS [57], a study also showed first trimester exposure to ultraviolet radiation reduced the infant's risk of developing MS [58]. Mclaughlin et al conducted a meta-analysis of 12 RCT's studying vitamin D supplementation and MS progression and found trends of improvement in relapse rate and MRI findings, though these findings were determined to be non-significant. Interestingly, these improvements were significantly reduced in higher doses (2850-10400 IU) compared to lower doses (800-1000 IU) [59].

In summary, though there is no shortage of studies examining vitamin D and its role in the prevention and disease control of MS. However, current data is mainly from case-control and epidemiological studies, existing RCT's studying the positive effects of vitamin D supplementation in MS outcomes are insufficient. Lastly, while positive trends of vitamin D supplementation in lower doses are demonstrated in MS progression, significant improvements have yet to be demonstrated.

Systemic Lupus Erythematosus (SLE)

SLE is a chronic autoimmune disease of unknown cause that can affect virtually any organ of the body. While onset of disease is technically possible at any age, only 35% of cases manifest before the age of 16 and after 55, making it a disease predominantly affecting the young adult population [60-61]. Multiple interventional studies have shown clinical improvement in patients supplementing with vitamin D, they also reported beneficial trends in inflammatory and hemostatic markers in patients taking vitamin D supplements [62-63]. A meta-analysis of 3 RCT's which studied vitamin D's effect on anti-dsDNA positivity reported a significant reduction in the experimental group [64]. One study supplemented 100.000 IU weekly for 4 weeks and subsequently monthly for half a year and there were 0 reported flares [65].

Overall, current data is rather limited but suggests low 25(OH)D can worsen disease burden and there is evidence for vitamin D supplementation in SLE patients. Worth mentioning, is the finding that the disease is more likely to worsen after ultraviolet radiation exposure. This makes supplementation the preferable method of vitamin D upregulation over natural exposure to sunlight [66].

Psoriasis

A chronic inflammatory skin diseases which exhibits a variety of clinical manifestations, it has a bimodal peak when looking at age of onset for women and men. For the former, this is between 20–29 and 50–59 years of age, whereas in men this tended to occur around 30– 39 and 60-79 years of age [67]. Keratinocytes are key players in the maintenance of the cutaneous barrier integrity when they differentiate, and this happens through vitamin D binding with their vitamin D receptors [68]. This mechanism has led to the usage of topical vitamin D analogs as treatment for psoriasis [69]. However, there is currently no literature that suggests vitamin D deficiency causes an increased risk of developing the disease [70-71]. Moreover, no correlation was shown between 25(OH)D titers after phototherapy and disease severity [71-72]. However, notable interventional studies did show two positive trends regarding vitamin D supplementation. A 60.000 IU dose of vitamin D supplemented every 2 weeks versus a placebo

group reported a significant reduction in the Psoriasis Area and Severity Index (PASI) [73]. When used in combination with corticoids, topical vitamin D analogues were reported to be effective in reducing the severity of plaque psoriasis [74-76].

In summary, current data does not prove a causative relationship between vitamin D titers and psoriasis. In addition, findings of correlation between severity of the disease and 25(OH)D levels are promising but we need larger high quality RCT's to confirm these trends.

Inflammatory bowel disease (IBD)

Crohn's disease and colitis ulcerosa are the two major disorders comprising the spectrum of IBD. UC tends to mainly affect the colon while Crohn's disease can involve any part of the gastro-intestinal tract, however there is considerable overlap and sometimes differentiation is impossible [77]. Although presentable at any age, the majority of onset happens between the ages of 15 and 30. A study using the data from the Rochester Epidemiology Project showed young adults aged 20 to 29 have the highest incidence of Crohn's disease [78]. Supplementation of vitamin D has been proven protective against IBD [79]. A relation between seasonal 25(OH)D titers and risk for IBD has been proposed with associations made between a more severe disease progression and vitamin D deficiency [80-81]. Moreover, a recent meta-analysis reported a two-fold risk of deficient vitamin D levels in patients with IBD and a more severe disease course in those who had 25(OH)D titers <20 ng/mL [82-83]. However, a considerable problem exists with these publications because its data is confounded by the fact that IBD inherently causes inflammatory malabsorption which is known to bring about vitamin deficiencies.

Interventional studies are scarce, one RCT assessed the impact of vitamin D supplementation in adolescents and children and found lower levels of inflammatory markers in those who received higher dose versus those on lower doses (400 vs 2000 IU daily) [84]. While some data currently exists that points to a positive effect of vitamin D on the course of inflammatory bowel disease, we still need much more data in the form of qualitative RCT's to draw hard conclusions.

Asthma and respiratory infections

Asthma bronchiale is a non-communicable and common disease of the lower airways, it is characterized by bronchial hyperresponsiveness and inflammation [85]. Its prevalence in the between the ages of 20-25 is around 9%, although disparities do exist due to demographical, environmental, and socio-economic differences [86]. An association has been made between worse disease control, increased airway inflammation, poor prognosis, and vitamin D deficiency [87]. A possible positive effect of vitamin D supplementation in improving exacerbations was reported in a literature review of interventional studies [88-89]. However, this positive trend was not shown with regards to the severity of asthma [89]. The meta-analysis of the adult populations in this review had similar findings. A reduction was found in asthma exacerbations requiring systemic corticosteroids and hospitalization. However, there was no observed reduction in the lung function testing or Asthma Control test Scores [90]. The positive trend in exacerbations were contributed to the decrease in respiratory tract infections [91]. Interventional studies assessing the effect of vitamin D supplementation (300-1200 IU

daily) found a potential reduction in influenza infections [92]. Of note, this risk reduction could only be observed in the first month of supplementation and was subsequently lost in the following month. Over the entire season, influenza prevalence was no different for both groups [93]. A large meta-analysis conducted in 2017 which reviewed 25 RCT's found an overall reduction in acute respiratory infections with significantly more benefit in those with severe deficient 25(OH)D titers (<10 mg/dL). This improved benefit was also observed in those receiving daily and weekly supplements over the patients receiving bolus doses [94-95]. The general interest in vitamin D and its potential effects on the prevention and severity of acute respiratory infection has grown substantially with the advent of the COVID-19 pandemic. This is due to the ease of access and use of vitamin D supplements and the observation that COVID-19 deaths were greater in populations at high risk for 25(OH)D deficiency [96]. A systematic review and meta-analysis consisting of 31 observational studies was performed and found a positive trend but no statistical significance in low 25(OH)D titers and COVID-19 related outcomes [96]. However, considering the low quality of the included studies and the very limited clinical drawbacks of supplementation, this preventative therapy should not be readily dismissed. Overall, data does seem to suggest an effect of 25(OH)D levels on reducing the risk of developing an acute respiratory infection. There is substantial evidence that supplementing vitamin D decreases asthma exacerbations, although it does not improve the disease severity.

4.2 Systematic Review of Vitamin D Prevalence in the young adult

Vitamin D deficiency in Europe was marked as a pandemic by Cashman et al in 2016, where they assessed the data of 55.844 European patients. They reported that 40% of individuals had deficient vitamin D titers. Although our study does not feature any European studies, it is apparent that this is a problem that extends beyond the borders of Europe. Approximately 40.2% of patients were found to be vitamin D deficient in this study. It is important to note there is no overlap in data, no studies included in Cashman et al were included in this review.

5. Limitations

None of the included studies of the systematic review were conducted in Europe, and 4 out of 5 were based in the US. Although all reviewed studies were above or below 40 degrees latitude to account for differences in UVB radiation, genetic and skin type differences are more difficult to correct for. The large diversity of the US population is likely to result in a higher prevalence of vitamin D deficiency. Though many studies were considered, only 5 made it to the full review with more than 94% of the included patients stemming from a single study. Consequently, the results of this review and the aforementioned study are close to identical with very little impact from the other included studies, despite large differences in findings. None of the included studies researched the exact age group 18-25, more than 94% of all patients were between 18-39 years old, it is possible that the deficiencies found in this age group are predominantly in the older end of the age interval, though evidence does not suggest a significant asymmetrical distribution.

6. Conclusion

High prevalence of vitamin D deficiency in the European population has been described for quite a few years. This endocrinological problem appears to extend beyond Europe into countries with similar UVB distribution. Individuals aged 18-25 seem to be no exception and appear to be affected in equal numbers relative to their population size. European data on vitamin D levels in young adults gathered in the last 10 years is almost non-existent.

The impact of a low vitamin D titer on the skeleton have been described and uniformly accepted for decades. However, it is proving difficult to assess the impact of vitamin D deficiency on the extraskeletal systems and particularly so in the young adult. It is possible that maintaining a sufficient 25(OH)D titer protects the young adult against the development of auto-immune, inflammatory, and neurological diseases. It could also help in reducing symptom severity in those who have already developed these diseases. However, despite positive trends reported in some studies, most interventional trials failed to report significant effects of vitamin D sufficiency on prevention or amelioration of disease processes. We need more qualitative interventional studies to conclusively determine the extraskeletal impact of vitamin deficiency.

7. Conflict of interest

The authors report no conflict of interest.

8. Funding

The authors declare no funding of any kind.

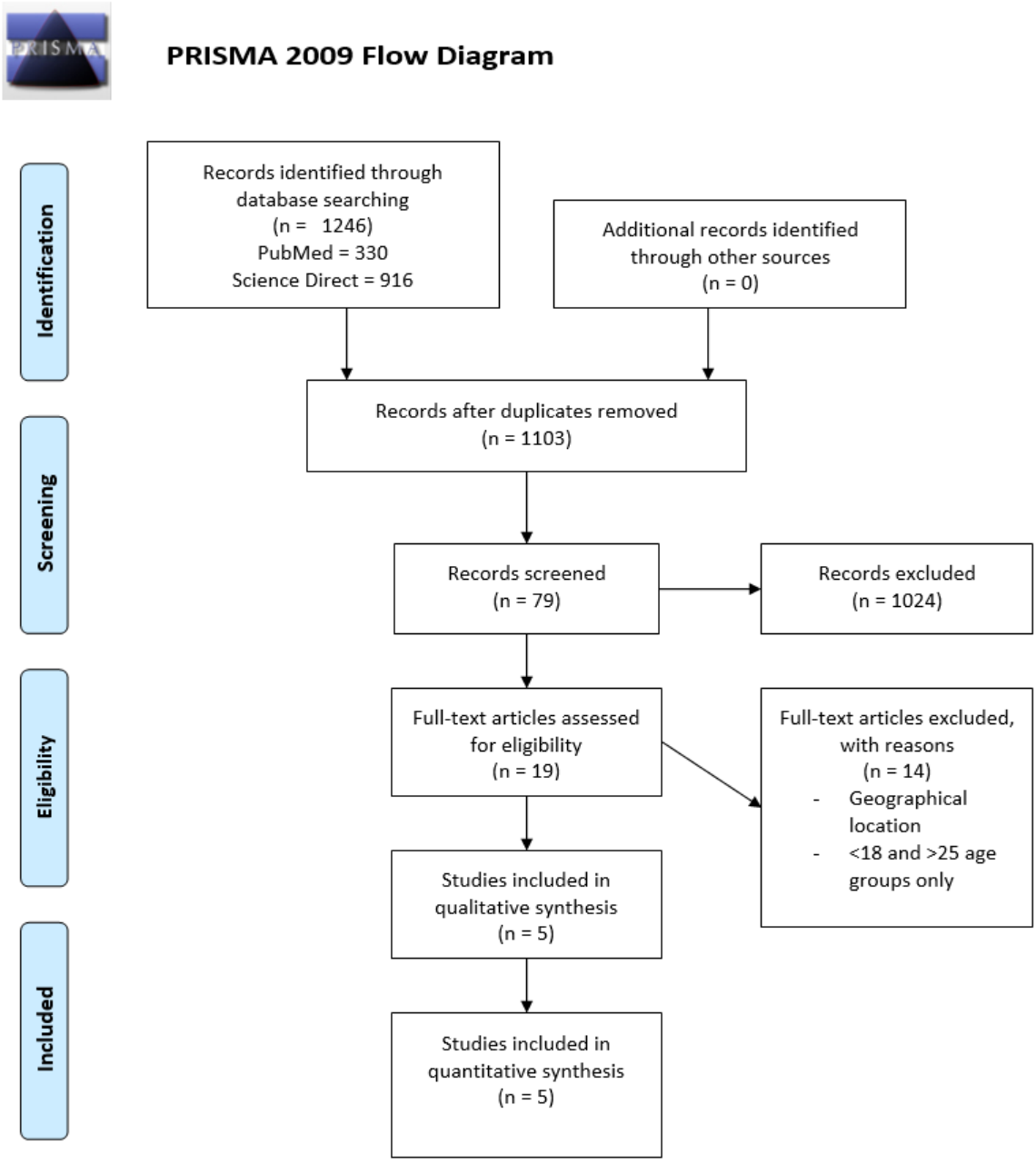


Figure 1 PRISMA flow diagram showing the process of data collection, selection, and organization.

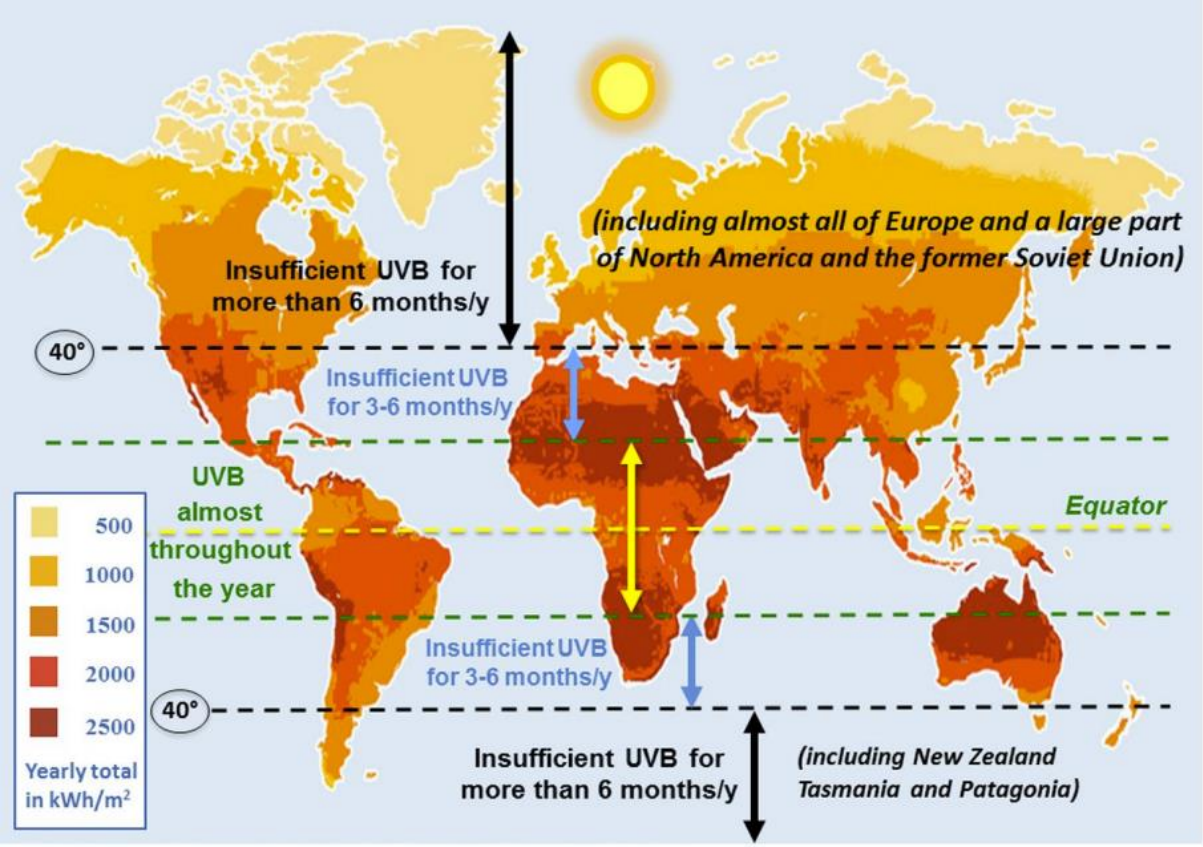


Figure 2 A world map of UVB radiation distribution.

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ABSTRACT

Background

The clinical impact of vitamin D deficiency has been the topic of intense debate for years, it poses a significant problem both in modern day Europe and globally. In January 2021 the high council of health in Belgium published advice to determine vitamin D status in all those who are at high risk to contract COVID-19 and those infected at time of presentation, and to supplement those who are found to be deficient. At present there are no official guidelines for vitamin D supplementation in Belgium, forcing most clinicians to rely on Dutch guidelines for daily practice. Although not ideal, the similarity in populations and culture in combination with geographical proximity makes them the most relevant guidelines available.

Notably, young adult patients are not systematically advised to supplement vitamin D regardless of seasonality in these guidelines. The arguments for not supplementing individuals from this age group are relatively poorly defined and do not seem to be based on high quality regional data.

Aims

To investigate the levels of serum vitamin D (25(OH)D) in young adults aged 18-25 and to identify the underlying risk factors for vitamin D deficiency and insufficiency.

Methods

This retrospective cohort study was conducted at 3 private primary care centers in Antwerp and Oost-Vlaanderen, Belgium. Patients between the ages of 18 and 25 whom had their vitamin D levels determined between October 2020 and October 2021 were included. Values were measured using electro chemiluminescence immunoassay (ECLIA). Definitions for the cut-off values pertaining to 25(OH)D insufficiency and deficiency were set at 20-30 ng/mL and <20 ng/mL. A questionnaire (attachment A1) designed to quantify risk factors was sent to all included patients via email or telephone. Data from blood analysis and questionnaires were then subsequently analyzed using various statistical models, primarily logistic regression.

Results

In the primary analysis, we included 221 patients, 54 from center A, 84 from center B and 83 from center C. The mean age was 21.9 ± 2.8 and most patients were female (65.2%). Only 45 (20.4%) patients had sufficient titers, 77 (34.8%) were insufficient and 99 (44.8%) were deficient in 25(OH)D. The majority (79.6%) was found to be at least insufficient (<30 ng/mL).

Both male and female patients had average insufficient average vitamin D levels, 20.2 ± 8.9 ng/mL and 22.9 ± 10.9 ng/mL respectively. We failed to demonstrate a significant difference between sexes when comparing vitamin D levels (p=0.059).

Seasonality was found to be significantly related to 25(OH)D levels (p<0.001). Winter and spring analyses had deficient average titers, 19.3 ± 8.3 ng/mL, and 18.1 ± 8.0 ng/mL respectively. Vitamin D levels determined during summer and autumn were insufficient on average and respectively 26.1 ± 10.8 ng/mL and 22.0 ± 10.6 ng/mL.

Major factors associated with deficiency and/or insufficiency were diary intake (OR 5.556 per point, 95% CI 1.071 to 28.821)

Conclusion

The European problem of widespread vitamin D deficiency also extends into the less studied group of young adults between the ages of 18-25. The current vitamin D supplementation strategy appears to be failing in this population if the target titer of >30 ng/mL is upheld, considering we found only 1 in 5 reach this threshold. More extensive research into this subject is necessary to determine if systematic supplementation in young adults is needed.

1. Introduction

Calciferol, also known as vitamin D, is a fat-soluble molecule which can be sourced either exogenously via dietary intake or through biochemical synthesis via skin exposure to sunlight [1]. Serum measurements are made with the biochemical marker 25-hydroxyvitamin D (25(OH)D), owing to its relatively long half-life [2-3]. The negative effects of severely low 25(OH)D titers in children and adults have been adequately researched, it manifests clinically as rickets in pediatric patients and osteomalacia for skeletally mature individuals. The titers required for these pathologies to arise, or rather to prevent then, have also been quite unanimously established [4-5]. In stark contrast, impact of vitamin D deficiency on extra skeletal health have been the topic of intense debate and no definite consensus has been reached despite the myriad of RCT's conducted in the last decade [6]. This is most unfortunate, considering deficiency in vitamin D has been associated with a wide range of debilitating and costly pathologies such as cancer, diabetes mellitus, cardiovascular disease [7], dementia [8-9], frailty [10] and (auto)immune disorder [11-12]. Though the associations with immune disorders are primarily based upon preclinical and in vitro models, they are substantial and demonstrate deficient vitamin D titers are associated with higher infection risk and increased odds of developing auto-immune diseases [13-14].

Vitamin D deficiency is a widespread and significant problem both globally and in Europe. In 2009, Grant et al estimated that it costs the EU around 187.000 million Euro per year. Subsequently, it can be considered a problem worth eliminating [15]. Despite the known economic problems posed by the endocrinological pandemic some countries in Western-Europe have not developed their own guidelines. Belgium, for example, does not have its own vitamin D supplementation guidelines and instead Belgian clinicians opt to follow those written by their colleagues from the Netherlands. Although not ideal, similar populations and latitudes make Dutch guidelines the most relevant option. Of note in these guidelines, young adults are not systematically advised to supplement vitamin D regardless of seasonality. The arguments presented for these recommendations are dated, few in numbers, poorly defined and do not seem based on high quality regional data.

In January 2021 the high council of health in Belgium published advice to determine vitamin D status in all those who are at high risk to contract COVID-19 and those infected at time of presentation, and to supplement those who are found to have deficient vitamin D titers (HGR). This advice mostly owing to the growing evidence that vitamin D plays a role in regulating the

immune response against viral infections, specifically the deficiency thereof conferring increased susceptibility to enveloped viruses [16].

In a literature review performed by our team prior to this study (cfr supra), we found that European data on vitamin D levels in young adults gathered in the last 10 years is nearly non-existent. Analysis of data outside of Europe, in countries with comparable UVB distribution, which included 5 studies and 10962 patients [17-21] showed that within the age group of 12-40, 35.4% had insufficient vitamin D titers and 40.2% were vitamin D deficient. In addition, in this review we researched the clinical effects of low vitamin D titers in young adults. We reaffirmed that nutritional osteomalacia manifests at prolonged titers below 10 ng/mL in this age group. Regarding the effect of vitamin D status on extra-skeletal health, positive trends were commonly described but most interventional trials failed to report significant effects of vitamin D sufficiency on prevention or amelioration of disease processes.

2. Aims

The primary aim of this study is to investigate whether young adults in Belgium between the ages of 18-25 have sufficient (>30 ng/mL) titers of 25(OH)D. The secondary aim is to determine the underlying risk factors associated with vitamin D deficiencies.

3. Ethics Approval

The research protocol was approved by the Ethics Committees of the Antwerp University Hospital/University of Antwerp on the 23rd of May 2022 and the study was conducted in compliance with the Declaration of Helsinki (local project ID 1964).

4. Methods

4.1 Study Population and Enrolment Procedure

Patients were eligible for this study if they were (i) aged 18 to 25 years old at the time of having their blood drawn, (ii) had 25(OH)D serum titers analyzed on blood samples taken between the 1st of October 2020 and the 1st of October 2021 and (iii) were not supplementing vitamin D at the time of their blood draw. Eligible patients were contacted through either email or telephone, this information was obtained from their contact records within the medical software from each primary care center. No explicit informed consent (IC) was needed as data was internally available and retrospectively retrieved by the study investigators. Only vitamin D titer, age and sex were extracted for patients who declined to complete the questionnaire or were unable to be contacted. Lastly, verbal informed consent was given by patients who completed the questionnaire, they were informed of the study specifics through an informative letter (attachment A2).

4.2 Data Collection

Following the approval of the research protocol by the ethics committees, all three participating primary care centers were visited by the researching physicians. Through medical program CareConnect™ all eligible patients were extracted from the databases using the 'statistics' module. Patients' medical data was retrieved through the voluntary questionnaire and cross-referenced with their electronic patient record. The questionnaire was based mainly on the

already identified possible risk factors described by Bolek-Berquist et al [22]. In case of missing or contradictory information the patients were subsequently contacted to complete or correct the data set. The data provided by participants which was then cross-referenced included: name, age, sex, skin type, history of chronic illnesses, medications, hobby's, BMI, smoking status, sunlight exposure, tanning bed exposure, usage of UV blocking lotion, dairy intake, fish intake, vitamin D supplementation and weight changes. If multiple blood analyses with 25(OH)D titer determination were found, the oldest one was withheld for analysis.

4.3 Sample Size

For the primary objective a sample size of 78 patients was calculated (alpha=0.05 and power=0.8). This was based on Liu et al's study of 10343 patients between the ages of 18-39, 65.6% of whom were found to have below 30 ng/mL levels of 25(OH)D [20]. Sample size calculation was performed as 'one study group vs. population' with a dichotomous primary endpoint, in this case the patient could have either sufficient or insufficient vitamin D levels. The anticipated incidence was set at 80%, based primarily on the fact that the HGR advice was published mid-winter, making it likely our titer data would be asymmetrically distributed towards winter and spring. Considering the content of the advice, the recommendation to test positive individuals, and the now known pandemic statistics this also predisposes our data to be asymmetrically distributed towards winter and fall. Consequently, we expect to find more vitamin D deficiencies in our population compared to that of our reference study.

For the secondary objective a sample size of 279 patients was calculated (alpha=0.05 and power=0.8). These numbers were based on Bolek-Berquist et al's study which found 3 of 11 questions in their questionnaire to be significant in predicting vitamin D deficiency in subjects with a mean age of 24 [22].

Unfortunately, we only reached the target inclusion rate for our primary objective. Failure to reach the estimated inclusion rate for statistically significant results for the secondary was to be expected, considering that only around 900 patients from all practices combined fulfilled the age criteria.

4.4 Statistical analysis

Data was analyzed using IBM SPSS version 28.0.1. Variables were described as numbers and percentages or as mean ± standard deviation, as appropriate. For continuous variables, differences between two (un)paired groups were compared using the paired-samples t test or independent samples t test. The chi-squared test, the McNemar test and Fisher's exact test were used for categorical variables, as appropriate. All comparisons were tested two-sided. P-values < 0.05 were considered statistically significant. The relative risks (RRs) and odds ratios (ORs) were calculated and reported with their 95% confidence intervals (CIs) for significant categorical predictors for vitamin D insufficiency (<30 ng/mL). For continuous variables, univariate logistic regression models were used to calculate the ORs (with their 95% CI), and p-values were derived from the likelihood-ratio test. Candidate variables, categorical as well as continuous, with a p-value <0.10 were considered for multivariate regression analysis and the optimal regression model was composed using a backward elimination strategy, based on the similarities between this study and the study performed by Zhang et al [23].

5. Results

5.1 Primary analysis

5.1.1 Patient selection and characteristics

For the primary objective the medical software statistics module detected 241 eligible candidates. Two patients from practice C were excluded for not having a blood sample registered on the system at the time of manual review. Moreover, through manual review 17 patients were also excluded for being on supplements at the time of their blood draw. In total, 221 patients were included, 54 from practice A (24.4%), 84 from practice B (38.0%) and 83 from practice C (37.6%) (Fig 1).



Figure 1 Primary analysis patient selection

Table 1 presents the baseline characteristics of the included patients. A total of 221 patients were included in our study, with a mean age of 21.9 ± 2.8. Of those patients 144 (65,2%) were female and 77 (34,8%) were male.

		Patients (N=221)
Sex	**Male, *n (%)***	77 (34.8%)
	Female, *n (%)*	144 (65.2%)
Age (yrs), *mean ± SD*		21.9 ± 2.8

Table 1 *Baseline characteristics of patients in the primary analysis*

One-sample Kolmogorov-Smirnov testing proved a normal distribution for the registered vitamin D titers (p=0.043). Subsequently, these values were evaluated by performing a one-sample t-test with the test value set at 15.99 based on the findings of Liu et al. This yielded a statistically significant result (p<0.001), indicating that the average 25(OH)D level we've measured is significantly different from Liu et al's determined average [20].

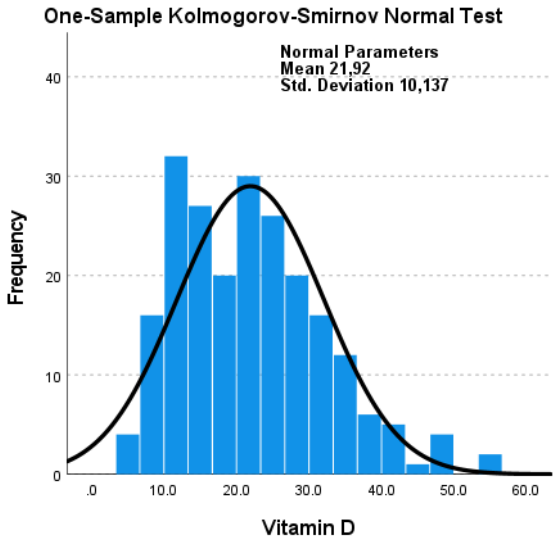


Figure 2 *1 sample K-S graph proving normal distribution in the primary analysis population vitamin D titers*

5.1.2 Sex and vitamin D levels

Of all males tested 36 were vitamin D deficient (46.8%), 31 were insufficient (40.3%) and only 10 had sufficient levels of vitamin D (13.0%). In the female population these were similar, we identified 63 deficient females (43.8%), 46 were insufficient and 35 were considered sufficient (24.3%).

Mean vitamin D titer for all included patients was found to be 21.9 ± 10.1 ng/mL. Male patients had a mean vitamin D level of 20.2 ± 8.9 ng/mL, slightly lower than the female patients who averaged at 22.9 ± 10.9 ng/mL 25(OH)D.

Interestingly, both male and female patients in this study had insufficient mean vitamin D levels and 79.6% of all patients had at best insufficient 25(OH)D titers (<30 ng/mL). Even though more than half of all blood analyses were performed during summer and autumn (53.0%).

Lastly, we failed to demonstrate a significant difference between sexes when comparing vitamin D levels by performing a one-way analysis of variance (ANOVA) test (p=0.059).

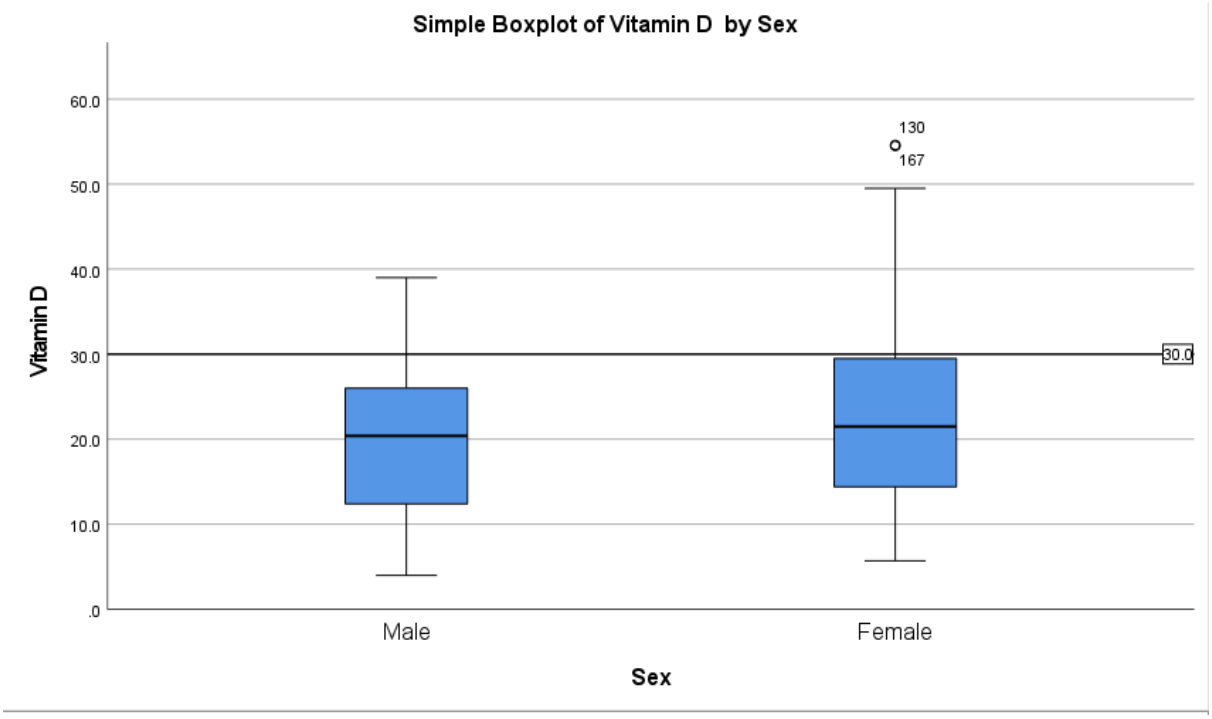


Figure 3 *Boxplot of male vs female vitamin D titer distribution*

5.1.3 Season and vitamin D levels

Winter analyses had a mean 25(OH)D level of 19.3 ± 8.3 ng/mL, blood samples drawn during springtime were found to have even lower levels at 18.1 ± 8.0 ng/mL. Summer and autumn showed slight improvements, in these seasons vitamin D titers were found to be 26.1 ± 10.8 and 22.0 ± 10.6, respectively.

To summarize, none of the seasons had a sufficient mean vitamin D titer (>30 ng/mL). Winter and spring had mean deficient 25(OH)D levels. (Table 2)

ANOVA testing determined that in our study, season was significantly related to vitamin D level (p<0.001).

	Patients (N=221)	Mean	95% Confidence Interval for Mean		Minimum	Maximum
			Lower Bound	Upper Bound		
Winter, n (%)	45 (20.4%)	19.3 ± 8.3	16.9	21.8	5.7	37.9
Spring, n (%)	59 (26.7%)	18.1 ± 8.0	16.0	20.2	5.5	36.8
Summer, n (%)	82 (37.1%)	26.1 ± 10.8	23.7	28.4	4.0	54.6
Autumn, n (%)	35 (15.9%)	22.0 ± 10.6	18.4	25.7	7.0	45.2

Table 2 *Distribution of patients and titers per season*

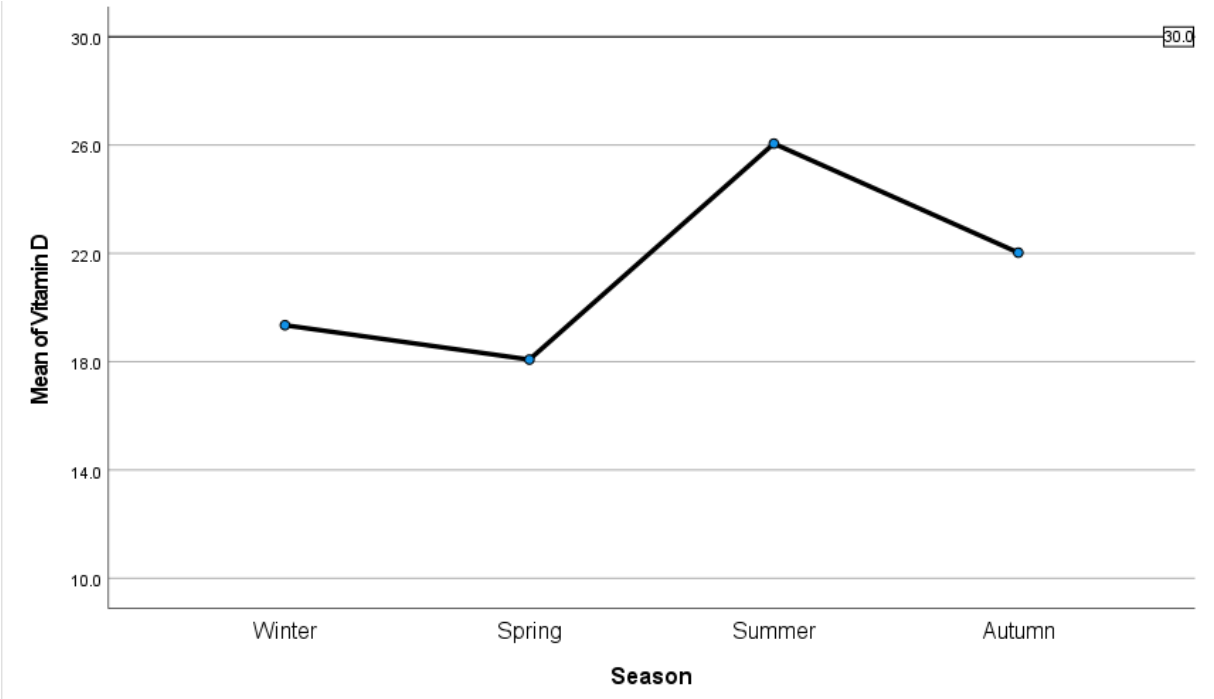


Figure 4 *Simple X-Y graph displaying vitamin D titer (Y) versus season (X)*

5.2 Secondary analysis

5.2.1 Survey response rate and selection

For the secondary analysis, 68 of 241 patients completed the questionnaire, indicating a 28.2% response rate. Two completed surveys were not withheld for further analysis due to the data not being available on the patient file at the time of manual review. Sixteen more surveys were discarded due to proven vitamin D supplementation at the time of their blood draw, either self-reported or through manual review. This resulted in a total of 50 surveys being withheld for statistical analysis (Figure 5).

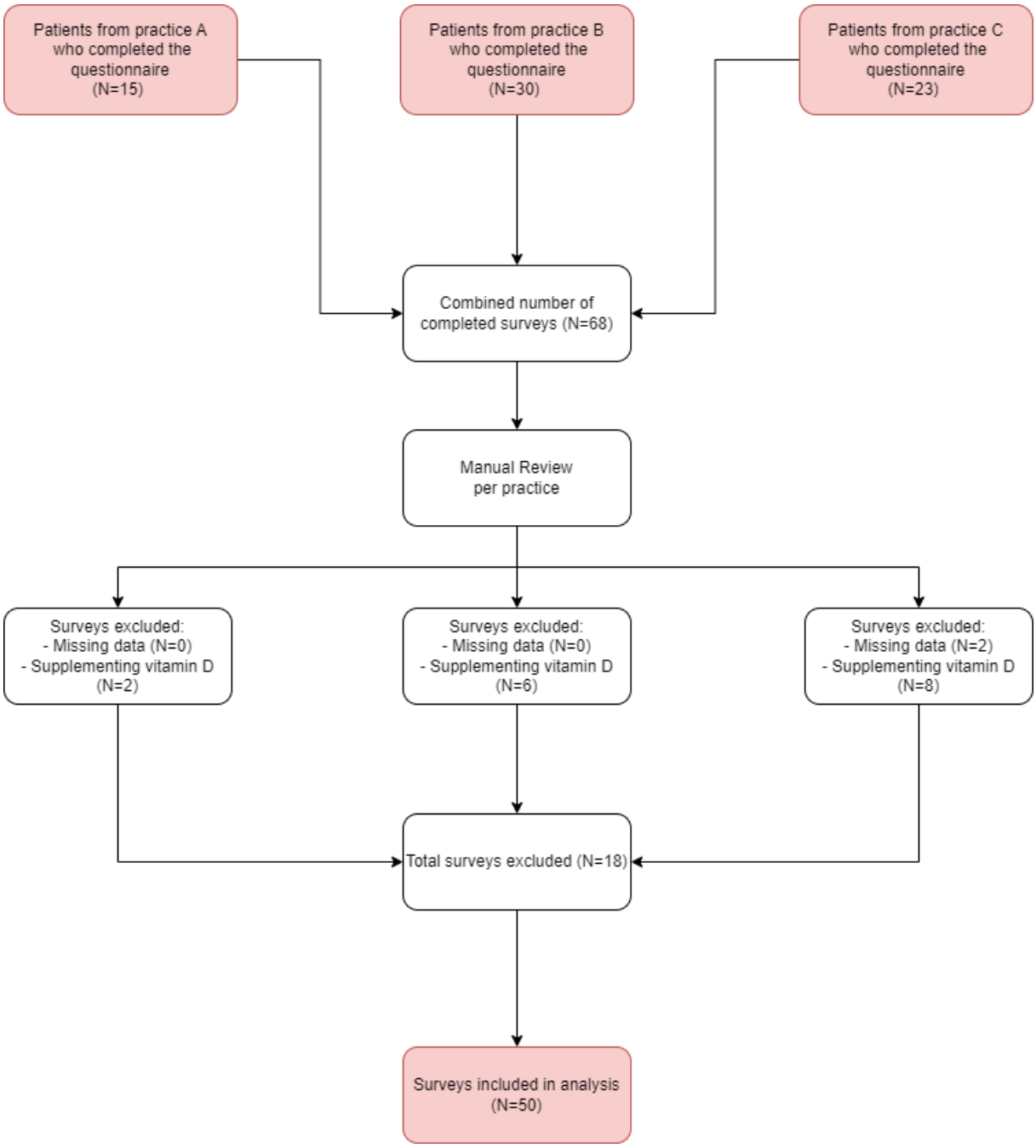


Figure 5 Flow chart displaying patient selection process for the secondary objective analysis

5.2.2 Survey population description

Table 3 describes the population description and their answers to the survey.

		Total Patients N=50 *N, (%)*	Vitamin D *ng/mL ± SD*
Practice	Practice A	13 (26.0%)	27.7 ± 16.4
	Practice B	24 (48.0%)	22.2 ± 11.4
	Practice C	13 (26.0%)	19.6 ± 10.1
Sex	Male	14 (28.0%)	17,1 ± 7.4
	Female	36 (72.0%)	25.2 ± 13.6
Skin Type	Type I & II	36 (72.0%)	23.2 ± 12.7
	Type III & IV	14 (28.0%)	22.2 ± 13.1
BMI	Underweight / Normal weight (<24,9 BMI)	38 (76.0%)	23.6 ± 12.8
	Pre-obesity / Obesity (≥25,0 BMI)	8 (16.0%)	19.5 ± 10.2
	Unknown	4 (8.0%)	23.8 ± 17.5
Medication and Supplements	Yes	22 (44.0%)	23.4 ± 11.6
	No	28 (56.0%)	22.6 ± 13.7
Smoking	Yes	9 (18.0%)	23.2 ± 17.4
	No	41 (82.0%)	22.9 ± 11.7
Weight	Yes, I lost weight	11 (22.0%)	25.6 ± 14.0
	Yes, I gained weight	9 (18.0%)	17.1 ± 6.1
	No	18 (36.0%)	22.4 ± 11.9
	No idea	12 (24.0%)	25.6 ± 15.8
Weight change	<5 kg lost	7 (36.8%)	23.5 ± 12.4
	≥5 kg lost	4 (21.1%)	30.0 ± 17.3
	<5 kg gained	5 (26.3%)	16.0 ± 7.9
	≥5 kg gained	3 (15.8%)	17.8 ± 3.7
Oily fish	Never	16 (32.0%)	25.4 ± 13.7
	Infrequently (≤1 per week)	33 (66.0%)	21.4 ± 12.2
	Frequently (>1 per week)	1 (2.0%)	34.5 ± N/A
Dairy products	Never	0 (0.0%)	
	Infrequently (<1 per day)	28 (56.0%)	26.0 ± 13.7
	Frequently (≥1 per day)	22 (44.0%)	19.1 ± 10.3
Chronic Disease	Yes	8 (16.0%)	28.0 ± 17.4
	No	42 (84.0%)	22.0 ± 11.6
Sunscreen	Never	5 (10.0%)	15.1 ± 11.3
	Infrequently	19 (38.0%)	24.2 ± 13.9
	Frequently	26 (52.0%)	23.5 ± 11.9

Tanning Bed	Yes	0 (0.0%)	
	No	50 (100.0%)	22.9 ± 12.7
Hobbies	None	11 (22.0%)	22.7 ± 14.3
	Mainly indoor hobbies (gaming, indoor fitness, card games, ...)	20 (40.0%)	20.6 ± 9.3
	Mainly outdoor hobbies (football, jogging, hiking, ...)	8 (16.0%)	25.3 ± 13.9
	Both	11 (22.0%)	25.7 ± 16.1
UV Exposure	Insufficient UV exposure	23 (46.0%)	22.1 ± 13.2
	Sufficient UV exposure	27 (54.0%)	23.7 ± 12.4
Vit D Status	Deficient (<20 ng/mL)	23 (46.0%)	13.1 ± 3.5
	Insufficient (20-30 ng/mL)	15 (30.0%)	22.9 ± 2.4
	Sufficient (>30 ng/mL)	12 (24.0%)	41.8 ± 9.6
Vit D Status	Insufficient (<30 ng/mL)	38 (76.0%)	17.0 ± 5.7
	Sufficient (>30 ng/mL)	12 (24.0%)	41.8 ± 9.6

Table 3 Descriptive table of all acquired variables for the secondary analysis

5.2.3 Vitamin D and practice

Practice A patients accounted for 26% of the completed surveys, practice B for 48% and practice C for 26%. Through binomial logistic regression, we determined that in our study practice was not able to significantly predict for vitamin D sufficiency (p=0.659).

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Practice			.460	2	.795			
	Practice(1)	-.524	.783	.448	1	.504	.592	.128	2.749
	Practice(2)	-.393	.891	.194	1	.659	.675	.118	3.873
	Constant	-.811	.601	1.821	1	.177	.444		

a. Variable(s) entered on step 1: Practice.

Table 4 SPSS output table from binomial regression analysis for variables practice & vitamin D status

5.2.4 Vitamin D and sex

Of all 50 included patients, 14 (28.0%) were male and 36 (72.0%) were female. Through binomial logistic regression, we determined that in our study sex was not able to significantly predict for vitamin D sufficiency (p=0.113).

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Gender	1.744	1.099	2.518	1	.113	5.720	.664	49.304
	Constant	-2.565	1.038	6.109	1	.013	.077		

a. Variable(s) entered on step 1: Gender.

Table 5 SPSS output table from binomial regression analysis for variables Sex & vitamin D status

5.2.5 Vitamin D and skin type

Of all 50 included patients, 36 (72.0%) reported having skin type I or II, and 14 (28.0%) reported having skin type III or IV. Through binomial logistic regression, we determined that in our study skin type was not able to significantly predict for vitamin D sufficiency (p=0.638).

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Skin Type	.336	.715	.222	1	.638	1.400	.345	5.681
	Constant	-1.589	.996	2.544	1	.111	.204		

a. Variable(s) entered on step 1: Skin Type.

Table 6 SPSS output table from binomial regression analysis for variables skin type & vitamin D status

5.2.6 Vitamin D and chronic disease

Of all 50 included patients, 8 (16.0%) reported suffering from a chronic disease, and 42 (84.0%) reported they did not. Through binomial logistic regression, we determined that in our study chronic disease was not able to significantly predict for vitamin D sufficiency (p=0.337).

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Chronic Disease	-.788	.821	.921	1	.337	.455	.091	2.274
	Constant	-.511	.730	.489	1	.484	.600		

a. Variable(s) entered on step 1: Chronic Disease.

Table 7 SPSS output table from binomial regression analysis for variables chronic disease & vitamin D status

5.2.7 Vitamin D and medication and/or supplements

Of all 50 included patients, 22 (44.0%) reported taking chronic medication and/or supplements, and 28 (56.0%) reported they did not. None of the reported substances were chronic medication or do not have any known mechanism of affecting vitamin D levels. Therefore, we did not perform any statistical testing for this particular variable.

5.2.8 Vitamin D and BMI

Of all 50 included patients, 38 (76.0%) reported having an underweight or normal BMI, 8 (16.0%) reported themselves as being within the category pre-obesity or obesity. For 4 (8.0%) patients BMI calculation was not possible due to missing variable(s). Through binomial logistic regression, we determined that in our study BMI was not able to significantly predict for vitamin D sufficiency (p=0.580).

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	MergeBMI	-.196	.354	.306	1	.580	.822	.411	1.646
	Constant	-.858	.612	1.963	1	.161	.424		

a. Variable(s) entered on step 1: MergeBMI.

Table 8 SPSS output table from binomial regression analysis for variables BMI & vitamin D status

5.2.9 Vitamin D and hobbies

Of all 50 included patients, 11 (22.0%) reported having no hobbies, 20 (40.0%) reported having mainly indoor hobbies, 8 (16.0%) claimed they mainly had outdoor hobbies and 11 (22.0%) reported having both. Through binomial logistic regression, we determined that in our study type of hobby was not able to significantly predict for vitamin D sufficiency (p=0.890).

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	Hobbies	.043	.312	.019	1	.890	1.044	.566	1.926
	Constant	-1.213	.550	4.862	1	.027	.297		

a. Variable(s) entered on step 1: Hobbies.

Table 9 SPSS output table from binomial regression analysis for variables hobbies & vitamin D status

5.2.10 Vitamin D and smoking status

Of all 50 included patients, 9 (18.0%) reported being smokers and 41 (82.0%) claimed they did not smoke. Through binomial logistic regression, we determined that in our study smoking status was not able to significantly predict for vitamin D sufficiency (p=0.473).

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	Smoking	-.575	.801	.515	1	.473	.563	.117	2.706
	Constant	-.693	.707	.961	1	.327	.500		

a. Variable(s) entered on step 1: Smoking.

Table 10 SPSS output table from binomial regression analysis for variables smoking & vitamin D status

5.2.11 Vitamin D and UV exposure

Of all 50 included patients, 23 (46.0%) reported having insufficient UV exposure and 27 (54.0%) claimed they had sufficient UV exposure. Through binomial logistic regression, we determined that in our study UV exposure status was not able to significantly predict for vitamin D sufficiency (p=0.104).

	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)

								Lower	Upper
Step 1[a]	UV Exposure	1.204	.742	2.636	1	.104	3.333	.779	14.261
	Constant	-1.897	.619	9.389	1	.002	.150		

a. Variable(s) entered on step 1: UV Exposure.

Table 11 SPSS output table from binomial regression analysis for variables UV exposure & vitamin D status

5.2.12 Vitamin D and tanning bed usage

Of all 50 patients included, none (0.0%) reported to using a tanning bed. Therefore, this variable was not used in statistical analysis.

5.2.13 Vitamin D and sunscreen usage

Of all 50 included patients, 5 (10.0%) reported to never using a sunscreen with factor 15 or higher, 19 (38.0%) claimed they used it infrequently and 26 (52.0%) reported they used it frequently. Through binomial logistic regression, we determined that in our study sunscreen usage was not able to significantly predict for vitamin D sufficiency (p=0.634).

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Sunscreen	.246	.517	.227	1	.634	1.279	.465	3.519
	Constant	-1.509	.832	3.289	1	.070	.221		

a. Variable(s) entered on step 1: Sunscreen.

Table 12 SPSS output table from binomial regression analysis for variables sunscreen & vitamin D status

5.2.14 Vitamin D and weight change

Of all 50 included patients, 7 (36.8%) reported having recently lost <5 kg, 4 (21.1%) claimed thy lost ≥5 kg, 5 (26.3%) reported having gained <5 kg around the time of the blood draw. Lastly, only 3 (15.8%) reported gaining ≥5 kg recently. Through binomial logistic regression, we determined that in our study weight change was not able to significantly predict for vitamin D sufficiency (p=0.669).

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Weight change	-.226	.528	.182	1	.669	.798	.283	2.248
	Constant	-1.067	.793	1.809	1	.179	.344		

a. Variable(s) entered on step 1: Weight change.

Table 13 SPSS output table from binomial regression analysis for variables weight change & vitamin D status

5.2.15 Vitamin D and dairy intake

Of all 50 included patients, 0 (0.0%) claimed to never eat dairy, 28 (56.0%) claimed they ate dairy products infrequently (<1 per day), and 22 (44.0%) reported eating dairy frequently (≥1

per day). Through binomial logistic regression, we determined that in our study dairy intake was able to significantly predict for vitamin D sufficiency (p=0.041). This model shows us that the odds of having vitamin D deficiency is 5.556 more likely for those with frequent dairy intake compared to those with infrequent dairy intake. (OR 5.556 per point, 95% CI 1.071 to 28.821)

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Dairy products(1)	1.715	.840	4.168	1	.041	5.556	1.071	28.821
	Constant	-2.303	.742	9.640	1	.002	.100		

a. Variable(s) entered on step 1: Dairy products.

Table 14 SPSS output table from binomial regression analysis for variables dairy intake & vitamin D status

		Vit D Status		
		Insufficient (<30 ng/mL)	Sufficient (>30 ng/mL)	Total
Dairy products	Infrequently (<1 per day)	18	10	28
	Frequently (≥1 per day)	20	2	22
Total		38	12	50

Table 15 Crosstabulation between dairy usage and vitamin D status

5.2.16 Vitamin D and oily fish intake

Of all 50 included patients, 16 (32.0%) claimed to never eat oily fish, 33 (66.0%) claimed they ate oily fish infrequently (≤1 per week), and 1 (2.0%) reported oily fish frequently (>1 per week). Through binomial logistic regression, we determined that in our study oily fish intake was not able to significantly predict for vitamin D sufficiency (p=0.791).

Variables in the Equation

								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1[a]	Oily fish	-.174	.658	.070	1	.791	.840	.231	3.050
	Constant	-1.033	.555	3.458	1	.063	.356		

a. Variable(s) entered on step 1: Oily fish.

Table 16 SPSS output table from binomial regression analysis for variables oily fish intake & vitamin D status

6. Discussion

6.1 Vitamin D levels in young adults

Per the Dutch guidelines on supplementation of vitamin D in persons aged 18-25, this is only deemed appropriate if they have hardly any exposure to sunlight when the sun is at its highest point or have dark skin. However, they firmly state that target titers should be above 30 ng/mL year-round for this population primarily predicated upon the idea that below this threshold the odds of developing osteomalacia increase to unacceptable levels. This is in line with the findings of our systematic review, performed prior to this study. We found a statistically significant association only exists for the skeletal complication of vitamin D deficiency in this age group. A myriad of studies does show positive trends with regards to extra-skeletal pathologies and insufficient vitamin D titers, but we currently lack hard evidence from large rigorous RCT's. Notably, these guidelines also do not describe any indication to determine vitamin D levels in this population.

Belgian guidelines for vitamin D supplementation do not exist, forcing clinicians to rely on the Dutch version. If we consider the findings of this study, this presents a novel problem. We found the majority of our study population did not have sufficient circulating levels of 25(OH)D, only about one in five (20.4%) had a vitamin D level above 30 ng/mL. If we looked at the population based on sex, both had insufficient titers on average. Seasonality was a factor found to be significantly related to vitamin D levels. However, seasons associated with the highest levels of 25(OH)D, due to optimal UVB distribution, still showed markedly insufficient vitamin D levels.

If we utilize the aforementioned target titer, it is evident that the majority of the young adults in this region have unacceptably low vitamin D levels, irrespective of season. Considering the relatively high cost of a doctor's visit with serum 25(OH)D testing, low risk of supplementing vitamin D and the prevalence of 25(OH)D insufficiency determined in our study, perhaps the criteria for supplementation should be revised. At the bare minimum this study indicates a need for more extensive research to establish up to date evidence-based guidelines.

6.2 Risk factors for hypovitaminosis D in young adults

Survey answers were denoted as categorical variables and values were pooled where possible to offset loss of statistical power due to low survey response. In contrast to the primary objective, vitamin D status was also reduced to either sufficient (>30 ng/mL) or not sufficient (<30 ng/mL). This allowed us to improve statistical power because it allows for binomial statistical regression instead of polynomial.

Out of all studied risk factors, only dairy intake was proven to have a significant ability to predict vitamin D status ($p<0.05$). In our study we observed that those with frequent dairy intake frequently (≥1 per day) have 5.556 greater odds of being vitamin D deficient than those who report infrequent dairy intake (<1 per day). This finding directly contradicts the findings of larger and more rigorous studies and is likely the result of an inadequately low sample size [24].

6.3 Limitations

Multiple limitations must be acknowledged. The accuracy and credibility or lack thereof regarding the answers to the questionnaire must be questioned. Upon manual review of patient reported data we found multiple contradictions with survey answers compared to patient file information. Although these were corrected, it is likely that a number of these were missed due to inadequate documentation within the primary care medical software. This recall bias is likely worsened by the rather large difference in time between blood draw and survey participation. Another limitation is that we cannot be absolutely certain that patients are not supplementing with vitamin D, due to the fact that 800 IE supplements do not necessitate a prescription. This indicates that our data on average 25(OH)D titers are likely to be overestimations. Risk factor analysis was performed on a patient population that did not meet the estimated sample size. Lastly, blood draws in young patients are usually performed only when there are significant symptoms present. We did not discern between patients who came in for a blood draw related to an acute or chronic pathology, and those who were solely tested for 25(OH)D titer determination with regards to the aforementioned HGR advice, which could have introduced some selection bias.

7. Conclusion

The European problem of widespread vitamin D deficiency also extends into the less studied group of young adults between the ages of 18-25. The current vitamin D supplementation strategy appears to be failing in this population if the target titer of >30 ng/mL is upheld, considering we found only 1 in 5 reach this threshold. More extensive research into this subject is necessary to determine if systematic supplementation in young adults is needed.

8. Conflict of interest

The authors report no conflict of interest

9. Funding

The authors declare no funding of any kind

10. Attachments

10.1 Attachment A1: Patient questionnaire

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Final edition, 30/11/2021

Enquete Patiënt

Vitamine D tekorten in personen tussen 18-25 jaar

Gelieve alle onderstaande vragen in te vullen.

1. Wat is uw geslacht?
   - ☐ Man
   - ☐ Vrouw

2. Wat voor een type huid heeft u? (Onderaan de vragenlijst kan u een afbeelding vinden ter referentie)
   - ☐ Type I
   - ☐ Type II
   - ☐ Type III
   - ☐ Type IV
   - ☐ Type V
   - ☐ Type VI

3. Bent u gediagnosticeerd met een chronische aandoening?
   - ☐ Neen
   - ☐ Ja, welke: .....

4. Neemt u op regelmatige basis medicatie/supplementen?
   - ☐ Neen
   - ☐ Ja, welke: .....

5. Wat zijn uw hobby's?
   - ☐ Voornamelijk binnenhobbies (gamen, indoor fitness, kaartspelletjes, ...)
   - ☐ Voornamelijk buitenhobbies (voetbal, joggen, hiken, ...)
   - ☐ Beiden
   - ☐ Geen van beiden

6. Wat is uw BMI? (formule: $\frac{\text{gewicht (in kilogram)}}{\text{lengte * lengte (in meters)}}$)
   .....

7. Rookt u?
   - ☐ Ja
   - ☐ Neen

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Bij de volgende vragen is het belangrijk aan te duiden in functie van uw gedrag in de **3 maanden voor uw bloedname.**

8. Hoeveel tijd spendeerde u buiten in de zon (tussen 10u en 15u)
   - ☐ <1u per maand
   - ☐ 1-3u per maand
   - ☐ 1u per week
   - ☐ 2-4u per week
   - ☐ 5-6u per week
   - ☐ 30 min a 1u per dag
   - ☐ Meer dan 2u per dag

9. Hoe vaak gebruikte u een zonnebank?
   - ☐ Nooit of <10 min per week
   - ☐ 10-20 min per week
   - ☐ 20-30 min per week
   - ☐ 30-40 min per week
   - ☐ 40-50 min per week
   - ☐ 50-60 min per week
   - ☐ >60 min per week

10. Hoe vaak gebruikt u zonnecreme met een factor hoger dan 15?
    - ☐ Nooit
    - ☐ Soms
    - ☐ Regelmatig
    - ☐ Vaak
    - ☐ Altijd

11. Hoe vaak eet u zuivelproducten? (yoghurt, kaas, pudding, platte kaas, melk,..)
    - ☐ Nooit of <1 keer per maand
    - ☐ 1-3 keer per maand
    - ☐ 1 keer per week
    - ☐ 2-4 keer per week
    - ☐ 5-6 keer per week
    - ☐ 1 keer per dag
    - ☐ 2-3 keer per dag
    - ☐ 4-5 keer per dag
    - ☐ 6+ keer per dag

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12. Hoe vaak eet u vette vis? (Makreel, sardines, eel, kabeljouw, herbot, ...)
    - ☐ Nooit of <1 keer per maand
    - ☐ 1-3 keer per maand
    - ☐ 1 keer per week
    - ☐ 2-4 keer per week
    - ☐ 5-6 keer per week

13. Neemt u vitamine D supplementen?
    - ☐ Ja
        - ○ 800 IE/dag (of minder)
        - ○ 25000 IE/week
        - ○ 50000 IE/week
    - ☐ Nee

14. Is uw gewicht veranderd?
    - ☐ Ja, ik ben bijgekomen
    - ☐ Ja, ik ben afgevallen
    - ☐ Nee

15. Indien ja, hoeveel was het verschil in gewicht?
    - ☐ <1 kg bijgekomen
    - ☐ 1-5kg bijgekomen
    - ☐ >5kg bijgekomen
    - ☐ <1 kg afgevallen
    - ☐ 1-5kg afgevallen
    - ☐ >5 kg afgevallen

Dank voor uw deelname aan onze studie.

Gelieve de ingevulde vragenlijst te mailen naar 1 van onderstaande onderzoekers, of de lijst in persoon af te geven in de praktijk waar u patiënt bent.
Indien u nog verdere vragen heeft kan u ons op onderstaande mailadressen bereiken.

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Score	Description	Female	Male
0–6 Type I	**Pale white skin** Extremely sensitive skin, always burns, never tans *Example: red hair with freckles*		
7–13 Type II	**White skin** Very sensitive skin, burns easily, tans minimally *Example: fair skinned, fair haired Caucasians, northern Asians*		
14–20 Type III	**Light brown skin** Sensitive skin, sometimes burns, slowly tans to light brown *Example: darker Caucasians, some Asians*		
21–27 Type IV	**Moderate brown skin** Mildly sensitive, burns minimally, always tans to moderate brown *Example: Mediterranean and Middle Eastern Caucasians, southern Asians*		
28–34 Type V	**Dark brown skin** Resistant skin, rarely burns, tans well *Example: some Hispanics, some Africans*		
35+ Type VI	**Deeply pigmented dark brown to black skin** Very resistant skin, never burns, deeply pigmented *Example: darker Africans, Indigenous Australians*		

10.2 Attachment A2: Informative patient letter

Final edition, 30/11/2021

Informatieformulier

Met de studie waaraan u vrijwillig deelneemt willen we onderzoeken of er een significant vitamine D tekort bestaat in de jongerenpopulatie tussen 18-25 jaar en welke factoren bijdragen tot dit eventuele tekort.

Volgens de actuele richtlijnen komt deze populatie niet in aanmerking voor het suppleren van vitamine D. Echter vermoeden we dat er een tekort is in deze populatie. Onze studie zou een aanleiding kunnen zijn om de richtlijnen te herzien.

Om dit onderzoek te kunnen uitvoeren zullen we gebruik maken van uw vitamine D status, die we terugvinden in een recente bloedname. We bewaren deze gegevens gepseudonimiseerd totdat we deze hebben kunnen koppelen aan de vragenlijst (zie verder). Indien we merken dat u een vitamine D tekort heeft communiceren we dit met u en raden we u, naar gelang de mate van het tekort, een gepaste behandeling aan volgens de actuele richtlijnen. Ook heeft u het recht op inzage van deze gegevens. Een weigering om deel te nemen aan deze studie zal in geen enkel opzicht een gevolg hebben op uw verdere behandeling.

Verder vragen we u ook een vragenlijst in te vullen. Nadat u ons deze heeft bezorgd koppelen we uw vitamine D waarde aan de door u ingevulde vragenlijst en zullen we daarna al uw gegevens anonimiseren. Bijgevolg zijn uw gegevens vanaf dit punt niet naar u te herleiden.

U heeft op elk moment van de studie het recht om uw deelneming in te trekken.

Voor verdere vragen of informatie kan u ons altijd bereiken via onderstaande mailadressen.

Dank bij voorbaat,

Dr. C. Hillegeer – 10274055006 – doktercedric@gmail.com

Dr. S. Wauters – 10394705006 – drwauters@hotmail.com

10.3 Attachment A3: Self-reflections of authors

Cedric Hillegeer

Een thesisproject ondernemen met Sebastien was een ongelooflijk lonende ervaring voor mij. We waren niet alleen in staat om een uitgebreid en goed onderzocht proefschrift te schrijven, maar we hebben ook een uitstekende werkrelatie opgebouwd die het proces plezierig en bevredigend maakte. Het was een heel mooi verlengstuk van onze al langdurige vriendschap. Vanaf het begin hebben we duidelijke communicatiekanalen opgezet en iedereen was toegewijd aan het succes van het project. Onze gedeelde visie en waarden maakten samenwerken tot een plezierige en productieve ervaring.

Een van de belangrijkste factoren die onze samenwerking tot een succes maakten, was de betrouwbare en snelle communicatie tussen ons. We hadden een duidelijk beeld van onze rollen en verantwoordelijkheden en we hielden elkaar regelmatig op de hoogte van onze vorderingen. Hierdoor konden we eventuele problemen snel en efficiënt identificeren en oplossen, zodat we op schema bleven en onze deadlines haalden.

Een andere kritische factor die bijdroeg aan ons succes was de flexibiliteit van alle betrokkenen. We werkten allemaal fulltime en het was een behoorlijke uitdaging om met onze verplichtingen om te gaan. We waren echter allemaal bereid om onze planningen aan te passen en compromissen te sluiten om ervoor te zorgen dat het project de aandacht kreeg die het verdiende. Deze flexibiliteit stelde ons in staat om de vaart in het project vast te houden en werk van hoge kwaliteit af te leveren.

Door dit project heb ik het belang ingezien van het stellen van zelfopgelegde deadlines en deze te beschouwen als officiële deadlines. Als HAIO die fulltime met patiënten werkt, was het essentieel om een gestructureerd tijdschema te hebben voor het voltooien van het onderzoeksproject. Het stellen van deadlines hielp ons om gemotiveerd, gefocust en op schema te blijven gedurende het hele proces, ondanks onze drukke schema's. Het stelde ons ook in staat om voldoende tijd te besteden aan onderzoek, analyse en interpretatie van de gegevens, waardoor de nauwkeurigheid en betrouwbaarheid van onze bevindingen werd gegarandeerd. Door onszelf verantwoordelijk te houden voor deze deadlines, hebben we het project efficiënt en kwalitatief kunnen afronden. Over het algemeen heeft deze ervaring mijn geloof versterkt in het belang van het vastleggen van zelfopgelegde deadlines en deze dan ook als officieel beschouwen, om succes te behalen in een onderzoeksproject.

Het werken aan dit proefschrift zorgde ook voor een aanzienlijke persoonlijke en interpersoonlijke groei. Ik ontwikkelde betere tijdmanagementvaardigheden, leerde veerkrachtiger te zijn bij uitdagingen en verbeterde mijn vermogen om samen te werken in een team. Deze vaardigheden en kwaliteiten zullen mij ongetwijfeld goed van pas komen in mijn toekomstige carrière als huisarts.

Dit proefschrift was een combinatie van vooronderzoek in de vorm van een systematische review gevolgd door zelf uitgevoerd retrospectief onderzoek. Door eerdere ervaring met deze soorten onderzoek voelde het proces natuurlijker en beheersbaarder aan dan de eerste keer. Ik

had immers al in de master geneeskunde exact deze twee types onderzoek uitgevoerd. Het kostte echter wel nog steeds een aanzienlijke hoeveelheid tijd om te voltooien, meer dan ik had verwacht.

Het uitvoeren van de systematische review was (opnieuw) een verhelderende ervaring. Het stelde ons in staat om de bestaande kennis over het onderwerp te begrijpen, hiaten in het onderzoek te identificeren en een onderzoeksvraag te formuleren die waarde zou toevoegen aan het veld. Het proces van het uitvoeren van de review was tijdrovend en vereiste een nauwgezette benadering van analyse, synthese en interpretatie van gegevens.

Het retrospectieve onderzoek dat erop volgde waar we data van eigen patientpopulaties moesten bekomen was zelfs nog meer veeleisend. We moesten gegevens uit patiëntendossiers verzamelen en analyseren en bovendien ervoor zorgen dat we ons aan ethische richtlijnen hielden en de vertrouwelijkheid van de patiënt handhaafden. Het onderzoek vereiste uitgebreide opschoning, analyse en interpretatie van gegevens om zinvolle inzichten en conclusies te genereren. Net als bij het laatste onderzoek dat ik heb uitgevoerd, was het meest tijdrovende deel het rekruteren van de patiënten en het controleren van de kwaliteit van hun antwoorden en vervolgens natuurlijk corrigeren waar nodig. Het menselijke element dat het onderzoek zo interessant maakt, maakt het ook 'traag'. Dit project leerde me dat hoewel eerdere ervaring en kennis het onderzoekstraject kunnen vergemakkelijken, het nog steeds een aanzienlijke hoeveelheid tijd en moeite kost om uitgebreid en kwalitatief onderzoek uit te voeren. Het benadrukte ook het belang van een duidelijke onderzoeksvraag en een goed ontworpen onderzoeksprotocol om ervoor te zorgen dat het onderzoek efficiënt en effectief is.

Als toekomstige huisartsen is het onze plicht om op de hoogte te blijven van het laatste onderzoek en de nieuwste ontwikkelingen op dit gebied om onze patiënten de best mogelijke zorg te bieden. Het voltooien van een proefschrift terwijl ik fulltime met patiënten werkte, was een uitdagende maar lonende ervaring. Het proces vereiste zorgvuldige planning, tijdmanagement en toewijding aan zowel het proefschrift als de verantwoordelijkheden voor de patiëntenzorg. De emotionele en mentale belasting was op sommige momenten aanzienlijk, maar de vaardigheden en kennis die ik tijdens het proces heb opgedaan, waren van onschatbare waarde in mijn nakende carrière als huisarts. De ervaring heeft me geleerd veerkrachtig te zijn en mijn tijd effectief te beheren. Uiteindelijk gaf het voltooien van de scriptie me een gevoel van voldoening en trots, wetende dat ik voor de tweede keer in mijn leven een instrumentele rol heb gespeeld in een meerjarig groot wetenschappelijk project.

Samenvattend, het werken aan dit proefschrift met Sebastien en onze begeleidende promotors, Veronique en Werner, was een ongelooflijk bevredigende ervaring voor mij. Ik heb waardevolle lessen geleerd over teamwerk, samenwerking en onderzoeksmethodologie, en ik heb een dieper inzicht gekregen in de klinische impact van vitamine D-spiegels bij jonge volwassenen. Bovendien heb ik waardevolle inzichten opgedaan op het gebied van huisartsgeneeskunde en haar rol bij het bevorderen van een goede gezondheid en het voorkomen van ziekten. Ik geloof dat de kennis en vaardigheden die ik via dit project heb opgedaan mij goed van pas zullen komen in mijn toekomstige praktijk.

Sebastien Wauters

Ik was zeer tevreden met het gekozen onderwerp van deze masterproef. De thesis die ik in de basisopleiding schreef met 2 andere studenten, de effecten van postoperatieve vitamine D suppletie na thyreoidectomie, heeft mijn interesse in dit domein opgewekt.

Hiernaast is er een duidelijk toenemende interesse over het belang van vitamine D. Dit maakte het voor mij zinvol om mijn kennis hieromtrent uit te breiden en eventueel iets bij te leveren aan het onderzoek dat nodig is in dit domein.

In het begin van de covid periode en het begin van onze masterproef publiceerde de hoge gezondheidsraad een advies om de vitamine D-status te bepalen bij al degenen die een hoog risico lopen om COVID-19 op te lopen en degenen die besmet zijn op het moment van aanmelding, en om degenen bij wie een tekort wordt vastgesteld te supplementeren. Het verschijnen van deze richtlijn was voor ons een bevestiging dat onze belangstelling in dit thema gegrond was. Door ons eigen onderzoek en dankzij de artikels die ik heb gelezen heb ik mijn kennis hieromtrent kunnen uitbreiden.

Hoewel we in onze master geneeskunde ook een masterproef hebben moeten schrijven heb ik het schrijven van deze thesis als een grotere uitdaging bevonden. Dit was voornamelijk te wijten aan het feit dat we mijns inziens veel minder tijd hadden door onder meer onze praktijkstages en andere taken verbonden aan de opleiding huisartsgeneeskunde. Naast de opleiding gebonden heb ik andere activiteiten waar ik ook belang aan hecht, wat maakte dat tijdsmanagment een belangrijke rol heeft gespeeld.

In verschillende zelfreflecties die we moesten schrijven doorheen de opleiding gaf ik aan dat de canmeds rol van manager een rol was waarin verbetering mogelijk was. Met deze masterproef heb ik mij op dit vlak verder ontwikkeld hoewel dit niet steeds makkelijk was.
Naast deze persoonlijke ontwikkeling heeft deze masterproef mij ook op professioneel vlak verandering gebracht. Door het schrijven van deze thesis en het lezen van de artikels tijdens de literatuurstudie is mijn kennis over het onderwerp uitgebreid. Ik merk dat ik steeds meer Vitamine D voorschrijf aan patiënten dan voor de aanvang van deze masterproef.

Ik baseerde me voordien op de NHG-richtlijnen waarbij niet wordt gesproken over een grote groep mensen. Nu raad ik aan steeds meer mensen vitamine D suppletie aan, ook al vallen zij niet in de populatie die volgens deze richtlijn gesupplementeerd zou moeten worden.

Ook heeft dit onderzoek mij geholpen met de hoeveelheid informatie ik kan meegeven wanneer patiënten vragen hebben over vitamine D. Ik merk dat er sinds covid hieromtrent steeds meer vragen komen en vind het dan ook fijn om hier een duidelijk antwoord op te kunnen geven.
Met deze masterproef leveren we ook bij aan de grote hoeveelheid onderzoek dat in dit domein nog nodig is. We zijn veel studies tegen gekomen die het belang van vitamine D onderbouwen in onderwerpen die goed zijn onderzocht. Hiernaast lazen we ook artikels waarbij een mogelijks effect tussen vitamine D en verschillende pathologiën wordt beschreven, maar waar verder onderzoek naar nodig is.

Naast deze bevindingen over de belangen van vitamine D werd ook duidelijk dat er een hypovitaminose bestaat in de onderzochte populatie jongeren in België. Deze populatie wordt niet geïncludeerd in de richtlijn van NHG over vitamine D suppletie waarop de meeste artsen zich baseren. Ik ben dan ook blij dat ik met deze achtergrondkennis deze patiënten beter kan adviseren in het nemen van Vitamine D supplementen.

De communicatie en samenwerking met de HAIO waarmee ik deze masterproef heb geschreven, Cédric Hillegeer, verliep tijdens het gehele proces zeer vlot. Dit mede omdat ik met hem bevriend was sinds de basisopleiding geneeskunde. Dit maakte dat we op een zeer laagdrempelige manier met elkaar konden communiceren over ideeën en dat we makkelijk feedback met elkaar konden uitwisselen. Dit terwijl het hele proces op een aangename manier kon verlopen.

Het schrijven van de masterproef gebeurde geleidelijk aan naarmate deadlines naderden. Cédric en ik waren goed op elkaar afgestemd qua timing waarbij we periodes hadden waarin we minder werk leverden en andere periodes waarin we intensiever bezig waren met het schrijven van de masterproef.

We hebben het hele proces stuk voor stuk aangepakt en communiceerden telkens met elkaar wie met welk deel van de masterproef bezig was opdat we alles zo efficiënt mogelijk konden laten gebeuren. Nadat iemand een stuk had geschreven bekeken we dit samen en wisselden we feedback uit tot we een eindproduct hadden waarmee beiden voldaan waren.
Ieders had zijn sterktes en zwaktes maar deze hebben elkaar aangevuld met een goed eindproduct als resultaat.

Ook de communicatie en samenwerking met onze promotoren, Veronique Verhoeven en Werner Van Peer, is in mijn opinie zeer goed verlopen. De opmars van online meetings sinds de covid periode heeft hier zeker ook zijn steentje tot bijgedragen. Al onze meetings verliepen online wat zorgde voor een zeer grote flexibiliteit langs ieders kant. Wanneer we gedurende het gehele proces vragen hadden, probeerde we deze eerst zelf eerst intern op te lossen. Indien we vastliepen konden we zeer laagdrempelig overleggen met beide promotoren op korte termijn.

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