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Determinants of plasma 25-hydroxyvitamin D levels in healthy adults in the Netherlands

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ABSTRACT

Background: Vitamin D plays a key role in maintaining skeletal health, but is also related to various non-skeletal health issues. Several determinants have been identified that influence blood plasma levels of 25-hydroxyvitamin D (25(OH)D), often in specific patients or elderly populations. This paper aims to replicate these findings in a healthy population.

Methods: Plasma levels of 25(OH)D were measured using tandem mass spectrometry. We examined the cross-sectional association of sociodemographic, health, lifestyle and sampling characteristics with 25(OH)D in a group of 539 adults, who were healthy control subjects in the NESDA study in the Netherlands (latitude 52 °N).

Results: Mean 25(OH)D levels were 68.0 (±27.2) nmol/l. Levels under 50 nmol/l occurred in 27% of the population; 40% reached levels above 75 nmol/l. Women had higher levels than men, and the use of oral contraceptives showed a significant positive association among females. Subjects with non-European ancestry had dramatically lower 25(OH)D levels. Other factors that were negatively associated were body mass index and the renal estimated glomerular filtration rate (eGFR). Meteorological data replaced season as a significant determinant. Moderate alcohol consumption and sports showed a positive association, while physical activity and the hepatic marker gamma-glutamyl transferase did not. Our results disconfirm the influence of age in this population of under 65 year olds.

Conclusion: Insufficient 25(OH)D levels were common in a healthy population. The set of eight variables that were significant in a multiple regression model (sex, ancestry, oral contraceptives, eGFR, BMI, sports, alcohol, sunshine) explained 29.5% of the variance.

KEYWORDS

25(OH)D, determinant, vitamin D

INTRODUCTION

In recent years, the interest in vitamin D has increased within several fields of medicine. Vitamin D is best known for its key role in maintaining the body's calcium homeostasis and thereby its involvement in skeletal health. Deficiency increases the risk of developing osteoporosis and, in severe cases, causes rickets or osteomalacia.[1,2] Vitamin D receptors are found in tissues throughout the entire human body, and recent findings indicate an involvement of vitamin D deficiency in other diseases, such as autoimmune diseases, certain types of cancer, cardiovascular diseases, and the metabolic syndrome.[3-5] Furthermore, vitamin D deficiency has been shown to be associated with psychiatric disorders such as depression.[6-9] Rickets and osteomalacia became increasingly common in European cities during the period of urbanisation and industrialisation from the 17th until the early 20th century, but were largely eradicated after the influence of sunlight exposure and cod liver oil was discovered.[10,11] These days, some foods are fortified with vitamin D. The only vitamin D-supplemented food in the Netherlands is margarine but in the United States and Canada, fortification of products such as cereals and milk is usual practice, resulting in increased circulating vitamin D levels in the population.[12] Nevertheless, vitamin D insufficiency and deficiency still commonly occur in environments with sufficient sunlight and dietary sources of vitamin D, primarily among vulnerable groups in the winter.[13,14]

Vitamin D occurs in the form of D2 and D3. Vitamin D2 is synthesised in plants; vitamin D3 is synthesised in humans and animals under the influence of ultraviolet radiation. Both forms are obtained by humans through the dietary intake of vegetables, eggs, dairy products, meat and fish, but the main source of vitamin D3 in the human body is *de novo* synthesis in the skin. Vitamin D is hydroxylised in the liver and stored as 25-hydroxyvitamin D (also called calcidiol, 25(OH)D or D25). In the kidneys, 25(OH)D is

converted into the hormonal form 1,25-dihydroxyvitamin D (also called calcitriol or 1,25$(OH)_2$D). Whereas 1,25$(OH)_2$D is the active form of vitamin D, the most reliable marker of vitamin D status is 25(OH)D (15). With a half-life of 2-3 weeks, 25(OH)D is much more stable than its derivative 1,25$(OH)_2$D, which has a half-life of only six hours.[16]

Although diet and sunlight exposure play an established role in vitamin D physiology, other determinants of vitamin D status have been identified. These include physical and sociodemographic characteristics (sex, age, sex hormones, ethnicity, socioeconomic status, urbanisation) and lifestyle (body mass index (BMI), physical activity, consumption of tobacco and alcohol, vitamin supplementation).[17,18] Further, as the liver and the kidneys play a crucial role in vitamin D physiology it can be expected that markers of hepatic and renal function, such as gamma-glutamyl transferase (GGT) and the estimated glomerular filtration rate (eGFR), are associated with plasma 25(OH)D levels. Finally, circumstantial sampling variables such as season and plasma storage time may influence 25(OH)D levels.

There has been a great interest in vitamin D and its involvement in various health problems in recent years. Vitamin D status is often studied in specific groups that have an increased risk of vitamin D deficiency or osteoporosis, such as hospitalised or elderly people. In such groups, confounding of variables makes it difficult to translate findings to the general population. Whereas several studies have described vitamin D determinants in healthy populations, countries differ in climate, dietary intake and supplementation of vitamin D. The aim of the current paper is to verify the independent importance of a wide range of sociodemographic, health, lifestyle and sampling characteristics for plasma levels of 25-hydroxyvitamin D in a group of healthy adults without clinically overt psychiatric or somatic diseases in the Netherlands (latitude 52 °N).

METHODS

Subjects

We used data from the baseline assessment of the Netherlands Study of Depression and Anxiety (NESDA),[19] an ongoing longitudinal cohort study, focusing on the course and determinants of depression and anxiety. The presence of depressive and anxiety disorders in this study was determined according to DSM-IV criteria using the World Health Organisation Composite International Diagnostic Interview (CIDI, version 2.1). The study was approved by our institution's medical ethics board and respondents signed a written informed consent form.

The NESDA cohort included 2981 subjects, aged 18-65, who visited one of our five research sites for a detailed interview. A total of 652 subjects did not have a lifetime history of anxiety, major depressive or dysthymic disorders as determined by the CIDI. For the present analyses, we used data from these control subjects only. Of these, 105 respondents were excluded because they reported clinically overt disease of the thyroid gland, kidney or liver, osteoporosis, stroke, lung emphysema, cancer, rheumatoid arthritis, multiple sclerosis, HIV or any other autoimmune disease. Furthermore, subjects were excluded due to unsuccessful blood draw (n = 7) or an extreme plasma 25(OH)D value (> 4 SD above the mean, n = 1). These exclusion criteria led to a total of 539 qualified subjects with a valid 25(OH)D measurement.

Vitamin D status

Fasting blood was collected at the time of baseline interview, between 8.15-10.10 hours. Vacutainers with EDTA (BD Diagnostics, NJ, USA) were used to collect plasma for later assessment of 25(OH)D and creatinine. Samples were spun down within one hour after blood draw and cryovials with portions of 500 µl of EDTA plasma were stored at -80 °C.

Measurements of 25(OH)D were performed at the Endocrine Laboratory of the VU University Medical Center. 25(OH)D was measured by isotope dilution/online solid-phase extraction liquid chromatography/tandem mass spectrometry (ID-XLC-MS/MS).[20] First, 25(OH)D was released from its binding proteins with a proprietary protein disruption buffer. Deuterated internal standard (IS) 25(OH)D3-d6 was added and samples were mixed. Samples were extracted and analysed by XLC-MS/MS (a Symbiosis online SPE system, Spark Holland) coupled to a Quattro Premier XE tandem mass spectrometer (Waters Corporation). We quantified plasma 25(OH)D by relating analyte/IS peak area ratios in patient plasma to analyte/IS peak area ratios in BSA-PBS buffer spiked with 25(OH)D2 and 25(OH)D3 at concentrations ranging from 0-100 µg/l (0-250 nmol/l) and IS at a fixed concentration. We established the accuracy of 25-hydroxyvitamin D results by measuring the standard and a control with a reference method.[21] 25(OH)D2 and 25(OH)D3 were measured separately but combined for the analyses.

We used continuous 25(OH)D measures for the majority of our analyses, but also describe the study population by classification into four groups: < 25 nmol/l, 25-50 nmol/l, 50-75 nmol/l and ≥ 75 nmol/l. Although there is no consensus on 25(OH)D classification, levels under 50 nmol/l are generally considered insufficient or deficient; levels of at least 75 nmol/l are recommended by e.g. the Institute of Medicine.[2,22,23]

DETERMINANTS

Sociodemographics

Sex, age, country of birth and educational level were assessed during the interview. We also obtained the birth

country of both biological parents for 501 respondents, allowing us to distinguish those with one and those with two non-European parents. We considered the educational level an indicator of socioeconomic status. The degree of urbanisation was based on the postal code of residence. Areas were considered urban when the surrounding address density is 1500 or more per square kilometre (Statistics Netherlands, www.cbs.nl).

Health and lifestyle indicators

Menopausal status and the use of oral contraceptives were assessed during the interview. Creatinine levels were measured at the Clinical Chemistry Laboratory of the University Medical Center Groningen with an enzymatic method (Roche Hitachi Modular, Roche Diagnostics, Basel, Switzerland). Estimated GFR (ml/min/1.73 m^2) was calculated using the CKD-EPI equation.[24] GGT was measured at each research site's local laboratory with an enzymatic method (Roche Hitachi Modular). Body height and weight were measured by the research assistant and used to calculate the body mass index (BMI = weight (kg) / height $(m)^2$).

Alcohol consumption (number of drinks/day) was reported by means of the written Alcohol Use Disorders Identification Test (AUDIT).[25] Consumption of up to 14 standard glasses per week for women or 21 for men was labelled as moderate; a greater consumption was considered heavy drinking. Physical activity was expressed in weekly Metabolic Equivalent of Task (MET) hours, based on the International Physical Activity Questionnaire (IPAQ)[26] and the frequency of sporting activities was assessed in the questionnaire. During the interview, participants were asked whether they smoked and whether they had used any vitamin supplements during the past 30 days.

Sampling

We regarded the period from October to March as winter and the period from April to September as summer. Although the season is an established determinant of vitamin D status, it is a rather crude measure of (potential) sunlight exposure. We therefore calculated the mean amount of sunshine during the ten-week period prior to each interview and at the relevant research site, using publicly accessible data from the Royal Netherlands Meteorological Institute (KNMI, www.knmi.nl). The weekly number of sunlight hours was defined as the sum of all sub-periods in that week for which the solar irradiance exceeded 120 W/m^2.

Statistical analyses

Univariate associations between individual characteristics and the continuous 25(OH)D variable were established using linear regression. All candidate predictors were entered into a multiple linear regression model in order to examine the independence of determinants. Sex and seasonal differences in the prevalence of vitamin D insufficiency were tested using chi-square tests. Data were analysed using IBM SPSS Statistics for Windows version 20 (IBM Corporation, Armonk, NY, USA).

Table 1. *Characteristics of the study population (n = 539)*

	Percentage or mean (± SD)
25-Hydroxyvitamin D levels	
Mean 25(OH)D (nmol/l)	68.0 (± 27.2)
Sufficiency:	
- < 25 nmol/l (<10 ng/ml)	5.0%
- 25-50 nmol/l (10 – 20 ng/ml)	21.5%
- 50-75 nmol/l (20 – 30 ng/ml)	33.4%
- > 75 nmol/l (>30 ng/ml)	40.1%
Sociodemographics	
Sex (female)	62.7%
Mean age (years)	39.2 (± 14.6)
Non-European ancestry:	
- One parent	4.45%
- Both parents	2.97%
Urban (> 1500/km^2)	72.6%
High educational level (college/university)	41.6%
Health and lifestyle characteristics	
Menopause (only among women)	30.1%
Oral contraceptives (only among women)	33.7%
Mean estimated GFR (ml/min/1.73 m^2)	106 (± 16.8)
Mean GGT (U/l)	24.2 (± 27.5)
Mean BMI	24.8 (± 4.53)
Mean sports activities (times/month)	3.80 (± 3.14)
Mean physical activity (MET-hours/week)	65.0 (± 52.0)
Mean alcohol use (drinks/week)	7.22 (± 9.13)
Smoking (yes)	27.3%
Vitamin supplements (yes)	13.0%
Sampling variables	
Mean storage of biobank sample (years)	5.22 (± 0.62)
Mean sunshine (hours/week over 10 weeks)	50.7 (± 19.3)
Season (winter)	49.0%

BMI = body mass index; GFR = glomerular filtration rate; GGT = gamma-glutamyl transferase; MET = metabolic equivalent task.

RESULTS

Description of the population

Table 1 shows the characteristics of the 539 subjects, of whom 62.7% were female. The mean age was 39 years (SD 14.6) and the mean level of 25(OH)D was 68.0 nmol/l (SD 27.2). Levels above 75 nmol/l were detected in 40.1% of our sample. Levels below 50 nmol/l were observed in 26.5%, and occurred more frequently in men (33.4%) than in women (22.5%, p = 0.006).

Determinants of 25(OH)D levels

Table 2 lists the association of the variables with 25(OH) D levels, both in a univariate and in a multiple regression analysis. The multiple regression model explained 30.2% of the variance; the variance explained by only those eight variables that were significant was 29.5%.

Sociodemographics

Mean levels of 25(OH)D were 61.8 nmol/l in men and 71.6 nmol/l in women, which was a significant difference ($F_{1,538}$ = 16.9, p < 0.001). In the multiple regression model, sex remained highly significant (p = 0.001). Whereas age is commonly considered a determinant of vitamin D levels, no such association was found in the univariate analysis (B = -0.123, p = 0.125). As age might have a differential effect in men and women, we explored the association in both sexes separately using a regression model including sex, age, and their interaction. We found that 25(OH)D decreased with age in women (B = -0.274, p = 0.009), but not in men (B = 0.139, p = 0.244). There was a significant interaction between sex and age (p = 0.011).

We further examined whether the effect of age in women was driven by menopausal status or the use of oral contraceptives. No significant difference was found between premenopausal and postmenopausal women ($F_{1,331}$ = 1.33, p = 0.250). Women who used oral contraceptives, however, had significantly higher 25(OH)D levels than those who did not (80.6 nmol/l vs. 67.1 nmol/l, $F_{1,337}$ = 19.0, p < 0.001) and they were also younger (29 years vs. 44 years, $F_{1,337}$ = 113.8, p < 0.001). After adjustment for the use of oral contraceptives, age was no longer significantly associated with 25(OH)D in women (p = 0.578). In the multiple model with all predictors, neither age (p = 0.831) nor the sex x age interaction (p = 0.167) remained significant.

Respondents with two non-European biological parents had significantly lower levels of 25(OH)D (34.6 nmol/l vs. 69.6 nmol/l respectively, $F_{1,514}$ = 27.4, p < 0.001) than those with two European parents. Respondents who were the child of a mixed couple had intermediate levels (57.3 nmol/l), differing significantly from both the European (F1,522 = 4.89, p = 0.027) and the non-European group ($F_{1,39}$ = 7.30, p = 0.010). Being the child of one or two non-European parents remained a significant predictor in the multiple regression analysis (p < 0.001). Respondents living in urban areas had lower 25(OH)D levels than those in less urban areas ($F_{1,535}$ = 9.19, p = 0.003). This association, however, lost significance in the multiple model (p = 0.175). Having a college or university degree was not related to 25(OH)D levels ($F_{1,537}$ = 0.15, p = 0.698).

Health and lifestyle indicators

A negative association of 25(OH)D with GGT was initially found (p = 0.001), but it did not remain significant in the multiple model (p = 0.122). The negative association of 25(OH)D with eGFR, which is calculated from creatinine levels, age, and sex, was not significant in the univariate analysis (p = 0.493), but highly in the multiple regression analysis (p = 0.005).

The BMI was significantly associated with lower 25(OH)D, both in the univariate and the multiple analyses (p < 0.001). The frequency of doing sports activities was a significant factor in both the univariate and the multiple regression analyses (p values < 0.001), whereas physical activity expressed in weekly MET-hours was not (p = 0.568 and p = 0.823). The consumption of alcohol appeared to have a positive association with 25(OH)D in the multivariate model only (p = 0.001). We further examined whether elevated levels of 25(OH)D could be found in moderate as well as in heavy drinkers. Both moderate and heavy alcohol users had 25(OH)D levels that were significantly higher than subjects who drank no more than one glass per week (p < 0.001 and p = 0.026, respectively). Vitamin D status did not differ between moderate and heavy drinkers (p = 0.959). Smoking was not associated with 25(OH)D (p = 0.664). The use of vitamin supplements was correlated with higher 25(OH) D levels (p = 0.023), but did not remain significant in the multiple regression (p = 0.124).

Sampling

Plasma samples were collected over a period of two and a half years and 25(OH)D levels were assessed four years after collection of the last sample. We found no evidence of degradation over time (p = 0.756). Concentrations of 25(OH)D were higher in summer than in winter (73.9 nmol/l vs. 62.3 nmol/l, respectively, p < 0.001). Levels of 25(OH)D were highest in August, when the amount of sunshine is at its peak (*figure 1*). The amount of sunshine during the ten weeks prior to blood draw was highly significant in the multiple model (p < 0.001).

DISCUSSION

Among a group of healthy adults in the Netherlands, at a northern latitude (52 °N), 26% had plasma 25(OH)D under 50 nmol/l and 40% had levels above 75 nmol/l. We identified eight determinants of plasma levels of 25(OH)

Table 2. *Associations of sociodemographic, health, lifestyle and sampling variables with 25(OH)D levels*

	Univariate			Multiple regression		
	B	**Significance**		**B**	**Significance**	
Sociodemographics						
Sex (female)	9.797	< 0.001	*	13.86	0.046	*
Age	-0.123	0.125		0.032	0.831	
Interaction age x sex	-	-		-0.218	0.167	
Non-European ancestry (per parent)	-16.12	< 0.001	*	-12.78	< 0.001	*
Urban (> 1500/km^2)	-7.904	0.003	*	-3.538	0.175	
High educational level (college/university)	0.921	0.698		-1.231	0.589	
Health and lifestyle indicators						
Oral contraceptives among women	13.58	< 0.001	*	8.587	0.007	*
eGFR (ml/min/1.73m^2)	-0.048	0.493		-0.250	0.005	*
GGT (U/l)	-0.142	0.001	*	-0.063	0.122	
BMI	-1.139	< 0.001	*	-0.921	< 0.001	*
Sports activities (times/month)	1.945	< 0.001	*	1.429	< 0.001	*
Physical activity (MET-hours/week)	0.013	0.568		-0.005	0.823	
Alcohol use (drinks/week)	0.053	0.678		0.403	0.001	*
Smoking	-1.141	0.664		-1.835	0.443	
Vitamin supplements	7.904	0.023	*	4.832	0.124	
Sampling variables						
Storage of biobank sample (years)	-0.718	0.703		0.540	0.756	
Sunshine (hours/week over 10 weeks)	0.521	< 0.001	*	0.485	< 0.001	*

BMI = body mass index; eGFR = estimated glomerular filtration rate; GGT = gamma-glutamyl transferase; MET = metabolic equivalent task; * p value < 0.05.

D: sex, oral contraceptive use, birth country of the parents, renal function, BMI, engagement in sports activities, alcohol consumption and the available amount of sunshine were independent significant determinants in a multiple regression model.

Sociodemographics

Since osteoporosis is most prevalent among postmenopausal women, it makes sense to examine the influence of sex and age on vitamin D status. Compared with several other European studies,[18,27,28] we found relatively high levels of circulating 25(OH)D and, surprisingly, levels were higher in women than in men. The higher levels in our population may be explained by the fact that we selected only healthy subjects instead of a random draw from the general population. Moreover, one study sampled in the winter,[28] when levels are lowest. Our finding that women had higher levels than men may be explained by the distribution of age in our sample: 42% of our subjects were under the age of 35, whereas the aforementioned studies included subjects with a minimum age of 35 years. Our result that 25(OH)D levels were higher in women than in men applied to these younger subjects, but not to the group above 35 years of age (results not shown).

Although it has been established that the ageing skin produces less vitamin D,[29] it does not explain the interaction of age with sex. The use of oral contraceptives, however, is age-dependent but also associated with 25(OH) D levels.[30] This finding is confirmed by our results: 25(OH)D levels were 20% higher in oral contraceptive users. Correspondingly, age was no longer significantly correlated with 25(OH)D when adjusting for the use of oral contraceptives, indicating that the age effect in women was driven by the use of oral contraceptives.

The association between the use of oral contraceptives and 25(OH)D levels has been shown repeatedly.[30-33] In addition to these cross-sectional studies, Harris also found that 25(OH)D dropped in women who discontinued oral contraceptives, although their number was small.[34] The inverse effect of vitamin D on oestrogen levels, however, is more controversial. A stimulating influence of 1,25$(OH)_2$D on oestrogen production in women has been found,[35] while others reported a negative association between the two.[18,36]

Clearly, the mechanisms of interplay between vitamin D and oestrogens needs further investigation.

Our results confirm that in the Netherlands, people with a non-European ancestry have significantly lower 25(OH)D levels than native Europeans, and that respondents with a mixed background have intermediate levels. Because skin colour was not recorded in the NESDA cohort, this finding must be interpreted with caution. But as the most commonly reported birth countries in this group were Surinam, Indonesia and the Dutch Antilles, it may be assumed that their average skin tone was darker than the average in the Netherlands. It has been recognised that a darker skin synthesises less vitamin D upon the same exposure to UV light,[37] but other explanations for the decreased 25(OH)D levels may be found in diet and sun-avoiding behaviour.[14,38]

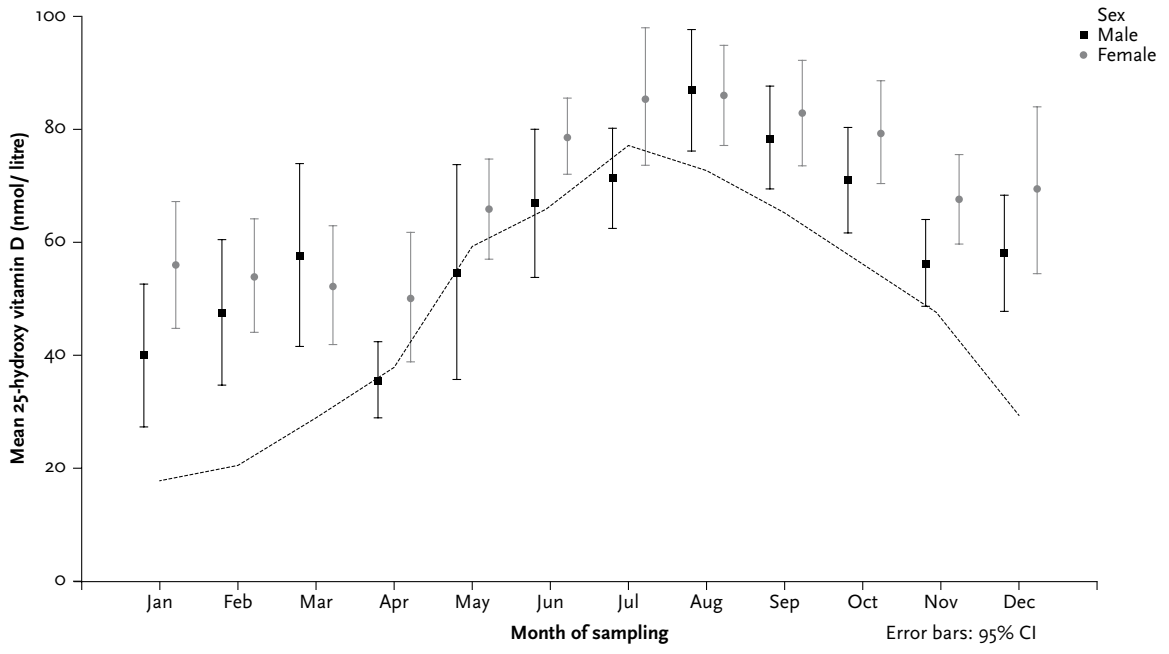
Clinical hypovitaminosis used to occur majorly at the time of industrialisation when people in the cities were insufficiently exposed to sunlight. In our study sample, urbanisation was not significantly correlated with 25(OH)D levels when ancestry was entered into the multiple regression model. Respondents with one or more non-European parents were more likely to live in urban areas than those with a European origin (97.5% vs. 71.7% respectively).

Socioeconomic status is generally considered to be a predictor for different health indicators. Whereas it has been reported that low-income groups have decreased 25(OH)D levels,[39] a recent study found an association with income but not educational level.[40] Similarly, our results do not reveal an association of 25(OH)D with educational attainment.

Health and lifestyle indicators

As the liver and kidney play an important role in the metabolism of vitamin D, we measured GGT and the eGFR as indicators of hepatic and renal function, respectively. Increased GGT levels can indicate impaired hepatic function, which might relate to a decreased production of 25(OH)D. This association was confirmed in the univariate analysis, but did not remain significant in the multiple regression model. Whereas impaired renal function is related to a decreased 25(OH)D,[41] our results show that an increased eGFR may correlate with decreased levels of 25(OH)D within healthy subjects. We suggest that an elevated eGFR may indicate a higher hydroxylation rate of 25(OH)D into 1,25$(OH)_2$D, resulting in lower 25(OH)D values, but an increase of readily available 1,25$(OH)_2$D. Support for this hypothesis can be found in a recent study that showed a direct relation between eGFR and circulating levels of 1,25$(OH)_2$D.[42]

Figure 1. *Fluctuation of 25(OH)D levels over the months of the year in men (n = 201) and women (n = 338). The grey line shows the mean weekly hours of sunshine in the ten weeks prior to sampling, ranging from 17 hours/week in January to 76 hours/week in July*



Evidence suggests that vitamin D status is associated with physical fitness. We confirm earlier findings that people with an increased BMI have lower 25(OH)D levels,[43,44] but the relation between 25(OH)D and physical activity is not so straightforward. Our results show that respondents who did sports had higher levels of 25(OH)D, while the association with physical activity expressed in weekly MET-hours was not significant. This corresponds with earlier indications that vigorous, but not moderate physical activity, is associated with a higher 25(OH)D.[45] Correspondingly, an association with physical fitness but not physical activity has been found,[46] although such cross-sectional results may be caused by the positive effect of vitamin D on muscular health.[47] Furthermore, as people who do sports might spend more time outdoors or have a healthier diet, we must conclude that the current results provide insufficient evidence to disentangle whether physical activity has a direct or indirect link with 25(OH)D. Although alcoholism is associated with a decrease in 25(OH)D, moderate consumption has been reported to have a positive influence on bone mineral density and 25(OH)D.[48] Correspondingly, we found that consumers of alcohol had higher 25(OH)D than those who drank no more than one glass per week, resulting in a positive effect of alcohol consumption in the multiple analysis. In spite of the fact that 10.8% of the study sample were labelled as heavy drinkers, excessive alcohol use was rare in our sample (1.1% showed alcohol dependence according to the AUDIT). Therefore, these data do not provide sufficient information to speculate about the consequences of alcoholism on vitamin D status.

Our results do not confirm previous findings that indicated an association between smoking and vitamin D intake and circulating levels, and bone density.[49-51] In general, however, these associations have only been found in postmenopausal women, while the majority of our sample consisted of men and premenopausal women.

The use of vitamin supplements was correlated with increased plasma 25(OH)D, but did not remain significant in the multiple model. It would, however, be premature to conclude that the use of vitamin supplements has no effect on vitamin D status at all. One limitation in the ascertainment of vitamin use is the fact that multivitamin tablets were included, while the presence and amount of vitamin D in such preparations was unknown. Also, since no more than 13% of the study population used these supplements, the statistical power is limited.

Sampling

Our data confirm earlier findings that samples do not show deterioration while stored at -80 °C for an extended period of time, but that the season is an important determinant.[52] In the summer months, 80.3% had levels of at least 50 nmol/l. This percentage was significantly lower in winter (66.9%, $p < 0.001$). As *figure 1* shows, the number of sunshine hours is around four times higher in July than in January but, in addition, the intensity of UV radiation is higher in the summer months, more time is being spent outside, and less skin is generally covered by clothes.

Strengths and limitations

Whereas vitamin D status is commonly studied in elderly populations, the present study examined determinants of plasma 25(OH)D in healthy adults between 18-65 years of age. The strengths of our analyses are the fact that mental and physical diseases were absent, and the simultaneous ascertainment of sociodemographic, health and lifestyle indicators, and sampling variables. In particular, the use of a quantified measure for availability of sunshine proved to be a much stronger predictor for 25(OH)D levels than merely the season. A limitation, however, was the lack of information about actual time spent in the sunshine and about dietary intake and supplementation of vitamin D.

CONCLUSION

Within a group of healthy adults at a northern latitude, 27% had insufficient 25(OH)D levels (< 50 nmol/l) and only 40% reached the recommended levels (> 75 nmol/l). A wide range of determinants influenced 25-hydroxyvitamin D levels: male gender, non-European ancestry, an elevated BMI, an increased eGFR and sampling in a season with less available sunlight were all associated with lower 25(OH)D levels. Those who participated in sports, consumed moderate amounts of alcohol and women who used oral contraceptives had higher 25(OH)D levels. This set of eight variables explained 29.5% of the variance. Our results confirm the role of these predictors, but disconfirm the influence of age and urbanisation in a healthy adult population under 65 years of age.

DISCLOSURES

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REFERENCES

1. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001;22:477-501.
2. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-81.
3. Armas LAG, Heaney RP. Vitamin D: The Iceberg Nutrient. J Renal Nutr. 2011;21:134-9.
4. Bouvard B, Annweiler C, Salle A, et al. Extraskeletal effects of vitamin D: Facts, uncertainties, and controversies. Joint Bone Spine. 2011;78:10-6.
5. Thacher TD, Clarke BL. Vitamin D Insufficiency. Mayo Clin Proc. 2011;86:50-60.
6. Bertone-Johnson ER. Vitamin D and the occurrence of depression: causal association or circumstantial evidence? Nutr Rev. 2009;67:481-92.
7. Hoogendijk WJ, Lips P, Dik MG, Deeg DJ, Beekman AT, Penninx BW. Depression is associated with decreased 25-hydroxyvitamin D and increased parathyroid hormone levels in older adults. Arch Gen Psychiatry. 2008;65:508-12.
8. Milaneschi Y, Shardell M, Corsi AM, et al. Serum 25-Hydroxyvitamin D and Depressive Symptoms in Older Women and Men. J Clin Endocrinol Metab. 2010;95:3225-33.
9. Milaneschi Y, Hoogendijk W, Lips P, et al. The association between low vitamin D and depressive disorders. Mol Psychiatry. 2014;19:444-51.
10. Holick MF. Mccollum Award Lecture, 1994 - Vitamin-D – New Horizons for the 21St-Century. Am J Clin Nutr. 1994;60:619-30.
11. Lips P. Vitamin D physiology. Progr Biophys Mol Biol. 2006;92:4-8.
12. Black LJ, Seamans KM, Cashman KD, Kiely M. An Updated Systematic Review and Meta-Analysis of the Efficacy of Vitamin D Food Fortification. J Nutr. 2012;142:1102-8.
13. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. Am J Clin Nutr. 2004;80:1710S-6S.
14. van Schoor NM, Lips P. Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab. 2011;25:671-80.
15. Jones G, Strugnell SA, Deluca HF. Current understanding of the molecular actions of vitamin D. Physiol Rev. 1998;78:1193-231.
16. Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr. 2008;88:507S-10S.
17. Daly RM, Gagnon C, Lu ZX, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol. 2012;77:26-35.
18. Janssen HCJP, Emmelot-Vonk MH, Verhaar HJJ, van der Schouw YT. Determinants of vitamin D status in healthy men and women aged 40-80 years. Maturitas. 2013;74:79-83.
19. Penninx BWJH, Beekman ATF, Smit JH, et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Meth Psych Res. 2008;17:121-40.
20. Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 Routine 25-Hydroxyvitamin D Assays: Influence of Vitamin D Binding Protein Concentration. Clin Chem. 2012;58:543-8.
21. Stepman HCM, Vanderroost A, Van Uytfanghe K, Thienpont LM. Candidate Reference Measurement Procedures for Serum 25-Hydroxyvitamin D-3 and 25-Hydroxyvitamin D-2 by Using Isotope-Dilution Liquid Chromatography-Tandem Mass Spectrometry. Clin Chem. 2011;57:441-8.
22. Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? J Steroid Biochem Mol Biol 2004;89-90:611-4.
23. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2011;96:1911-30.
24. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-12.
25. Saunders JB, Aasland OG, Babor TF, Delafuente JR, Grant M. Development of the Alcohol-Use Disorders Identification Test (Audit) - Who Collaborative Project on Early Detection of Persons with Harmful Alcohol-Consumption. Addiction. 1993;88:791-804.
26. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35:1381-95.
27. Kuhn T, Kaaks R, Teucher B, et al. Dietary, lifestyle, and genetic determinants of vitamin D status: a cross-sectional analysis from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Germany study. Eur J Nutr. 2014;53:731-41.
28. Touvier M, Deschasaux M, Montourcy M, et al. Determinants of Vitamin D Status in Caucasian Adults: Influence of Sun Exposure, Dietary Intake, Socio-Demographic, Lifestyle, Anthropometric and Genetic Factors. J Invest Dermatol. 2014 [Epub ahead of print].
29. Maclaughlin J, Holick MF. Aging Decreases the Capacity of Human-Skin to Produce Vitamin-D3. J Clin Invest. 1985;76:1536-8.
30. Hedlund L, Brembeck P, Olausson H. Determinants of Vitamin D Status in Fair-Skinned Women of Childbearing Age at Northern Latitudes. Plos One. 2013;9:8.
31. Gagnon C, Baillargeon JP, Desmarais G, Fink GD. Prevalence and predictors of vitamin D insufficiency in women of reproductive age living in northern latitude. Eur J Endocrinol. 2010;163:819-24.
32. Shirazi L, Almquist M, Malm J, Wirfalt E, Manjer J. Determinants of serum levels of vitamin D: a study of life-style, menopausal status, dietary intake, serum calcium, and PTH. BMC Womens Health. 2013;13:33.
33. Sowers MR, Wallace RB, Hollis BW, Lemke JH. Parameters Related to 25-Oh-D Levels in A Population-Based Study of Women. Am J Clin Nutr. 1986;43:621-8.
34. Harris SS, Dawson-Hughes B. The association of oral contraceptive use with plasma 25-hydroxyvitamin D levels. J Am Coll Nutr. 1998;17:282-4.
35. Parikh G, Varadinova M, Suwandhi P, et al. Vitamin D Regulates Steroidogenesis and Insulin-like Growth Factor Binding Protein-1 (IGFBP-1) Production in Human Ovarian Cells. Horm Metab Res. 2010;42:754-7.
36. Knight JA, Wong J, Blackmore KM, Raboud JM, Vieth R. Vitamin D association with estradiol and progesterone in young women. Cancer Causes Control. 2010;21:479-83.
37. Lo CW, Paris PW, Holick MF. Indian and Pakistani Immigrants Have the Same Capacity As Caucasians to Produce Vitamin-D in Response to Ultraviolet-Irradiation. Am J Clin Nutr. 1986;44:683-5.
38. Webb AR. Who, what, where and when – influences on cutaneous vitamin D synthesis. Prog Biophys Mol Biol. 2006;9:17-25.
39. Hirani V, Mosdol A, Mishra G. Predictors of 25-hydroxyvitamin D status among adults in two British national surveys. Br J Nutr. 2009;101:760-4.
40. Kant AK, Graubard BI. Race-ethnic, family income, and education differentials in nutritional and lipid biomarkers in US children and adolescents: NHANES 2003-2006. Am J Clin Nutr. 2012;96:601-12.
41. Damasiewicz MJ, Magliano DJ, Daly RM, et al. 25-hydroxyvitamin D Levels and chronic kidney disease in the AusDiab (Australian Diabetes, Obesity and Lifestyle) study. BMC Nephrol. 2012;13:55.
42. Borgermann J, Lazouski K, Kuhn J, et al. 1,25-Dihydroxyvitamin D fluctuations in cardiac surgery are related to age and clinical outcome. Crit Care Med. 2012;40:2073-81.
43. Compston JE, Vedi S, Ledger JE, Webb A, Gazet JC, Pilkington TRE. Vitamin-D Status and Bone Histomorphometry in Gross Obesity. Am J Clin Nutr. 1981;34:2359-63.
44. Wortsman J, Matsuoka LY, Chen TC, Lu ZR, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690-3.
45. Dong YB, Pollock N, Stallmann-Jorgensen IS, et al. Low 25-Hydroxyvitamin D Levels in Adolescents: Race, Season, Adiposity, Physical Activity, and Fitness. Pediatrics. 2010;125:1104-11.
46. Valtuena J, Gonzalez-Gross M, Huybrechts I, et al. Factors Associated with Vitamin D Deficiency in European Adolescents: The HELENA Study. J Nutr Sci Vitaminol. 2013;59:161-71.
47. Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. Osteoporosis Int. 2002;13:187-94.
48. Ilich JZ, Brownbill RA, Tamborini L, Crncevic-Orlic Z. To drink or not to drink: How are alcohol, caffeine and past smoking related to bone mineral density in elderly women? J Am Coll Nutr. 2002;21:536-44.
49. Morabia A, Bernstein MS, Antonini S. Smoking, dietary calcium and vitamin D deficiency in women: a population-based study. Eur J Clin Nutr. 2000;54:684-9.
50. Brot C, Jorgensen NR, Sorensen OH. The influence of smoking on vitamin D status and calcium metabolism. Eur J Clin Nutr. 1999;53:920-6.
51. Law MR, Hackshaw AK. A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. BMJ. 1997;315:841-6.
52. Agborsangaya C, Toriola AT, Grankvist K, et al. The Effects of Storage Time and Sampling Season on the Stability of Serum 25-Hydroxy Vitamin D and Androstenedione. Nutr Cancer. 2009;62:51-7.