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ENDOCRINE METHODS AND TECHNIQUES

# Development of an algorithm to predict serum vitamin D levels using a simple questionnaire based on sunlight exposure

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**Abstract** Sun exposure is the main determinant of vitamin D production. The aim of this study was to develop an algorithm to assess individual vitamin D status, independently of serum 25(OHD) measurement, using a simple questionnaire, mostly relying upon sunlight exposure, which might help select subjects requiring serum 25(OHD) measurement. Six hundred and twenty adult subjects living in a mountain village in Southern Italy, located at 954 m above the sea level and at a latitude of 40°50′11″76N, were asked to fill the questionnaire in two different periods of the year: August 2010 and March 2011. Seven predictors were considered: month of investigation, age, sex, BMI, average daily sunlight exposure, beach holidays in the past 12 months, and frequency of going outdoors. The statistical model assumes four classes of serum 25(OHD) concentrations: ≤10, 10–19.9, 20–29.9, and ≥30 ng/ml. The algorithm was developed using a two-step procedure. In Step 1, the linear regression equation was defined in 385 randomly selected subjects. In Step 2, the predictive ability of the regression model was tested in the remaining 235 subjects. Seasonality, daily sunlight exposure and beach holidays in the past 12 months accounted for 27.9, 13.5, and 6.4 % of the explained variance in predicting vitamin D status, respectively. The algorithm performed extremely well: 212 of 235 (90.2 %) subjects were assigned to the correct vitamin D status. In conclusion, our pilot study demonstrates that an algorithm to estimate the vitamin D status can be developed using a simple questionnaire based on sunlight exposure.

**Keywords** Statistical model · Predictors · 7-Dehydrocholesterol · Hypovitaminosis D · Seasonality

## Introduction

Vitamin D deficiency is a common finding, particularly in the elderly, but it may be present at any age [1–3]. The great majority of vitamin D in the body (up to 95 %) derives from sun exposure of the skin. The skin is penetrated by ultraviolet–B (UVB) radiation and is able to convert 7-dehydrocholesterol (7-DHC) to previtamin $D_3$ and subsequently to vitamin $D_3$. Circulating Vitamin $D_3$ is metabolized in the liver to 25-hydroxyvitamin $D_3$ and then metabolized in the kidneys by the enzyme 1α-hydroxylase to its active form, 1,25-dihydroxyvitamin $D_3$. Plasma parathyroid hormone (PTH), serum calcium and phosphate, and FGF23 closely regulate renal production of 1,25-dihydroxyvitamin D [4, 5].

Therefore, wise sun exposure can provide an adequate amount of vitamin $D_3$, which is stored in body fat and released during the winter, when vitamin $D_3$ cannot be produced. Individual (skin type, age, duration of sun exposure) and environmental (latitude, season, time of day, skin protectors, pollution) factors are the major determinants of the effectiveness of 7DHC conversion to vitamin $D_3$ [6–8]. It has been estimated that for white elderly people living in Boston, the exposure of face, arms, and legs two to three times a week to sunlight for 5–10 min in

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summer months in the afternoon might be a strategy to optimize vitamin $D_3$ production [9, 10].

Low vitamin D status has been associated with osteomalacia/rickets, osteoporosis, and, if severe, with myopathy and sarcopenia [11]. Moreover, suboptimal vitamin D status has been associated with lower efficacy of anti-resorptive therapy for osteoporosis [12–14]. Finally, extrarenal production of 1,25-dihydroxyvitamin D has been involved in the control of the immune response [15], cell proliferation [5], mortality [16, 17], and many other extraskeletal effects [18].

The measurement of total serum 25-hydroxyvitamin D [25(OH)D, i.e., the sum of $25(OH)D_2$ from vegetable sources and endogenous and exogenous $25(OH)D_3$ from animal sources, with $25(OH)D_3$ prevailing on $25(OH)D_2$ in humans] is the more reliable marker to define the individual vitamin D status. The gold standards to measure serum 25(OH)D would be liquid chromatography and tandem mass spectroscopy, but these techniques are costly and available only in few specialized centers [19, 20]. Alternatively, serum 25(OH)D can be measured by immunometric assays, which show however wide variability (up to 30 %) and lack of standardization, and may not always be available [21, 22].

Vitamin D status can be defined on the basis of serum 25(OH)D levels [1, 23–25]. The Endocrine Society recommends the following cut-offs: deficiency <20 ng/mL (50 nmol/L), insufficiency 20–30 ng/mL (50–75 nmol/L), and sufficiency ≥30 ng/mL (75 nmol/L). With such boundaries, it has been estimated that globally 1 billion people have vitamin D deficiency or insufficiency [4, 25].

Several guidelines agree on recommending screening for vitamin D deficiency only in at risk individuals and not in the whole population, because there is no evidence demonstrating benefits of screening for vitamin D deficiency at population level [23, 25, 26].

Because of the limitations of 25(OH)D assay and the fact that age and sun exposure are the major determinants of vitamin D production, prior studies have been designed to identify potential predictor of vitamin D status, independently of serum 25(OH)D measurement. Self-administered questionnaires related to sun exposure have been the method most widely used [27–29]. Some, but not all, studies have shown a statistically significant correlation between sunlight exposure assessed by questionnaires and the UV radiation reaching the skin, measured by digital UV dosimeters [30]. Most studies have been performed in selected populations, particularly in women, and only a few in large samples representative of the general population [31, 32].

The aim of this study was to develop an algorithm to estimate individual vitamin D status, independently of serum 25(OH)D measurement, using a simple questionnaire, mostly relying upon indirect measurement of sunlight exposure, administered to a sample of Italian adult population which might correctly forecast its vitamin D status and might help select the subjects in whom serum 25(OH)D needs to be measured.

## Methods

The questionnaire was developed including simple questions, which address relevant determinants of vitamin D status: age, body mass index (BMI), and sunlight exposure. The questionnaire included anthropometric parameters (age, sex, BMI) and the following 4 items:

- How much time do you expose your face, arm, and legs to sunlight every day? (<10, 10–20, 21–30, >30 min). The 30-min value is closed to the value that, under this condition, has been shown to give the maximal dermal conversion of 7-DHC to previtamin $D_3$ in fair skinned individuals [33].
- Did you take a beach holiday in the past 12 months? (yes/no).
- How often do you go outdoors? (often/sometimes, seldom).
- Month of investigation (August/March).

The study was performed in Pescopagano, a Southern Italian village located in the Lucan Apennines, at 954 m above the sea level, and at a latitude of 40°50′11″76 N, a value which is close to that of Italian central latitude (about 45°).

Adult subjects participating in a larger study on the prevalence of thyroid and parathyroid disorders [34, 35] were asked to fill in themselves the questionnaire in 2 different periods of the year: the midsummer (August 2010, mean UV index 3.57) and the early spring (March 2011, mean UV index 2.0), which correspond to the maximal and minimal skin synthesis of vitamin $D_3$, respectively. Different subjects participated in the two surveys. All participants underwent a medical interview in order to exclude subjects taking vitamin D supplements, drug interfering with vitamin D metabolism, as well as subjects with severe kidney and liver disorders. To what extent a subclinical liver disorder could affect the predictive value of our questionnaire remains to be established [16]. Informed consent was obtained from all participants.

Blood samples were obtained by venipuncture, and serum aliquots were stored at −20C for further analyses. Serum 25(OH)D was measured in all individuals by RIA (DiaSorin Inc, Sillwater, MN). The intra- and inter-assay CV at 10 and 30 ng/mL were 8.1 and 10.1, and 7.8 and 9.0 %, respectively.

### Statistical analysis

Normality was assessed using the Shapiro–Wilk test, histograms and Q–Q plots. Continuous variables were expressed as mean ± SD and compared by the two-tailed independent-samples $t$ test and ANOVA test, as appropriate. Discrete variables were compared using the $\chi^2$ test. Value <0.05 was considered as statistically significant.

The algorithm was developed using a two-step procedure. In Step 1, the linear regression equation was defined, considering a randomly selected cohort of 385 subjects. This stratified sample sub-cohort consisted of 62.1 % of the entire population ($N = 620$). The subjects included in this cohort were similar with regard to age, gender, BMI, 25(OH)D concentration, and parameters related to sun exposure to patients included in the second cohort (data not shown). In Step 2, the predictive ability of the regression model was tested in the remaining 235 subjects (37.9 % of the entire population).

Model selection was carried out using the Bayesian Information Criterion, by which the potential predictors were analyzed by assessing the top ten models as generated by the above Criterion and by including the variables that in the univariate analyses were associated with the 25(OH)D serum concentration ($p$ value <0.10).

We quantified the fit of the model in several ways. We calculated $R^2$ to quantify the proportion of the variance in 25(OH)D concentrations explained by the model. We then calculated a root mean square prediction error to quantify the average absolute difference between observed and predicted serum 25(OH)D concentrations. We finally calculated two cross-validated coefficients of correlation between observed and estimated concentrations.

The resultant linear regression residual plots were checked for violations of linear relations. A quadratic term was computed for age and was included in the regression analysis to further test for nonlinearity.

The modeling goal aimed to predict which of the following group of vitamin D status the subjects belong to: serum 25(OH)D concentrations—≤10, 10–19.9, 20–29.9, and ≥30 ng/ml.

All tests were 2 sided. Statistical analyses were performed by using SAS package (version 9.2).

## Results

The study group consists of the majority of the 685 subject included in a previously reported series [34, 36]. Seven subjects with hyperparathyroidism and 58 with hyper- or hypothyroidism were excluded. The remaining 620 subjects were included in the present study: 523 were evaluated in August 2010 and 97 in March 2011. The demographic characteristics of the study sample are reported in Table 1. The two groups of subjects evaluated in August and March were comparable for sex, age, and BMI. There was no statistically significant difference in the mean age and BMI between males and females in the whole group as well as in the subjects evaluated in August or March. In the whole group of subjects, as well as in the subgroups evaluated in August or March, average daily sunlight exposure was significantly lower in females than in males. Mean age in the whole group and in both sexes was inversely correlated with average daily sunlight exposure, beach holidays in the last year, and frequency of time outdoors. The mean serum level of 25(OH)D was 24.0 ± 11.7 ng/ml in the whole group, and it was significantly higher in males than females (26.6 ± 11.6 vs. 22.8 ± 11,5, $p < 0.0001$). As expected, mean serum 25(OH)D levels were significantly higher in subjects evaluated in August than those in subjects evaluated in March (26.9 ± 10.5 vs. 9.7 ± 4.8 ng/ml, $p < 0.0001$), with levels higher in males than in females.

In the whole group of subjects, serum concentration of 25(OH)D was inversely correlated with age ($p < 0.0001$, $r = 0.225$) and BMI ($p < 0.0001$, $r = 0.201$). There was a statistically significant positive relationship between mean serum 25(OH)D and average daily sunlight exposure, beach holidays in the past 12 months, and frequency of going outdoors in the whole groups and in subjects evaluated in August ($p < 0.0001$ for all comparisons), but not in those examined in March (Table 2). The same findings were also observed when males and females were separately evaluated (data not shown). Figure 1 shows the distribution of examined subjects in the four subgroups with respect to serum 25(OH)D concentrations (≤10, ≥10–19.9, ≥ 20–29.9, ≥30 ng/ml). Most subjects (68.5 %) examined in August had values ≥20 ng/ml, whereas almost all (96.9 %) examined in March had values <20 ng/ml and more than half (56.7 %) <10 ng/ml. Both sexes had a similar distribution.

## Development of an algorithm

Variables included in the algorithm consisted of anthropometric and lifestyle parameters. We have no data on the reproducibility of the response to the lifestyle items included in the questionnaire, since the questionnaire was administered only once. However, from a statistical point of view, a sample size of 385 achieves a 86.0 % power to detect a change in slope from 0.20 under the null hypothesis to 0.50 under the alternative hypothesis when the standard deviation of X is 1.00, the standard deviation of Y is 2.00, and the two-sided significance level is 0.05 [37] ].

**Table 1** Demographic characteristics and other items included in the questionnaire of the population studied

| Item | Time of evaluation | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | March + August | | | March | | | August | | |
| | All $n = 620$ | Females $n = 384$ | Males $n = 236$ | All $n = 97$ | Females $n = 59$ | Males $n = 38$ | All n = 523 | Females $n = 325$ | Males $n = 198$ |
| Age (yearrs) | 48.2 ± 17.9 | 48.2 ± 17.7 | 47.9 ± 18.2 | 43.6 ± 14.2 | 43.9 ± 13.1 | 43.2 ± 15.9 | 49.0 ± 18.4 | 49.0 ± 18.4 | 48.9 ± 18.5 |
| BMI | 27.7 ± 5.3 | 27.6 ± 5.7 | 28.0 ± 4.7 | 27.4 ± 4.9 | 27.8 ± 5.6 | 26.7 ± 3.5 | 27.8 ± 5.4 | 27.5 ± 5.7 | 28.3 ± 4.8 |
| Sunlight exposure (min) | | | | | | | | | |
| <10 | 166 (26.8) | 152 (39.6) | 44 (18.6) | 30 (30.9) | 21 (35.6) | 9 (23.7) | 166 (31.7) | 131 (40.3) | 35 (17.1) |
| 10–20 | 131 (21.0) | 84 (21.8) | 47 ((20.0) | 28 (28.9) | 18 (30.6) | 10 (26.3) | 103 (19.7) | 66 (20.3) | 37 (18.7) |
| 21–30 | 87 (14.0) | 56 (14.6) | 31 (13.1) | 16 (16.5) | 10 (16.9) | 6 (15.8) | 71 (13.6) | 46 (14.2) | 25 (12.6) |
| >30 | 206 (33.2) | 92 (24.0) | 114 (48.3) | 23 (23.7) | 10 (16.9) | 13 (34.2) | 183 (35.0) | 82 (25.2) | 101 (51.0) |
| Beach holidays | | | | | | | | | |
| Yes | 310 (50) | 194 | 116 | 52 | 32 | 20 | 258 | 162 | 96 |
| No | 310 (50) | 190 | 120 | 45 | 27 | 18 | 265 | 163 | 102 |
| Times going outdoors | | | | | | | | | |
| Often | 502 (81.0) | 298 (77.6) | 204 (86.4) | 80 | 48 | 32 | 422 | 250 | 172 |
| Sometimes | 79 (12.7) | 56 (14.6) | 23 (9.8) | 14 | 9 | 5 | 65 | 47 | 18 |
| Rarely | 39 (6.3) | 30 (7.8) | 9 (3.8) | 3 | 2 | 1 | 36 | 28 | 8 |

The percentage is indicated *in parenthesis*

Sunlight exposure of the whole population: females versus males: $p = 0.0013$

Sunlight exposure March versus August $p = 0.27$

March sunlight exposure: females versus males: $p = 0.036$

August sunlight exposure: females versus males: $p < 0.0001$

Times going outdoors of the whole population: females versus males: $p = 0.2$

Times going outdoors March versus August $p = 0.4$

**Table 2** Relationship between serum 25(OH)D concentration and determinants of exposure to sunlight

| Determinants | Whole group $n = 620$ | August $n = 523$ | March $n = 97$ |
|---|---|---|---|
| Sunlight exposure (min) | | | |
| <10 | 21.1 ± 11.0 | 23.1 ± 10.5 | 5.9 ± 5.4 |
| 10–20 | 23.7 ± 12.1 | 27.8 ± 10.1 | 8.7 ± 4.0 |
| 21–30 | 24.0 ± 11.2 | 27.4 ± 9.3 | 9.0 ± 5.2 |
| >30 | 27.6 ± 11.4 | 29.7 ± 10.3 | 11.1 ± 4.5 |
| | $p < 0.0001$ | $p < 0.0001$ | $p = 0.3$ |
| Beach holidays | | | |
| Yes | 27.6 ± 0.6 | 31.1 ± 10.5 | 10.1 ± 0.6 |
| No | 20.9 ± 12.5 | 22.8 ± 8.8 | 9.3 ± 0.3 |
| | $p < 0.0001$ | $p < 0.0001$ | $p = 0.4$ |
| Times going outdoors | | | |
| Often | 25.5 ± 11.6 | 17.2 ± 8.7 | 8.6 ± 4.3 |
| Sometimes | 19.8 ± 10.8 | 21.8 ± 10.7 | 10.5 ± 4.8 |
| Rarely | 16.6 ± 8.7 | 17.2 ± 8.7 | 9.6 ± 4.9 |
| | $p < 0.0001$ | $p < 0.0001$ | $p = 07$ |

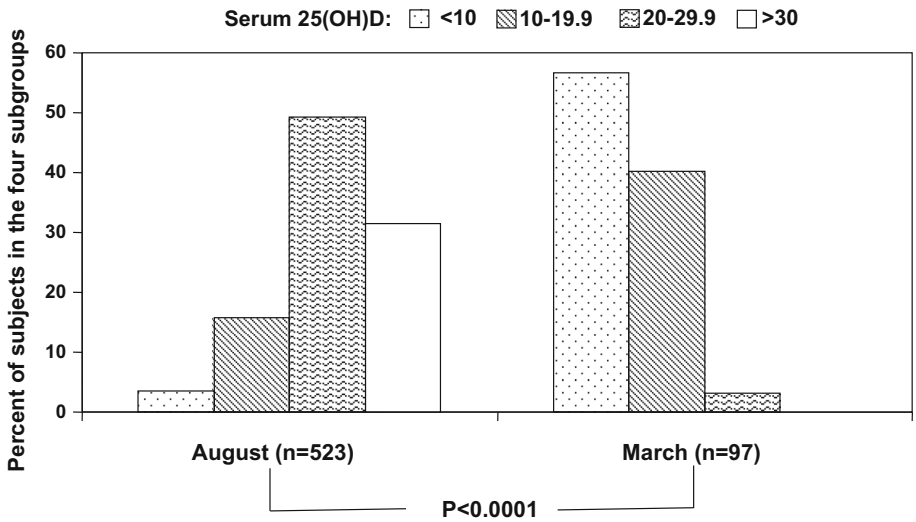


**Fig. 1** Distribution of subjects according to different vitamin D status defined by measurement of serum 25(OH)D

All variables significantly associated with serum 25(OH)D concentration, namely age, sex, BMI, average daily sunlight exposure, beach holidays in the past 12 months, frequency of going outdoors, and month of evaluation, were included in a multivariate regression analysis (performed in 385 randomly selected subjects) as covariates, and vitamin D status as dependent variable. All correlations remained statistically significant. This set of parameters explained 55.7 % of the variance of the dependent variable [serum 25(OH)D concentration]. Seasonality, daily sunlight exposure, and beach holidays in the past 12 months accounted for 27.9, 13.5, and 6.4 % of the explained variance in the prediction of vitamin D status, respectively. Each remaining variable accounted for <2 %. The linear regression equation, which relates vitamin D status to the predictors (items of the questionnaire), is the following:

$$\begin{aligned}[\text{Group of vitamin D status}] &= -10.864 + \text{``Age''} \times 0.914 \\ &+ \text{``Sex''} \times 1.888 + \text{``BMI''} \\ &\times 0.888 + \text{``Month of evaluation''} \\ &\times 13.683 + \text{``Average daily sunlight exposure''} \\ &\times 2.713 + \text{``Beach holidays in the past 12months''} \\ &\times 4.755 + \text{``Frequency of going outdoors''} \times 2.657.\end{aligned}$$

In our model, low levels of collinearity are present (condition index <21), and therefore, it is possible to exclude that the coefficients of each independent variable included in the equation are biased by collinearity.

The validity of the equation model to predict the vitamin D status was tested in the second cohort of 235 subjects.

The results are shown in Table 3. The algorithm performed extremely well, and 212 of 235 (90.2 %) subjects were assigned to the correct vitamin D status. The predictive abilities of the regression model were 90.2 and 85.9 % for concentrations of 25(OH)D <10 and ≥30 ng/mL, respectively.

## Discussion

The measurement of serum 25(OH)D is not widely available, and variable results are obtained with different assay methods. Moreover, it is debated whether it is necessary to measure serum 25(OH)D in each individual to establish whether vitamin D supplementation is needed and which supplementation schedule should be used. As a matter of fact, different strategies of vitamin D supplementation have been established on the basis of serum 25(OH)D concentration [23, 38].

In the present study, performed in a large sample of adult population, we observed that serum 25(OH)D is higher in males than in females, and, independently of sex, in younger than older individuals. This is likely explained by differences in lifestyle between men and women living in rural areas, particularly the longer time spent outdoors by men.

Using an easy-to-use questionnaire, based on anthropometric data and items related to sun exposure, an algorithm was developed, in order to provide an estimate of vitamin D status without measurement of serum 25(OH)D and may help select subjects in whom serum 25(OH)D needs to be measured. The best predictor was the season of evaluation (end of summer vs end of winter). This is in keeping with the concept that season, together with latitude and time of the day, is a determinant of the solar zenith angle, which represents the ultimate parameter responsible of the quality of the UVB reaching the skin and therefore of the cutaneous conversion of 7-DHC to previtamin $D_3$. As matter of fact, the significant associations between serum 25(OH)D concentration and sunlight exposure, beach holidays in the past 12 months, and times going outdoors observed in subjects evaluated at the end of summer (August) were no longer present in those evaluated at the end of winter (March). This observation is in agreement with the findings of Bolek-Berquist et al., who showed that sun exposure was not a determinant of 25(OH)D concentration at the end of winter and early spring [39]. Similarly, Hanwell et al. found no correlation between a sun exposure score, based on time spent outdoor and extent of skin exposed each day over the previous week, and serum 25(OH)D concentration during the winter [33].The algorithm herein developed is able to explain about 55.7 % of the variance in serum 25(OH)D concentration. Other variables, including vitamin D intake, phototype, use of sunscreen, clothing, pollution, individual difference in the activity of the 25-hydroxylase enzyme, and polymorphism in the D-binding protein, likely account for the rest of the variability [5].

Prior attempts to develop model to predict serum 25(OH)D concentrations have mostly been performed in selected populations (patients with multiple sclerosis, cancer, and postmenopausal osteoporotic women) and were able to predict from 19 to 39 % of the variation of 25(OH)D concentration [40], being age, anthropometric parameters, sun exposure, season of blood withdrawal, and diet supplement use the most common significant factors.

The main strengths of our algorithm are (a) the simplicity of the questionnaire on which it was based; (b) the inclusion in the questionnaire of demographic and anthropometric items, other than those evaluating sunlight exposure, which increased the predictive value of the algorithm; and (c) its validation in a sample of the general population. Additional advantages of assessing vitamin D status by a questionnaire rather than measurement of serum 25(OH)D could be as follows: (i) the 25(OH)D assay is not widely available; (ii) it overcomes the problem of the variability (up to 30 %) of the

**Table 3** Individual assignment to different vitamin D statuses by measurement of serum 25(OH)D and by the questionnaire-based algorithm

| Algorithm-predicted vitamin D status | Serum 25(OH)D vitamin D status | | | |
|---|---|---|---|---|
| | <10 ng/mL ($n = 41$) | 10–19.9 ng/mL ($n = 47$) | 20–29.9 ng/mL ($n = 69$) | ≥30 ng/mL ($n = 78$) |
| <10 ng/mL | 37 | 1 | 1 | 0 |
| | **90.2** % | 2.1 % | 1.4 % | |
| 10–19.9 ng/mL | 4 | 45 | 5 | 0 |
| | 9.8 % | **95.8** % | 7.2 % | |
| 20–29.9 ng/mL | 0 | 1 | 63 | 11 |
| | | 2.1 % | **91.3** % | 14.1 % |
| ≥30 ng/mL | 0 | 0 | 0 | 67 |
| | | | | **85.9** % |

Bold values is to better point out the high performance of our regression model at the different classes of vitamin D status

results of serum 25(OH)D measurements using different immunological assays [15]; (iii) the result could be immediately available using an "ad hoc" developed smartphone application; and (iv) there is no cost.

This algorithm, however, has several limitations: (a) the study population consisted only of adult Caucasian subjects; (b) the population examined is living in a rural area, with minimal air pollution; (c) data were collected only in two periods of the year, August (end of summer) and March (the end of winter); and (d) the study was performed at 954 m above the sea level, at a latitude of 40°50′11″76 N.

As far as the latter point is concerned, experimental in vitro evidence indicates that, at a latitude of 27°N, up to 1400 m, the production of previtamin $D_3$ from 7-DHC is not influenced by altitude, whereas an almost linear increase is observed above 2000 m. Regarding the latitude, under similar experimental condition, an increase in the latitude from 42 to 52°N decreases by about 50 % the production of previtamin $D_3$. Therefore, we can argue that our algorithm might keep its validity in predicting vitamin D status in individual living from sea level to about 1500 m of altitude at a latitude representative of the Mediterranean countries, but not at any latitude.

The algorithm cannot be used to check the effect of vitamin D supplementation.

In conclusion, our pilot study shows that an algorithm to estimate the vitamin D status can be developed using a simple questionnaire. We acknowledge that, because of the limitations, the predictive value of the current algorithm needs to be validated in other experimental conditions. In this regard, a larger study will be performed in order to develop a more widely usable algorithm in which the questionnaire will be administered at different latitudes, in different months of the year, and in urban and rural communities.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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