# Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women\*

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### ABSTRACT

The aim of this study was to investigate modifiable predictors of vitamin D status in healthy individuals, aged 55–74, and living across the USA. Vitamin D status [serum 25-hydroxyvitamin D (25(OH)D)] was measured along with age and season at blood collection, demographics, anthropometry, physical activity (PA), diet, and other lifestyle factors in 1357 male and 1264 female controls selected from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) cohort. Multivariate linear and logistic regression analyses were used to identify associations with vitamin D status. Three%, 29% and 79% of the population had serum 25(OH)D levels <25, <50 and <80 nmol/L, respectively. The major modifiable predictors of low vitamin D status were low vitamin D dietary and supplement intake, body mass index (BMI) >30 kg/m², physical inactivity (PA) and low milk and calcium supplement intake. In men, 25(OH)D was determined more by milk intake on cereal and in women, by vitamin D and calcium supplement and menopausal hormone therapy (MHT) use. Thus targeting an increase in vigorous activity and vitamin D and calcium intake and decreasing obesity could be public health interventions independent of sun exposure to improve vitamin D status in middle-aged Americans.

## 1. Introduction

Established determinants of vitamin D status, as measured by serum 25-hydroxyvitamin D (25(OH)D) are exposure to sunlight and intake of vitamin D, either from foods or vitamin supplements [1–3]. Decreased physical activity (PA) and obesity and low social status have also been associated with low vitamin D levels in Europe and the USA [4–6]. The aim of the present study was to investigate predictors of vitamin D status within a large non-elderly population of healthy men and women living across the USA.

## 2. Materials and methods

The subjects in this study were healthy controls, age-matched to case distributions, selected for five case-control studies of serum vitamin D and cancer nested within the original Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) cohort. The PLCO Cancer Screening Trial is a large randomized controlled multicenter trial in the United States of approximately 155 000 men and women at sites in Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO; and Washington, DC, that was designed to evaluate selected methods for the early detection of these four cancers as well as non-Hodgkins lymphoma (NHL), breast and pancreatic cancer: enrollment began November 1, 1993, and ended June 30, 2001 [7]. Details of these studies of colorectal adenoma, non-Hodgkins lymphoma (NHL) and prostate, breast, and pancreatic cancer are described elsewhere [8–12]. Briefly, we included 399 controls used

for a study of colorectal adenoma (matched to cases by gender and race) [10], 286 controls used for a study of non-Hodgkin lymphoma [11], 713 controls used for a study of prostate cancer (matched to cases by age, time since screening, and year of follow-up) [8], 932 controls used for a study of breast cancer (matched to cases by age and year of blood draw) [9], and 350 used for a study of pancreatic cancer (matched to cases by age, gender, race and date of blood draw) [12]. Of these controls, 59 were included in more than one study; thus, in total 2621 control subjects from PLCO were included in this present data analysis. At the initial screening, all participants were asked to complete a questionnaire including demography, anthropometry, lifestyle factors (including smoking history and vigorous physical activity (PA) during the last year), and usual dietary intake over the 12 months before enrolment (137item food frequency questionnaire and 14 questions about intake of vitamin and mineral supplements [13]). Daily nutrient intake from foods was calculated by multiplying the reported frequency of consumption of each food item by the nutrient composition of the imputed gender-specific portion size using the nutrient database from the U.S. Department of Agriculture [14]. Calcium and vitamin D intake were measured both from food and supplemental sources. Serum samples were collected during the baseline visit, and stored at -70 °C. Levels of the serum 25(OH)D for subjects were determined using a radio-iodinated tracer assay in the laboratories of Hollis and Horst [8-12,15]. Replicate blinded quality control samples from 2 to 4 different individuals were included in all 25(OH)D batches. The overall coefficients of variation were 16.3% for the colorectal adenoma, 11.4% for NHL, 5.9% for prostate, 8.2% for the breast study, and 4.7% for the pancreas study. Separate variables, representing each of the ten study centers across the USA and each of the five nested cancer case-control studies were entered as confounders in all models. Age at blood draw and pack-years of smoking, current smoking and educational level were entered as a priori confounders in all models. Total dietary energy was also added as a confounder in order to adjust for individual variation in total energy intake. As the aim of this study was to identify predictors of 25(OH)D levels, initial data screening was performed in order to identify statistically significant and biologically meaningful variables associated with continuous and categorical vitamin D status. T-tests for continuous variables and chi-square tests for categorical variables were used to determine statistical significance with two-sided *p*-values less than 0.01. Those unadjusted factors found to be significant were then included in a forward stepwise multiple linear regression analysis in order to ascertain the independent predictors of serum 25(OH)D either continuously or <50 nmol/L 25(OH)D, respectively. Linear trends of ordered categorical variables were assessed using ordinal values consisting of the mid-range values for each category and applying a likelihood ratio test [16]

### 3. Results

Descriptive characteristics of the population by 25(OH)D level are presented in Table 1. There was very little overt clinical vitamin D deficiency in this study population, with only 3% of the population having serum 25(OH)D <25 nmol/L; 12%, 29%, 79% and 95% had levels of serum 25(OH)D <37 nmol/L, <50 nmol/L, <80 nmol/L, <100 nmol/L, respectively. The average age was  $63 \pm 5$  years (males (M):  $64 \pm 5$  years; females (F):  $63 \pm 5$  years), 6% were of non-Caucasian origin, 8% were current smokers and 36% had education above college level. Forty % (M: 41%; F: 39%) had engaged in vigorous activity  $\geq 3$  h/week during the last year and average body mass index (BMI) was  $27 \pm 5$  kg/m<sup>2</sup> (M:  $27 \pm 4$  kg/m<sup>2</sup>; F:  $27 \pm 6$  kg/m<sup>2</sup>).

Serum 25(OH)D levels varied by season of blood collection with the highest levels during the summer and autumn and the lowest levels during winter and spring. Mean 25(OH)D levels were significantly higher in females than males (p < 0.001) (Fig. 1). However, when mean 25(OH)D levels of females not taking MHT were compared to those of males, there were no significant differences between females and males (p = 0.5). Similarly there were significant differences between 25(OH)D levels by vitamin D supplement use between females and males over the year (p < 0.001) (Fig. 2). However, when mean 25(OH)D levels of females not taking vitamin D supplement were compared to the mean of males, there were no significant differences.

In univariate analysis, twelve factors were found to be significantly (p < 0.01) associated with vitamin D status (Table 1). Table 2 presents the categorical analyses for those factors that remained significant (p < 0.01) on step-down multivariate linear regression analyses of 25(OH)D. The factors associated with low vitamin D status (adjusted for confounders and mutually) were: donating blood in winter, being of non-Caucasian background, being female,

Table 1
Characteristics of seasonal, demographic, anthropometric, lifestyle and dietary factors by serum 25(OH)D in US men and women living across the USA.

| Predictor variables                                      | Total<br>n = 2621 | 25(OH)D<br><37 nmol/L<br>n = 305 | $25(OH)D \ge 37$<br>to $<50 \text{ nmol/L}$<br>n = 466 | $25(OH)D \ge 50$<br>to <80 nmol/L<br>n = 1306 | $25(OH)D \ge 80$<br>to <100 nmol/L<br>n = 403 | $25(OH)D$ $\geq 100 \text{ nmol/L}$ $n = 141$ |
|--|-------------------|----------------------------------|--|---|---|---|
| Percentage   |                   |                                  |  |   |   |   |
| Season: Winter (December-May)*** %                       | 47                | 72                               | 62   | 43  | 31  | 23  |
| Demography race: White*** %                              | 94                | 88                               | 95   | 95  | 95  | 94  |
| Current menopausal hormone therapy (MHT)*** % yes        | 53                | 43                               | 44   | 52  | 60  | 74  |
| Current smoking* % yes                                   | 8                 | 11                               | 8  | 8   | 6   | 4   |
| Education college or above % yes                         | 36                | 35                               | 35   | 36  | 37  | 37  |
| Current vigorous physical activity (PA) (≥3 h/week)*** % | 40                | 29                               | 32   | 41  | 49  | 55  |
| Mean (SD)  |                   |                                  |  |   |   |   |
| Height (in.)***  | 67(4)             | 67(4)                            | 68(4)  | 67(4)   | 66(4)   | 66(4)   |
| Current body mass index (BMI kg/m <sup>2</sup> )***      | 27(5)             | 29(6)                            | 28(5)  | 27(5)   | 26(4)   | 25(4)   |
| Red meat group (g/day)***                                | 77(67)            | 82(76)                           | 88(78)   | 76(66)  | 66(54)  | 69(61)  |
| Milk, on cereal (g/day)***                               | 92(90)            | 64(76)                           | 84(81)   | 99(94)  | 99(91)  | 105(94)                                       |
| Energy from diet (kcal/day)*                             | 2059(856)         | 2054(882)                        | 2137(905)  | 2073(884)                                     | 1964(714)                                     | 1974(757)                                     |
| Dietary calcium (mg/day)*                                | 966(518)          | 865(453)                         | 961(499)   | 989(564)                                      | 981(450)                                      | 940(410)                                      |
| Dietary vitamin D (µg/day)**                             | 5.3(3.6)          | 4.3(3.0)                         | 5.3(3.3)   | 5.6(4.0)                                      | 5.4(3.1)                                      | 5.2(2.6)                                      |
| Supplemental calcium (mg/day)**                          | 281(385)          | 146(279)                         | 222(354)   | 281(379)                                      | 406(421)                                      | 426(479)                                      |
| Supplemental vitamin D (µg/day)**                        | 6.4(8.0)          | 3.1(6.1)                         | 4.9(7.4)   | 6.6(7.9)                                      | 8.6(8.6)                                      | 9.7(9.9)                                      |
| Supplemental vitamin E (IU/day)**                        | 168(254)          | 106(218)                         | 160(259)   | 168(251)                                      | 212(267)                                      | 213(279)                                      |

<sup>\*</sup> p < 0.05.

<sup>\*\*</sup> p < 0.01.

<sup>\*\*\*</sup> p < 0.001.

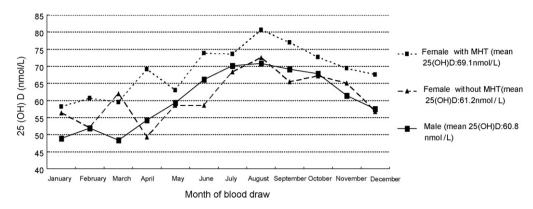


Fig. 1. Seasonal variation of serum 25(OH)D concentrations in US men and women: including with and without use of menopausal hormone therapy (MHT) in women.

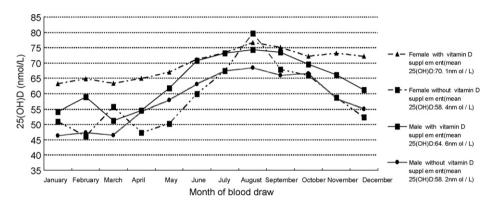


Fig. 2. Seasonal variation of serum 25(OH)D concentrations in US men and women showing intake or not of vitamin D supplement use.

being obese (BMI  $\geq$  30 kg/m<sup>2</sup>), not being physically active, having low dietary vitamin D intake and vitamin D and Ca supplement use. It is interesting that, independent of dietary vitamin D and calcium intake, consuming less milk on cereal remained a significant predictor as did current MHT use in women. There were gender differences in this population: women had a higher 25(OH)D levels when they either took MHT or vitamin D or calcium supplements ( $p_{\text{interaction for vitamin D supplement intake}} \times \text{gender} = 0.005$ ). Vitamin D supplement and MHT use were found to be significantly associated in women: those who took vitamin D supplements were 1.5 (1.2-1.9) more likely to take MHT as were those who took Ca supplements OR = 1.9 (1.5-2.4). It is interesting that the association between MHT and serum 25(OH)D strengthens when women took supplements (the odds for low vitamin D status (<50 nmol/L 25(OH)D) for those using both MHT and vitamin D supplements = 0.5; 95% CI = 0.3-0.8 versus those who did not take supplements = 0.7 (95% CI = 0.5-1.1)). Similar risk factor associations were seen with 25(OH)D <80 nmol/L and linear regression analyses of continuous serum vitamin D (data not shown).

## 4. Discussion

Our observation that 25(OH)D serum levels are higher in summer and autumn than in winter and spring is consistent with studies where date of blood draw has been ascertained [17–18]. Mean serum 25(OH)D levels in our study of middleaged men and women (men = 60.8 nmol/L; women = 65.4 nmol/L) were similar to the overall mean data from the latest US National Health and Nutrition Examination Survey (men = 62.9 nmol/L;

women = 61.5 nmol/L) [19] and higher than most European studies [20–24] (British men: 53.8; women: 51.5) [5]; except in Northern Europe, where there is a high consumption of fatty fish (e.g. Norway:74.1 nmol/L [22]). We found on initial analysis that women had higher serum 25(OH)D levels than men, a finding that was not found in other studies, where women tended to have lower values [17]. In our sample, the gender effect disappeared when the effect of MHT and vitamin D supplement use was taken into account; the MHT effect as has previously been reported [25,26].

Dietary vitamin D has consistently been reported as a determinant of vitamin D status in all reported predictor studies, especially in Nordic and northern European countries [5,17,20–24,27] with high intake of fatty fish and low sunlight exposure. Milk intake is a significant source of vitamin D in the diet in the US where supplementation of milk with vitamin D has been standard for many decades [6,17,19]. As with our findings, in all other studies where vitamin D supplement intake was high there was a strong association with serum 25(OH)D levels [17]. To our knowledge the interesting and divergent associations of serum 25(OH)D with intake of vitamin D and calcium supplements in women and milk on cereal in men has not been previously reported.

We also found vigorous physical activity to be a strong and modifiable contributor to vitamin D status, consistent with other studies [11,17,20–24,28–31]. This association has often been attributed to physical activity being a surrogate for sun exposure; however, in the few studies in which both exposures were measured simultaneously [28,31], the vigorous activity-vitamin D relationship persisted. Some aspect of exercise might be contributing to the maintenance of vitamin D status, other than by increasing exposure of skin to sunlight; indeed, this is supported by small clinical

**Table 2**Categorical analysis of those predictors of vitamin D status, as measured by serum 25(OH)D (less than 50 nmol/L) that remained significant after stepwise multiple logistic regression analysis in men and women living in the USA.

|                                 | · o                                     |                                       |   |  |  |  |  |
|---------------------------------|---|---------------------------------------|---|--|--|--|--|
|                                 | Total = 2621<br>OR <sup>a</sup> (95%CI) | Men = 1357<br>OR <sup>a</sup> (95%CI) | Women = 1264<br>OR <sup>a</sup> (95%CI) |  |  |  |  |
| Season                          |   |                                       |   |  |  |  |  |
| Summer                          | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| Winter                          | 3.4 (2.8-4.1)                           | 2.7(1.9-3.7)                          | 3.4 (2.3–5.0)                           |  |  |  |  |
| Race                            |   |                                       |   |  |  |  |  |
| Other                           | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| Caucasian                       | 0.2 (0.1-0.4)                           | 0.2 (0.1-0.6)                         | 0.2 (0.1-0.4)                           |  |  |  |  |
| MHT (current use                | e)                                      |                                       |   |  |  |  |  |
| No                              | -,                                      |                                       | 1.0                                     |  |  |  |  |
| Yes                             |   |                                       | 0.8 (0.5–1.0)                           |  |  |  |  |
| Vicenessa DA (h/s               | I-)                                     |                                       | , ,                                     |  |  |  |  |
| Vigorous PA (h/w<br>None        | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| <3 h                            | 0.7 (0.5–0.9)                           | 0.7 (0.4–1.0)                         | 0.7 (0.5–1.1)                           |  |  |  |  |
| ≥3 h                            | 0.5 (0.4–0.7)                           | 0.5 (0.3-0.7)                         | 0.7 (0.3–1.1)                           |  |  |  |  |
| ≥511<br>p trend                 | ***                                     | 0.5 (0.5-0.7)                         | 0.5 (0.5-0.8)<br>**                     |  |  |  |  |
| •                               |   |                                       |   |  |  |  |  |
| BMI (kg/m <sup>2</sup> )        | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| <25                             | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| ≥25 to <30                      | 1.4 (1.1–1.8)                           | 1.2 (0.9–1.7)<br>2.0 (1.4–3.0)        | 1.6 (1.1–2.3)<br>3.6 (2.4–5.4)          |  |  |  |  |
| $\geq$ 30 p trend               | 2.7 (2.0–3.5)                           | 2.0 (1.4-3.0)                         | 3.0 (2.4-3.4)                           |  |  |  |  |
| •                               |   |                                       |   |  |  |  |  |
| Milk (g/day)                    |   |                                       |   |  |  |  |  |
| <13                             | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| ≥13-80                          | 0.8 (0.6–1.1)                           | 0.8 (0.5–1.2)                         | 0.7 (0.5–1.1)                           |  |  |  |  |
| ≥80–159                         | 0.8 (0.6–1.0)                           | 0.6 (0.4–0.9)                         | 1.0 (0.7–1.6)                           |  |  |  |  |
| $\geq 159$ p trend              | 0.6 (0.5–0.9)                           | 0.6 (0.4–1.0)                         | 0.6 (0.3-0.9)<br>ns                     |  |  |  |  |
| p tienu                         |   |                                       | 115                                     |  |  |  |  |
| Dietary vitamin D (µg/day)      |   |                                       |   |  |  |  |  |
| <3.0                            | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| ≥3.0-4.5                        | 0.9 (0.6–1.2)                           | 0.9 (0.6–1.5)                         | 0.8 (0.5–1.3)                           |  |  |  |  |
| ≥4.5–6.7                        | 0.6 (0.4–0.9)                           | 0.7 (0.4–1.2)                         | 0.6 (0.3–1.1)                           |  |  |  |  |
| $\geq$ 6.7 p trend              | 0.4 (0.3–0.7)                           | 0.5 (0.2–0.9)                         | 0.5 (0.2–1.0)                           |  |  |  |  |
| •                               |   |                                       |   |  |  |  |  |
| Supplemental calcium (mg/day)   |   |                                       |   |  |  |  |  |
| None                            | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| ≥0-162                          | 1.1 (0.7–1.9)                           | 1.2 (0.6–2.3)                         | 1.2 (0.5–2.6)                           |  |  |  |  |
| ≥162-500                        | 0.8 (0.6–1.2)                           | 0.8 (0.5–1.3)                         | 0.8 (0.5–1.3)                           |  |  |  |  |
| ≥500                            | 0.7 (0.5–1.0)                           | 0.8 (0.4–1.6)                         | 0.7 (0.4–1.1)                           |  |  |  |  |
| ≥ <i>p</i> trend                |   | ns                                    |   |  |  |  |  |
| Supplemental vitamin D (µg/day) |   |                                       |   |  |  |  |  |
| 0–10                            | 1.0                                     | 1.0                                   | 1.0                                     |  |  |  |  |
| ≥10-20                          | 0.5 (0.4–0.7)                           | 0.6 (0.4–1.0)                         | 0.5 (0.3–0.7)                           |  |  |  |  |
| ≥20–30                          | 0.4 (0.3–0.7)                           | 0.7 (0.3–1.5)                         | 0.3 (0.1-0.5)                           |  |  |  |  |
| ≥30                             | 0.5 (0.2–1.2)                           | 0.3 (0.1–1.4)                         | 0.7 (0.2–2.0)                           |  |  |  |  |
| p trend                         |   |                                       |   |  |  |  |  |

OR: odds ratio; BMI: body mass index; MHT: menopausal hormone therapy; PA: vigorous physical activity.

studies [32]. Further investigation into the independent role of PA in vitamin D bioavailability and metabolism would appear warranted

Our findings of high BMI being associated with low vitamin D levels is consistent with other studies in the US [6,11,17,30] and in other countries [17,21,27]. The inverse 25(OH)D and obesity relationship has been explained by "trapping" of the vitamin D parent compound, cholecalciferol, in adipose tissue [33]. Our study is limited, by not having a direct measure of sun exposure or indoor or outdoor exercise patterns, although our measures are adjusted for date of blood draw. Strengths of the present investigation are its relatively large sample size and the fact that the vitamin D

analyses were all performed with the same assay. Thus targeting an increase in vigorous activity and vitamin D and Ca intake and decreasing obesity could be public health interventions independent of sun exposure to improve vitamin D status in middle-aged Americans.

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<sup>&</sup>lt;sup>a</sup> Adjusted for age at blood draw, gender, smoking pack-years, vitamin D laboratory analysis, PLCO center, total energy intake, educational level and mutually for listed predictor variables.

<sup>\*</sup> p < 0.05.

<sup>\*\*</sup> p < 0.01.

<sup>\*\*\*</sup> p < 0.001.

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