Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter\textsuperscript{1,2}

Ann Burgaz, Agneta Åkesson, Annette Öster, Karl Michaëlsson, and Alicja Wolk

ABSTRACT

Background: Vitamin D is produced endogenously after sun exposure but can also be obtained from natural food sources, food fortification, and dietary supplements.

Objective: We aimed to determine the vitamin D status of women (61–86 y old) living in central Sweden (latitude 60°) during winter and its relation with vitamin D intake and exposure to ultraviolet B radiation.

Design: In a cross-sectional study, we assessed the vitamin D status (serum 25-hydroxyvitamin D [25(OH)D]) of 116 women by using an enzyme immunoassay. The women completed questionnaires covering food habits, use of dietary supplements, and sun-related behavior.

Results: In a multiple linear regression model, the main determinants of serum 25(OH)D concentrations (\( \bar{x} \pm SD: 69 \pm 23\) nmol/L) were dietary vitamin D (6.0 \( \pm 1.8\) \( \mu\)g/d), travel to a sunny location during winter within the previous 6 mo (26\%), and the use of dietary supplements (16\%). There was no association between serum 25(OH)D status during the winter and age, time spent outdoors, the use of sunscreen, or skin type. Serum 25(OH)D concentrations increased by 25.5 nmol/L with 2–3 servings (130 g/wk) fatty fish/wk, by 6.2 nmol/L with the daily intake of 300 g vitamin D–fortified reduced-fat dairy products, by 11.0 nmol/L with regular use of vitamin D supplements, and by 14.5 nmol/L with a sun vacation during winter. Among nonsupplement users without a wintertime sun vacation, 2–3 servings fatty fish/wk increased serum vitamin D concentrations by 45\%.

Conclusion: Fatty fish, vitamin D–fortified reduced-fat dairy products, regular supplement use, and taking a sun vacation are important predictors for serum concentrations of 25(OH)D during winter at a latitude of 60°.

KEY WORDS Vitamin D status, ultraviolet B radiation exposure, 25-hydroxyvitamin D, 25(OH)D, vitamin D intake, dietary vitamin D, vitamin D fortification, vitamin D supplements

INTRODUCTION

The role of vitamin D in calcium absorption and metabolism for bone health is well known. However, there is accumulating evidence that low vitamin D status is associated with greater risk of cancer (1, 2), multiple sclerosis (3), and type 1 diabetes mellitus (4). In addition, vitamin D is important for an optimally functioning immune system (5) and has a favorable influence on blood pressure (6).

Vitamin D is unique, because the human body’s meeting of biological requirements can depend on both endogenous production of vitamin D\(_{3}\), formed from activation of 7-dehydrocholesterol through exposure of the skin through ultraviolet B (UVB) radiation, and exogenous sources, D\(_{3}\) and D\(_{2}\), that are found in diet, vitamin D–fortified foods, and supplements. In Sweden, all fortified foods and dietary supplements currently contain vitamin D\(_{3}\).

The sunlight exposure of the skin is important, and limited sunlight exposure may result in vitamin D deficiency. Several factors influence the intensity and duration of exposure to UVB radiation, including geographic location, season, atmospheric conditions, and time spent outdoors (7). Factors such as age, melanin content of skin, sunscreen use, clothing, and obesity may influence the skin production of vitamin D (8). In the Swedish latitudes (55–69°), the cutaneous synthesis of previtamin D is not detectable during the winter season (9), which leaves the population dependent on dietary sources of vitamin D during a considerable part of the year (8).

The purpose of this study was to investigate the relative importance of dietary intake of vitamin D and vitamin D intake from supplements and from UVB exposure to the serum concentration of 25(OH)D during winter (January–March) in a general population of elderly women living in central Sweden (latitude: 60°).

SUBJECTS AND METHODS

Subjects

Subjects were recruited from the Swedish Mammography Cohort, a population-based cohort established during a mammography-screening program that was introduced in 2 counties in central Sweden between 1987 and 1990. All women born between 1914 and 1948 (\( n = 90 069\) were invited to participate in the study; 74\% of that group returned a completed food-frequency questionnaire (FFQ). A second questionnaire was mailed to those who still lived in the study area in 1997 (response rate: 70\%).

During the period from January 12 to March 10, 2006, we contacted 122 noninstitutionalized women in the cohort, and 118

\textsuperscript{1} From the Division of Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden (AB, AA, AO, and AW), and the Department of Surgical Sciences, Section of Orthopaedics, and Uppsala Clinical Research Center, University Hospital, Uppsala, Sweden (KM).

\textsuperscript{2} Reprints not available. Address correspondence to A Wolk, The National Institute of Environmental Medicine, Karolinska Institute, PO Box 210, SE-171 77 Stockholm, Sweden. E-mail: alicija.wolk@ki.se.

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of those women agreed to participate in this study. Two women were excluded because of incomplete dietary information, which left 116 subjects for the final analysis.

Written informed consent was obtained from all participants. The Ethics Committees of the Karolinska Institute approved the study.

Sampling

We collected information on dietary intake by using a mailed self-administered validated FFQ containing questions on the consumption of 123 food items over the past year and use of dietary supplements (regular, occasional, or no use). The FFQ included the following fortified reduced-fat dairy products: low-fat (0.1% and 0.5% fat) milk, medium-fat (1.5%) milk, and low-fat cultured milk (sour milk and yogurt); for fortified margarine, the FFQ included reduced-fat margarine, soft margarine, regular margarine, and liquid margarine. Fatty fish included in the FFQ were herring, mackerel, and salmon.

We performed a methodologic study to determine the validity of the FFQ in 129 randomly selected women from the cohort. Pearson correlation coefficients between data from four 1-wk diet records (obtained 3–4 mo apart) and the FFQ were 0.6–0.7 for the intake of different types of vitamin D–fortified reduced-fat dairy products, 0.3–0.7 for vitamin D–fortified margarines, and 0.5 for different types of fatty fish (A Wolk, unpublished observations, 1992).

The estimated mean content of vitamin D was 7.5 μg in multivitamins and 10 μg in specific vitamin D preparations. In addition, weight and height were measured, and these variables were used to calculate body mass index (BMI; in kg/m²). A BMI ≤ 25 was considered normal-weight, and a BMI > 25 was considered overweight.

At the time of blood collection, the women completed questions on various aspects of UVB exposure, such as duration of sunlight exposure between 0900 and 1800 every week (during both summer and winter), tanning-bed use, the frequency of sun vacations during winter (ie, travel to a latitude with high UVB radiation during the past 6 mo of wintertime), and most (59%) of the women spent >6 h/wk outside during the summer (May–September). Two-thirds of the women self-reported having skin type III, which corresponds to "sometimes burns mildly and gradually tans," after sun exposure, and only a few study participants always used sun-protection products. Tanning beds were rarely used (Table 1).

Serum concentrations of 25(OH)D were significantly correlated with the dietary vitamin D intake (r = 0.22, P = 0.02). Furthermore, concentrations of 25(OH)D were on average 17% higher in those who used dietary supplements regularly than in those who did not (78 and 67 nmol/L, respectively; P = 0.06), 16% higher in those who had taken a sun vacation during the previous 6 mo than in those who had not (77 and 66 nmol/L, respectively; P = 0.03), and 13% higher in those who were normal-weight than in those who were overweight women according to BMI (73 and 64 nmol/L, respectively; P = 0.05).

We did not find any significant correlation of serum 25(OH)D with age (P = 0.90). We also found no significant differences between 25(OH)D concentrations with respect to sun exposure factors, such as the number of hours spent outside in daylight during summer (P = 0.69) or winter (P = 0.99), a preference for sun or shadow (P = 0.59), skin type (P = 0.35), or use of sun protection products (P = 0.96).

Biochemical analyses

To evaluate vitamin D status, we measured 25(OH)D in serum by using an enzyme immunoassay (IDS Ltd, Boldon, United Kingdom) that quantifies 25(OH)D₂ and 25(OH)D₃. Cross-reactivity to 25(OH)D₃ is 100% and that to 25(OH)D₂ is 75%. The immunoassay is based on the binding of highly specific sheep antibodies to vitamin D. To ascertain analytic quality, we analyzed all standards, controls, and samples in duplicate and reanalyzed all duplicates with a CV > 10%. The control samples provided by the manufacturer were within the recommended range. The interassay CV based on 3 different concentrations of control samples was <8.3%.

Statistical analysis

Univariate associations were assessed by using Pearson’s correlation coefficient (r), because the normal probability plot of the residuals indicated no deviation from a linear pattern. Data from 2 independent groups of subjects were compared by using an independent t test. In multiple linear regression, all variables associated with serum 25(OH)D concentrations in univariate analyses (P < 0.1) were included. Only significant (P ≤ 0.05) covariates were included in the final model. All tests were 2-sided. Statistical analyses were performed by using SPSS software (version 14.00; SPSS Inc, Chicago, IL).

RESULTS

Among the 116 women aged 61–86 (mean age: 69 y), the mean serum concentration of 25(OH)D was 69 nmol/L (range: 35–147 nmol/L) (Figure 1). The estimated mean dietary intake of vitamin D, including vitamin D–fortified foods, was 6.0 μg/d (240 IU/d). Dietary supplements containing vitamin D were used regularly by 16% of the women. One-quarter of the women had taken a vacation to a sunny location during winter (ie, had traveled to a latitude with high UVB radiation during the previous 6 mo of wintertime), and most (59%) of the women spent >6 h/wk outside during the summer (May–September). Two-thirds of the women self-reported having skin type III, which corresponds to "sometimes burns mildly and gradually tans," after sun exposure, and only a few study participants always used sun-protection products. Tanning beds were rarely used (Table 1).

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TABLE 1
Descriptive characteristics of the participating women

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>69 ± 23²</td>
</tr>
<tr>
<td>Dietary vitamin D intake (µg/d)</td>
<td>6.0 ± 1.8</td>
</tr>
<tr>
<td>(IU/d)</td>
<td>240 ± 72</td>
</tr>
<tr>
<td>Regular supplement use [% (n)]</td>
<td>19 (16)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 ± 4.1</td>
</tr>
</tbody>
</table>

Sun or other UVB exposure
- Sun vacation during the previous 6 mo [% (n)] 30 (26)
- Outside in daylight ≥6 h/w [% (n)]
  - Summer 68 (59)
  - Winter 45 (39)
- Preference [% (n)]
  - Sun 9 (8)
  - Sun and shadow 94 (81)
  - Shadow 13 (11)
- Sun bed during the previous 3 mo [% (n)] 4 (3)
- Skin type [% (n)]
  - I 4 (3)
  - II 24 (21)
  - III 73 (63)
  - IV 15 (13)
- Use of sun-protection products [% (n)]
  - Always 13 (11)
  - Sometimes 66 (57)
  - Never 37 (32)

¹ n = 116 women 61–86 y old. 25(OH)D, 25-hydroxyvitamin D; UVB, ultraviolet B light.
² ± SD (all such values).
³ Supplements not included.
⁴ Indicates use of vitamin D or multivitamins (± minerals).
⁵ Classifications: I, always burns and never tans; II, usually burns or tans with difficulty; III, sometimes burns mildly or tans gradually; IV, rarely burns and tans with ease (10).

Of the dietary factors, the main food groups that best correlated with 25(OH)D serum concentrations were fatty fish (r = 0.21, P = 0.02) and vitamin D–fortified reduced-fat dairy products (r = 0.20, P = 0.04). There was no correlation with vitamin D–fortified margarine (P = 0.59). The major food source contributing 28% of the vitamin D intake was salmon, herring, or both (ie, fatty fish) that were consumed ≥1 time/wk by 84% of the participants.

To further investigate the relative importance of the determinants of serum 25(OH)D concentrations, we analyzed vitamin D intake as a continuous variable, sun holiday (yes versus no), supplement use (regular, occasional, or no use), and BMI in a multiple linear regression model. On the basis of this model, which explained 13% of the variance (adjusted R²) (Table 2), serum 25(OH)D increased by 13.1 nmol/L for every 3.5-µg (140-IU) increment in dietary vitamin D intake (approximately corresponding to a 2-SD increase in vitamin D intake in the study group). In addition, a history of a recent sun vacation increased the serum 25(OH)D concentrations in this model by 14.8 nmol/L, whereas regular use of dietary supplements was associated with an increase of 11.8 nmol/L (Table 2). The relative importance was greater for vitamin D intake and sun vacation than for supplement use, as evaluated by standardized coefficient (Table 2). The BMI was not significantly associated with 25(OH)D in this multivariate model. We could not evaluate the association with tanning bed use because a very small proportion of persons in the population (3%) used tanning beds. Combining the vitamin D intake from supplements with the dietary intake in the multiple regression model had no major effect on the results.

Instead of total dietary vitamin D intake, we restricted the multiple linear regression analyses to the major dietary sources of vitamin D. The most important relative effect of dietary intake on the vitamin D status in the study group was that of consumption of fatty fish; each additional 130 g fatty fish/wk was associated with a 10.2 nmol/L increment in serum 25(OH)D concentrations. The corresponding increase for 2 daily servings (300 g) of fortified reduced-fat dairy products was 6.2 nmol/L (Table 3).

In a subgroup of 72 women with no supplement use and no recent sun vacation, the correlation between serum 25(OH)D status and vitamin D intake was higher (r = 0.35) than that in the entire study population of 116 women (r = 0.22) (Figure 2), and the P value for the difference between these 2 correlation coefficients was 0.07. Among these 72 women, each 3.5-µg (140-IU) increase in dietary vitamin D intake resulted in an increase of 14.8 nmol/L (95% CI: 5, 24 nmol/L) in serum 25(OH)D concentrations. Consumption of fatty fish 2–3 times/wk increased their serum 25(OH)D concentrations by 45% from those in women with no fatty fish consumption (83 and 57 nmol/L, respectively).

TABLE 2
Major factors predicting serum 25-hydroxyvitamin D [25(OH)D] concentrations in 116 middle-aged to elderly women assessed by multiple linear regression analysis

<table>
<thead>
<tr>
<th>Serum 25(OH)D nmol/L</th>
<th>Independent variables</th>
<th>Unstandardized coefficient¹</th>
<th>Standardized coefficient¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dietary vitamin D intake²</td>
<td>13.1 (4.9, 21)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Regular supplement use</td>
<td>11.4 (0.5, 22)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Sun vacation during winter³</td>
<td>14.8 (5.4, 24)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

¹ β coefficient.
² Increment of 3.5 µg/d or 140 IU/d in dietary vitamin D intake correspond to 2 SDs in the study group.
³ 95% CI in parentheses (all such values).

TABLE 3
Dietary and other factors predicting serum 25-hydroxyvitamin D [25(OH)D] concentrations assessed by multiple linear regression

<table>
<thead>
<tr>
<th>Serum 25(OH)D nmol/L</th>
<th>Independent variables</th>
<th>Unstandardized coefficient¹</th>
<th>Standardized coefficient¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fatty fish (130 g/wk)</td>
<td>10.2 (2.6, 18)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Vitamin D–fortified reduced-fat dairy products (300 g/d)</td>
<td>6.2 (0.8, 12)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Regular supplement use</td>
<td>11.0 (0.1, 22)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Sun vacation during winter⁴</td>
<td>14.5 (5.3, 24)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

¹ β coefficient.
² 95% CI in parentheses (all such values).
³ Travel to a latitude with high ultraviolet B radiation during the previous 6 mo of winter.
DISCUSSION

In this study of middle-aged and elderly women, performed during the winter in central Sweden (latitude 60°), the main determinants of 25(OH)D status in serum were dietary intake of vitamin D, taking a vacation to a sunny location during the preceding autumn or during the winter, and the use of dietary supplements. Among the dietary factors, fatty fish and fortified reduced-fat dairy products were of importance in predicting the concentrations of 25(OH)D in serum.

The estimated mean dietary vitamin D intake in this population was 6.0 μg/d (240 IU/d) (not including supplements). Although this intake was >40% lower than the 10 μg/d (400 IU/d) recommended in the Nordic countries (11), it is still higher than the estimated average dietary intake based on 10 European studies [mean: 3.4 μg/d (136 IU/d)] (12). A higher dietary vitamin D intake in Scandinavian countries than in Central and Southern European countries was previously reported for both young adults and elderly (13). The higher vitamin D intake in Sweden than in Southern Europe may be due to the general vitamin D fortification of all low-fat dairy products [0.1%- and 0.5%-fat milk, medium-fat (1.5%) milk, and low-fat cultured milk] and margarines (soft margarine, reduced-fat margarine, regular margarine, and liquid margarine), as well as to the more frequent consumption of fatty fish such as salmon and herring and the greater use of dietary supplements (14). In general, very few foods contain an appreciable amount of vitamin D, and future studies of vitamin D intake could simplify their dietary evaluation of study participants.

The major determinant of vitamin D status usually is skin exposure to sunlight (15). During winter at a latitude of 60°, it is not possible to produce previtamin D from UVB radiation (16). As a consequence, taking a sun vacation during the previous 6 mo had a considerable effect on the serum 25(OH)D status of subjects in the present study. However, we were able to show a notable importance of the diet during the winter. For subjects who had no recent sun vacation and did not use vitamin D supplements, vitamin D intake was of substantial value, and fatty fish consumption had the most important effect on vitamin D status—2–3 weekly portions of fatty fish increased serum 25(OH)D concentrations by 45%. One serving of fatty fish (130 g) contains 16 μg vitamin D. For comparison, one serving (130 g) of lean codfish contributes only 1 μg vitamin D, and one serving of fortified reduced-fat dairy products (150 g) contains 0.5 μg vitamin D. In line with previous studies (17, 18), we observed an increase in serum 25(OH)D concentrations among regular users of dietary supplements containing vitamin D.

Aging has been shown to affect vitamin D synthesis, primarily through a lesser capability of skin biosynthesis (19). The relatively narrow age range in the present study, 61–86 y, may explain the absence of an association between serum 25(OH)D and age. Unlike studies showing an inverse association between 25(OH)D concentration and obesity (20, 21), the present study did not find such significant association after adjustment for other factors. The relatively narrow BMI range also could explain the absence of an association.

Controversy exists regarding the concentration of circulating 25(OH)D that is appropriate for vitamin D sufficiency (1, 22–24). There seems to be no doubt, however, that 25(OH)D concentrations <12.5 nmol/L can result in bone diseases such as rickets in infants and osteomalacia in adults (14). The suggested optimal 25(OH)D concentrations for protection against osteoporosis (22) range between 40 and 100 nmol/L (1, 22, 24–27). In the present study, no subjects had serum 25(OH)D concentrations <12.5 nmol/L, only 5% had concentrations <40 nmol/L, and 19% had concentrations <50 nmol/L. Previously published data suggest a relatively high prevalence of vitamin D deficiency among the elderly in Europe (19). However, serum 25(OH)D concentrations seem to be generally higher in Swedish women than in women in the rest of Europe (Table 4).

These findings have some limitations. In particular, a high variability in the 25(OH)D assay results between laboratories and between methods has been reported. It should be noted that there is no gold standard or internationally accepted calibration of 25(OH)D assays, which may impair comparisons between studies (39, 40). In addition, a larger number of subjects and a less homogenous group might have improved our results.

Strengths of our study are the population-based random selection of noninstitutionalized women, the use of a detailed questionnaire about sun exposure habits together, and comprehensive information on diet and the use of dietary supplements. The lack of sun exposure during the study period allowed us to calculate vitamin D concentrations in women on the basis of dietary factors, supplements, and sun holidays. Therefore, parallels can be drawn to populations who have very little or no vitamin D from
sun exposure—eg, people of color, veiled women, or those who spend most of their time indoors.

In conclusion, dietary sources of vitamin D such as fatty fish and vitamin D–fortified reduced-fat dairy products are important to the vitamin D status of older Swedish women during winter. Travel to southern locations can also improve vitamin D status during the winter. Likewise, regular supplementation helps to increase the serum values of 25(OH)D when there is a lack of UVB radiation.

The authors’ responsibilities were as follows: AB, AÅ, KM, and AW: the study concept and design; AW and KM: data collection; AB and AO¨: the interpretation of results; AE, AO¨, KM, and AW: the statistical analyses; AB, AÅ, KM, and AW: the draft of the manuscript; and all authors: review of the final manuscript. None of the authors had any personal or financial conflict of interest.

REFERENCES


**TABLE 4**

<table>
<thead>
<tr>
<th>Country (reference)</th>
<th>Age (sex)</th>
<th>Subjects</th>
<th>Mean serum 25(OH)D</th>
<th>Analytic method</th>
<th>Season; latitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceland (28)</td>
<td>70 (F)</td>
<td>308</td>
<td>53</td>
<td>Competitive RIA</td>
<td>Winter; 63–67°</td>
</tr>
<tr>
<td>Norway (29)</td>
<td>&lt;40–60 (M)</td>
<td>258</td>
<td>50</td>
<td>Competitive RIA</td>
<td>Winter; 58–71°</td>
</tr>
<tr>
<td>Sweden</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>61–86 (F)</td>
<td>116</td>
<td>69</td>
<td>Competitive EIA</td>
<td>Winter; 55–69°</td>
</tr>
<tr>
<td>(30)</td>
<td>&gt;80 (M, F)</td>
<td>81</td>
<td>65</td>
<td>Competitive RIA</td>
<td>Winter; 55–69°</td>
</tr>
<tr>
<td>(31)</td>
<td>41–96 (F)</td>
<td>150</td>
<td>87</td>
<td>Competitive assay</td>
<td>All seasons; 55–69°</td>
</tr>
<tr>
<td>Denmark (32)</td>
<td>45–58 (F)</td>
<td>2016</td>
<td>63</td>
<td>Competitive assay</td>
<td>All seasons; 54–57°</td>
</tr>
<tr>
<td>Ireland (33)</td>
<td>69 (M, F)</td>
<td>30</td>
<td>21</td>
<td>Protein-binding assay</td>
<td>Winter; 52–55°</td>
</tr>
<tr>
<td>Netherlands (34)</td>
<td>76 (M, F)</td>
<td>74</td>
<td>33</td>
<td>Competitive assay</td>
<td>All seasons; 51–54°</td>
</tr>
<tr>
<td>United Kingdom (35)</td>
<td>&gt;65 (F)</td>
<td>800</td>
<td>57</td>
<td>Competitive assay</td>
<td>All seasons; 50–58°</td>
</tr>
<tr>
<td>Hungary (36)</td>
<td>41–91 (F)</td>
<td>319</td>
<td>48</td>
<td>Competitive RIA</td>
<td>Winter; 46–49°</td>
</tr>
<tr>
<td>France (37)</td>
<td>80 (F)</td>
<td>440</td>
<td>43</td>
<td>Competitive RIA</td>
<td>Winter; 42–51°</td>
</tr>
<tr>
<td>Italy (38)</td>
<td>59 (F)</td>
<td>570</td>
<td>45</td>
<td>Competitive RIA</td>
<td>All seasons; 36–46°</td>
</tr>
</tbody>
</table>

1 | 25(OH)D, 25-hydroxyvitamin D; RIA, radioimmunoassay; EIA, enzyme immunoassay. |