The effects of seasonal variation of 25-hydroxyvitamin D and fat mass on a diagnosis of vitamin D sufficiency[1−3]

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ABSTRACT

Background: The effect of season on vitamin D status is often overlooked in studies of optimal vitamin D concentrations and in clinical practice.

Objectives: We aimed to determine the effects of seasonal variation of 25-hydroxyvitamin D [25(OH)D] on a previously selected minimum concentration for vitamin D sufficiency (50 nmol/L) and to determine whether fat mass modifies these effects.

Design: A cross-sectional study evaluated 1606 healthy postmenopausal women and 378 older men from Auckland, New Zealand, who were undergoing single measurements of 25(OH)D.

Results: Concentrations of <50 nmol 25(OH)D/L were seen in 49% (range: 23%–74%) of women and 9% (range: 0%–26%) of men when measured, but 73% of women and 39% of men were predicted to have trough 25(OH)D concentrations < 50 nmol/L, according to the demonstrated seasonal variation. The predicted duration of 25(OH)D concentrations < 50 nmol/L was 250 d/y in women and 165 d/y in men.

Conclusion: Seasonal variation significantly affects the diagnosis of vitamin D sufficiency, which requires seasonally adjusted thresholds individualized for different locations. Clinicians should consider the month of sampling and the amount of body fat when interpreting 25(OH)D measurements. *Am J Clin Nutr* 2007;86:959–64.

KEY WORDS Vitamin D, vitamin D deficiency, insufficiency, seasonal variation, fat mass

INTRODUCTION

Vitamin D insufficiency in adults causes myopathy, osteopenia, secondary hyperparathyroidism, and osteomalacia (1). The serum concentration of 25-hydroxyvitamin D [25(OH)D] is considered to be the best estimate of body stores of vitamin D (1). Estimates of the serum concentration of 25(OH)D above which vitamin D stores are considered adequate vary widely, from 25 nmol/L to 100 nmol/L (2). The 2 major biological determinants of 25(OH)D concentrations are ultraviolet (UV) B exposure and fat mass (3). In countries distant from the equator, there is seasonal variation in UV-B exposure because of the lower angle of the sun and the greater cloud cover in the winter months. In addition, more clothes are worn in winter, which reduces skin exposure to UV-B. As a result of this seasonal variation in UV-B radiation, there also is seasonal variation in 25(OH)D concentrations, such that concentrations are highest in late summer and early autumn and lowest in late winter and early spring. The

concentrations of 25(OH)D are inversely associated with fat mass. This association has been attributed to the sequestration into adipocytes of fat-soluble vitamin D generated in the skin or orally ingested, before it can be transported to the liver and converted to 25(OH)D (4). Previously, we showed seasonal variations in 25(OH)D concentrations in healthy, independent-living, middle-aged and older men and postmenopausal women living in Auckland, New Zealand (37°S) (3, 5). Such seasonal variations in 25(OH)D concentrations mean that a person could have adequate 25(OH)D concentrations in the summer and autumn months but suboptimal concentrations in the winter and spring. We set out to determine the effects of seasonal variations in 25(OH)D on a previously selected minimum threshold for diagnosis of vitamin D sufficiency (50 nmol/L) and to establish whether fat mass or body weight modifies these effects.

PARTICIPANTS AND METHODS

Participants

Healthy, independent-living, postmenopausal women (*n* = 1606) and healthy, independent-living, middle-aged and older men (*n* = 378) volunteered for 2 separate studies of calcium supplementation. The protocols and methods for these studies were reported previously (3, 5). In brief, men aged ≥40 y and women aged ≥55 y who were > 5 y past menopause were eligible to participate. Potential participants were ineligible if they had significant renal, hepatic, or thyroid dysfunction or any other major ongoing disease, including malignancy, undiagnosed diabetes mellitus, and metabolic bone disease. Further exclusion criteria included the use of medication known to affect calcium metabolism, the use of cholecalciferol supplements in doses of >1000 IU/d (cholecalciferol is the only vitamin D supplement available in New Zealand), the current use of glucocorticoids, or the use of testosterone, hormone replacement therapy, anabolic steroids, fluoride, or bisphosphonates in the previous

year. In addition, men were ineligible if they had coronary heart disease or an estimated 5-y cardiovascular risk of >15% (6), or if they were undergoing therapy for hyperlipidemia.

Written informed consent was obtained from all participants. Both studies received ethics approval from the Auckland Ethics Committee.

Measurements

Height was measured at baseline by using a Harpenden stadiometer (Holtain Ltd, Crosswell, United Kingdom), weight was recorded by using electronic scales (Champ II; Ohaus, Pine Brook, NJ), and all participants supplied a fasting blood sample. Body composition was measured by using a Lunar Prodigy dual-energy X-ray absorptiometer (GE Lunar, Madison, WI) in the men and a Lunar Expert dual-energy X-ray absorptiometer (GE Lunar) with proprietary LUNAR EXPERT software (version 1.7; GE Lunar) in the women. Serum 25(OH)D was measured by radioimmunoassay (DiaSorin, Stillwater, MN) in all the women and the first 252 men; a chemiluminescent assay (Nichols, San Juan Capistrano, CA) was used in the last 126 men. All 25(OH)D samples from both studies were measured in one laboratory which takes part in, and meets the performance targets for, the Vitamin D External Quality Assessment Scheme (7). Because there was a change in assay during the studies, we developed equations to allow the conversion of results obtained from the 2 assays, as previously described (3). All 25(OH)D measurements obtained with the Nichols assay were converted to predicted Diasorin values by using the following equation:

$$\text{Diasorin} = \text{Nichols} \times 0.75 + 5.6 \qquad (1)$$

Statistical analysis

The concentrations of 25(OH)D were plotted against the day of the year on which the blood sample was taken in each of the cohorts, and a sine curve was fitted. It was assumed that each participant's 25(OH)D concentrations throughout the year would follow a sine curve similar to that of the overall population. Therefore, the sine curve for any participant would be the sine curve for the population translated along the y axis until it intersected with the known 25(OH)D concentration on the known day of the year for that person. By solving the equation for the sine curve for each participant, we were able to predict the peak 25(OH)D concentration, the trough 25(OH)D concentration, and the number of days on which the 25(OH)D concentration was <50 nmol/L. We used the following equation for each sine curve:

$$25(\text{OH})\text{D} = \text{baseline} + \text{amplitude} \times \text{sine}$$
$$(\text{angular frequency} \times \text{day of year} + \text{phase shift}) \qquad (2)$$

The amplitude of the sine curve is the maximal deviation from the baseline [ie, (peak value − trough value)/2]; the angular frequency is $2 \times \pi$/period (ie, $2 \times \pi/365$); and the phase shift is the amount of translation along the x axis.

To determine the effect of fat mass, we divided the cohorts into quartiles of fat mass and fitted a separate sine curve for each quartile of fat mass. We used analysis of variance to compare the baseline, amplitude, and phase shift coefficients of the sine curves between men and women and between fat mass quartiles. We also used analysis of variance to compare the amplitude, peak, and trough of the sine curves between men and women and

TABLE 1
Baseline characteristics of the study population[1]

	Men ($n = 378$)	Women ($n = 1606$)
Age (y)	57 ± 11	74 ± 4
Height (cm)	176 ± 7	159 ± 6
Weight (kg)	82 ± 13	67 ± 11
BMI (kg/m^2)	26.4 ± 3.4	26.4 ± 4.3
Lean mass (kg)	59 ± 7	36 ± 4
Fat mass (kg)	19 ± 8	27 ± 10
Percentage fat (%)	24 ± 7	41 ± 8
25-hydroxyvitamin D (nmol/L)	85 ± 31	51 ± 19

[1] All values are $\bar{x} \pm$ SD. Data from both cohorts were published previously (3, 5). All of the differences between the men and women were significant ($P < 0.001$) except the differences in BMI ($P = 0.9$) (Student's t test).

between fat mass quartiles. We then repeated these analyses by using body weight quartiles instead of fat mass quartiles. Finally, to explore whether the differences in the amount of seasonal variations in 25(OH)D concentrations between the sexes were due to differences in fat mass between men and women, we used path analysis with F statistics on analysis of variance models of peak 25(OH)D or trough 25(OH)D or amplitude versus sex or versus sex and fat mass quartile.

All sine curve fitting was performed by using GRAPHPAD PRISM for WINDOWS software (version 4.00; GraphPad Software, San Diego, CA). All other statistical calculations were carried out with SAS software (version 9.1; SAS Institute, Cary, NC). All tests were 2-tailed, and statistical significance was set at $P < 0.05$.

RESULTS

The baseline characteristics of each cohort were published previously and are shown in **Table 1**. The sine curve fitted for the cohort of women is shown in **Figure 1**. For the fitted sine curve, we used the following equation:

$$y = 50.99 + 10.67 \times \text{sine(frequency} \times x + 0.41) \qquad (3)$$

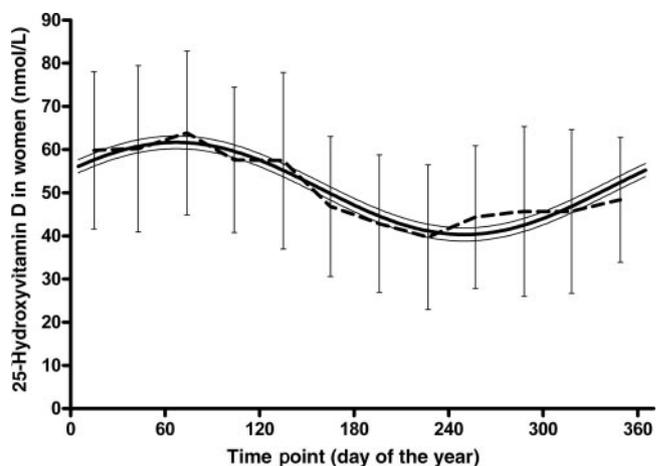where $r^2 = 0.15$. The sine curve fitted for the cohort of men is

FIGURE 1. Sine curve of best fit for 25-hydroxyvitamin D (—) in the cohort of women ($n = 1606$) with measured mean monthly 25-hydroxyvitamin D (− −) for comparison. 95% CIs are shown by the thin lines and standard deviations by the error bars.

FIGURE 2. Sine curve of best fit for 25-hydroxyvitamin D (—) in the cohort of men ($n = 378$) with measured mean monthly 25-hydroxyvitamin D (– –) for comparison. 95% CIs are shown by the thin lines and standard deviations by the error bars.
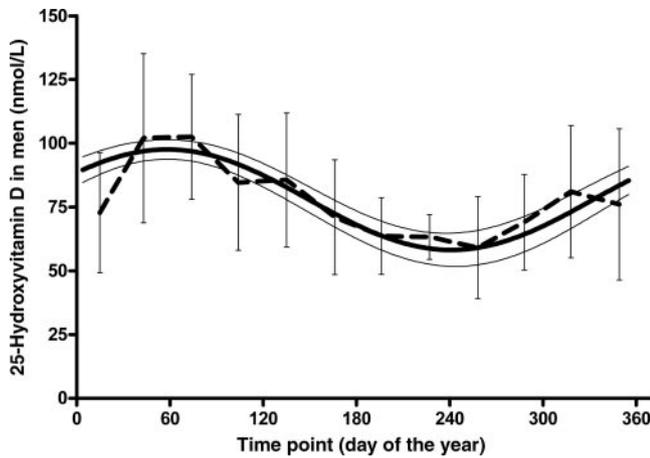
TABLE 2
The minimum 25-hydroxyvitamin D concentration required to have a predicted 25-hydroxyvitamin D trough of >50 nmol/L, by month of measurement[1]

Month	Men ($n = 378$)	Women ($n = 1606$)
	nmol/L	
January	81 (76, 86)	65 (63, 67)
February	87 (82, 92)	69 (67, 71)
March	87 (82, 92)	71 (69, 73)
April	79 (74, 84)	67 (65, 69)
May	69 (64, 74)	62 (60, 64)
June	59 (53, 65)	57 (55, 59)
July	52 (46, 58)	52 (50, 54)
August	50 (44, 56)	50 (48, 52)
September	50 (44, 56)	50 (48, 52)
October	53 (47, 59)	51 (49, 53)
November	61 (55, 67)	54 (52, 56)
December	71 (66, 76)	60 (58, 62)

[1] All values are estimates; 95% CIs in parentheses.

shown in **Figure 2**. For the fitted sine curve, we used the following equation:

$$y = 77.89 + 19.67 \times \text{sine(frequency} \times x + 0.57) \quad (4)$$

where $r^2 = 0.19$. There was excellent agreement between the fitted sine curve and the mean monthly 25(OH)D concentrations in both cohorts (Figures 1 and 2).

The predicted sine curves indicated that 73% of the women had a trough 25(OH)D concentration of <50 nmol/L. In comparison, the observed prevalence of 25(OH)D concentrations < 50 nmol/L was 49%, and it ranged from 23% to 74%, depending on the month of blood sampling (5). Thirty-nine percent of the men had a predicted trough 25(OH)D concentration of <50 nmol/L, whereas the observed prevalence of 25(OH)D concentrations < 50 nmol/L was 9% (range: 0%–26%) (3). Of the women predicted to have trough 25(OH)D concentrations of <50 nmol/L (73% of the total), 28% were predicted to have 25(OH)D concentrations < 50 nmol/L for the entire year, and the mean ± SD number of days with predicted 25(OH)D concentrations < 50 nmol/L was 250 ± 108. Of the men predicted to have nadir 25(OH)D concentrations of <50 nmol/L (39% of the total), 3% were predicted to have 25(OH)D concentrations < 50 nmol/L for the whole year, and the mean number of days with 25(OH)D concentration < 50 nmol/L was 165 ± 89. From the sine curves, we determined the minimum 25(OH)D concentration for each month required to ensure that the 25(OH)D concentration was maintained at >50 nmol/L throughout the year (**Table 2**). During summer (December–March in New Zealand), this value approached 90 nmol/L in men and 70 nmol/L in women.

To determine the effect of fat mass on seasonal variations in 25(OH)D, we divided the cohorts into quartiles of fat mass and fitted a sine curve to each quartile. Baseline and amplitude coefficients for these sine curves differed significantly between men and women ($P < 0.01$) and across fat mass quartiles ($P < 0.01$), but there were no significant sex × quartile interactions ($P > 0.14$). Phase shift coefficients did not differ significantly between men and women or between fat mass quartiles ($P > 0.35$). The peak 25(OH)D concentration and amplitude of the sine curves decreased with increasing fat mass, whereas the

trough 25(OH)D concentrations did not differ significantly (**Table 3**). Thus, subjects in the highest quartile of fat mass had smaller seasonal variations in 25(OH)D concentrations and lower peak 25(OH)D concentrations than did subjects in the lowest quartile of fat mass (**Figure 3**). In women, each 1-kg difference in fat mass was associated with changes of −0.52 nmol/L, −0.05 nmol/L, and −0.23 nmol/L in peak and trough 25(OH)D concentrations and in amplitude, respectively. In men, each 1-kg difference in fat mass was associated with changes of −1.3 nmol/L, −0.27 nmol/L, and −0.51 nmol/L in peak and trough 25(OH)D and in amplitude, respectively. When we repeated these analyses by using weight quartiles instead of fat mass quartiles, we obtained similar trends. Each 1-kg difference in body weight was associated with changes of −0.34 nmol/L, −0.09 nmol/L, and −0.13 nmol/L in peak and trough 25(OH)D concentrations and in amplitude, respectively, in women and changes of −0.25 nmol/L, 0.13 nmol/L, and −0.19 nmol/L in peak and trough 25(OH)D concentrations and in amplitude, respectively, in men. There was no association between fat mass quartile and the number of days per year of predicted 25(OH)D concentrations < 50 nmol/L in men or women.

Finally, to determine whether the differences between the amplitudes and the peak and trough values of the sine curves in men and women were due to differences in fat mass between the sexes, we restricted our analyses to men ($n = 162$) and women ($n = 725$) in the same fat mass range (15–25 kg). We found that the amplitudes and the peak and trough values of the sine curves for these subgroups did not differ significantly from the values for the entire cohorts. Thus, the sine curve for the subgroup of men had larger amplitude (19 nmol/L), peak value (96 nmol/L), and trough value (58 nmol/L) than did the curve for the subgroup of women (amplitude 11 nmol/L, peak 64 nmol/L, trough 42 nmol/L). In a complimentary path analysis of all participants, including fat mass quartiles in models of peak 25(OH)D, trough 25(OH)D, and amplitude versus sex did not attenuate the relation between sex and these variables. Taken together, these findings suggest that fat mass differences between the sexes could not fully explain the sex differences in the amount of seasonal variations in 25(OH)D.

TABLE 3
The effect of fat mass on seasonal variations in 25-hydroxyvitamin D concentrations[1]

	Quartile				P^2		
	1	2	3	4	Sex	Quartile	Sex × quartile
Fat mass (kg)							
Men	10.4 ± 3.0[3]	16.3 ± 1.1	20.7 ± 1.6	29.3 ± 5.5			
Women	16.0 ± 3.3	23.2 ± 1.6	29.1 ± 1.8	39.6 ± 7.0			
Peak 25(OH)D (nmol/L)							
Men	111 (102, 120)[4]	101 (91, 110)	100 (91, 110)	86 (78, 93)	<0.01	<0.01	0.13
Women	67 (64, 70)	63 (60, 66)	61 (59, 64)	55 (52, 58)			
Trough 25(OH)D (nmol/L)							
Men	62 (46, 78)	61 (44, 78)	56 (42, 70)	58 (47, 69)	<0.01	0.72	0.75
Women	41 (37, 44)	42 (39, 45)	42 (39, 45)	40 (37, 43)			
Amplitude (nmol/L)							
Men	24 (14, 35)	20 (9, 31)	22 (13, 32)	14 (6, 22)	<0.01	<0.01	0.10
Women	13 (11, 16)	10 (8, 13)	10 (8, 12)	7 (5, 10)			

[1] 25(OH)D, 25-hydroxyvitamin D. $n = 77$ men and 374 women in each quartile.

[2] The main effects in the models of peak 25(OH)D, trough 25(OH)D, or amplitude compared with sex or fat mass quartile and sex × quartile interaction (ANOVA).

[3] $\bar{x}$ ± SD (all such values).

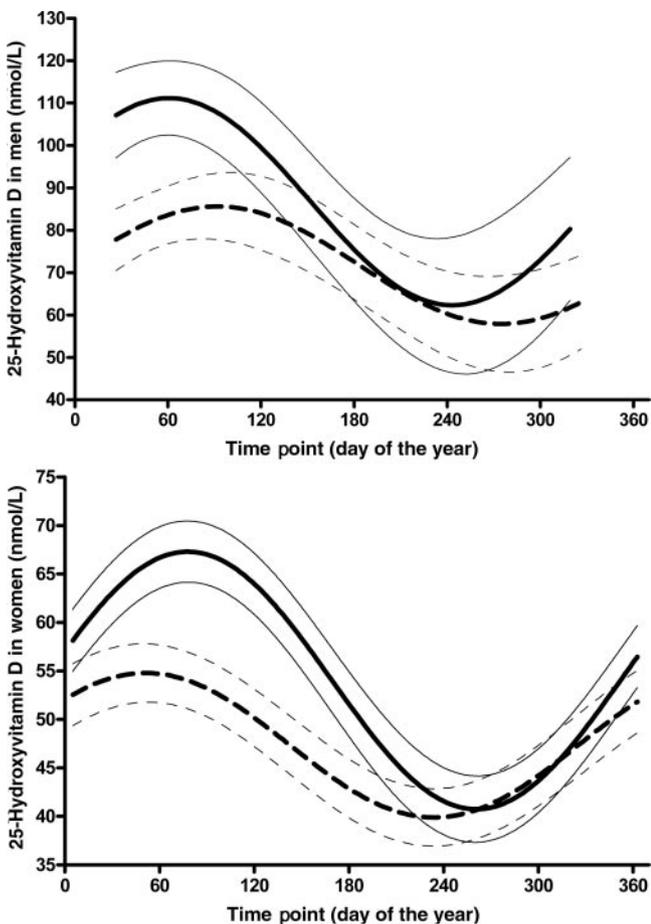[4] $\bar{x}$; 95% CI in parentheses (all such values).

FIGURE 3. The effect of fat mass on seasonal variation in 25-hydroxyvitamin D concentrations in men and women. $n = 88$ men and 374 women in the lowest and the highest quartiles of fat mass. Data are shown from the lowest (—) and highest (– –) quartiles for clarity only. 95% CIs are shown by the thin dashed and continuous curves.

We repeated all these analyses in the men, restricting the analyses to the 252 men who had 25(OH)D measured by using the Diasorin assay. In all cases, the results were similar to those we obtained for the entire cohort.

DISCUSSION

Seasonal variations in 25(OH)D concentrations have a substantial effect on a diagnosis of vitamin D insufficiency. We found that 49% (range: 23–74%) of postmenopausal women and 9% (range: 0–26%) of middle-aged and older men had 25(OH)D concentrations < 50 nmol/L in the month of measurement. However, the current analysis predicts much higher prevalences of 25(OH)D concentrations < 50 nmol/L (73% in women and 39% in men) in late winter and early spring. In those persons predicted to have 25(OH)D concentrations < 50 nmol/L, the mean predicted duration was 250 days/y in women and 165 days/y in men. Thus, many people are predicted to have suboptimal 25(OH)D concentrations for a substantial proportion of the year, despite having apparently adequate concentrations at the time of testing. We found that summer and fall 25(OH)D concentrations of ≥70–90 nmol/L in men and ≥60–70 nmol/L in women are required to ensure 25(OH)D concentrations > 50 nmol/L throughout the year. Currently, there is no universally accepted definition of vitamin D sufficiency, and estimates of 25(OH)D concentrations considered to be adequate range from as low as 25 nmol/L to as high as 100 nmol/L; the most commonly recommended thresholds are between 50 and 80 nmol/L (2). The need to consider seasonal variations when interpreting these thresholds has not been widely discussed. Although not stated explicitly, it can be inferred that the definition of vitamin D sufficiency refers to the lowest 25(OH)D concentration during the year. Vitamin D insufficiency is associated with adverse effects such as secondary hyperparathyroidism, increased bone turnover, decline in bone density, and increased risk of fractures (1, 2). Population studies have reported higher rates of bone remodelling (8, 9) and fragility fracture (9–11) during the winter, which

suggests that any period of vitamin D insufficiency is undesirable. Thus, in locations where seasonal variations in 25(OH)D occur, thresholds for diagnosis of vitamin D sufficiency also will vary by season.

Seasonal variations in 25(OH)D concentrations have been widely reported (12), even in subtropical locations with sunny weather year-round (13). In our cohorts, who reside in a subtropical location at latitude 37°S, there were seasonal variations in 25(OH)D [peak 25(OH)D − trough 25(OH)D] of 21 nmol/L in women and 39 nmol/L in men. The amount of seasonal variation of 25(OH)D is likely to be determined by the latitude and the climate. In countries at higher latitudes, lower angles of incidence of incoming solar radiation during winter mean that UV rays travel a greater distance through the atmosphere, which results in greater atmospheric absorption of UV radiation. Seasonal changes in cloud cover also may contribute to the greater atmospheric absorption of UV radiation. In addition, exposure of the skin to UV-B is generally decreased during the colder months because more clothes are worn. Thus, adjustment for the effects of seasonal variation on thresholds for diagnosis of vitamin D sufficiency should be individualized to the latitude and climate of a location.

The effect of fat mass on seasonal variations in 25(OH)D concentrations has not been reported previously. Fat mass is a small but important determinant of 25(OH)D concentrations in cross-sectional analyses, in which fat mass and 25(OH)D concentrations are inversely associated even after adjustment for potential confounding factors such as exercise levels (3–5, 14–21), which are a surrogate measure of sunlight exposure. This relation may occur because vitamin D and its metabolites are fat soluble, which leads to greater sequestration in the adipose tissue of obese persons (4, 15). Other possible explanations would be that overweight persons have less exposure to sunlight because of their choice of clothing or because of lower exercise levels and less mobility. Low 25(OH)D concentrations promote secondary hyperparathyroidism (1), which may in turn increase hepatic catabolism of 25(OH)D, thereby further lowering 25(OH)D concentrations (22). Our findings that greater fat mass was associated with lower peak 25(OH)D concentrations and smaller seasonal variations in 25(OH)D— but no change in trough 25(OH)D concentrations—are consistent with an effect of both reduced sunlight exposure in heavier individuals and the role of adipose tissue as a reservoir of vitamin D and its metabolites. Regardless of fat mass, reduced exposure to sunlight will lead to lower peak 25(OH)D concentrations. If adipocytes act as a reservoir of vitamin D and its metabolites and as a buffer against both higher 25(OH)D concentrations in the summer and lower concentrations in the winter, then greater adiposity would be associated with lower peak 25(OH)D concentrations, less seasonal variation, and higher-than-expected trough 25(OH)D concentrations. A combination of these 2 hypotheses—less sunlight exposure and a greater reservoir of vitamin D and its metabolites—would therefore explain our findings. These findings are congruent with analyses of 25(OH)D concentrations after controlled inputs (23).

Fat mass modifies the effect of seasonal variation on thresholds for diagnosis of vitamin D sufficiency. Persons in the highest quartile of fat mass have lower peak 25(OH)D concentrations and smaller amounts of seasonal variation in 25(OH)D concentrations than do persons in the lowest quartile of fat mass, whereas trough 25(OH)D concentrations are similar across fat mass quartiles. Thus, seasonally adjusted thresholds for a diagnosis of vitamin D sufficiency decrease with increasing fat mass and increase with decreasing fat mass. However, this effect is small and may be clinically relevant only in persons whose body weight or fat mass is >1 SD from the mean and who have 25(OH)D measurements taken in summer. The differences in 25(OH)D concentrations and seasonally adjusted thresholds for a diagnosis of vitamin D sufficiency between men and women are not fully explained by the differences in fat mass between the sexes (3). It is likely that sex-related differences in behavioral and cultural factors associated with any or all of sunlight exposure, physical activity, skin-protective practices (3), and sex-related differences in vitamin D metabolism (16, 17) underpin the differences between men and women in 25(OH)D concentrations and seasonally adjusted thresholds for a diagnosis of vitamin D sufficiency.

The present study has some limitations. Participants were healthy independent volunteers and were predominantly of European descent. The findings therefore may not be applicable to persons with pigmented skin, to different population groups such as the frail elderly, or to persons living in different latitudes or different climates. We have assumed that persons would have seasonal variations in 25(OH)D the same as the population average. Therefore, the findings may not be applicable to persons whose seasonal variations in 25(OH)D are markedly different from the average, such as persons who have very limited sunlight exposure or who use artificial tanning devices during the winter. Finally, as previously discussed, there is no consensus on the optimal circulating 25(OH)D concentration. Whereas we selected a concentration of 50 nmol/L, other authors have recommended a concentration of 75–80 nmol/L (2). Our findings can be readily applied to such thresholds by adding the difference between any selected concentration for vitamin D sufficiency and 50 nmol/L to the values in Table 2. For example, to maintain 25(OH)D concentrations > 80 nmol/L year-round, men would need concentrations > 100–120 nmol/L and women 90–100 nmol/L in the summer months.

In summary, we found that seasonal variations in 25(OH)D concentrations have a significant effect on thresholds for a diagnosis of vitamin D sufficiency. Levels of fat mass affect peak 25(OH)D concentrations and the amount of seasonal variation in 25(OH)D [peak − trough 25(OH)D concentrations], but not trough 25(OH)D concentrations, and they potentially affect the thresholds for a diagnosis of vitamin D sufficiency. Because seasonal variations in 25(OH)D are primarily dependent on latitude and climate, seasonally adjusted thresholds for a diagnosis of vitamin D sufficiency will have to be individualized for different locations. Clinicians should take into account the season of sampling when determining whether a patient is at risk of vitamin D insufficiency at any time during the year.

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