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The effects of seasonal variation of 25-hydroxyvitamin D on diagnosis of vitamin D insufficiency

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Abstract

Aims To explore the effects of seasonal variation on the diagnosis of vitamin D sufficiency and to determine whether age, gender, and ethnicity modify these effects.

Methods 21,987 adults had a measurement of serum 25-hydroxyvitamin D (25OHD) at Labplus, Auckland City Hospital, between January 2002 and September 2003, and sine curves were fitted for 25OHD versus day of year to predict the 25OHD nadir for each individual.

Results 48% (range: 30–63%) of individuals had 25OHD <50 nmol/L in the month of measurement, but 63% were predicted to have 25OHD <50 nmol/L in late winter or early spring based on expected seasonal variation. The 25OHD levels required to ensure 25OHD levels >50 nmol/L throughout the year varied substantially by season (in summer at least 60–75 nmol/L), and tended to be higher in men than women, decrease with age, and vary with ethnicity. Mean 25OHD levels were very low (<40 nmol/L) in people of Indian, Middle Eastern, and African descent.

Conclusion Seasonal variation in 25OHD affects the diagnosis of vitamin D sufficiency. Clinicians should consider the month of sampling when interpreting the results of 25OHD measurements. In New Zealand, a summertime 25OHD >60–75 nmol/L is generally required to ensure year-round 25OHD levels >50 nmol/L.

Vitamin D insufficiency is common in adults living in New Zealand[1] and can cause myopathy, osteopenia, secondary hyperparathyroidism, and osteomalacia.[2] The serum level of 25-hydroxyvitamin D (25OHD) is considered to be the best estimate of body stores of vitamin D,[2] but estimates of the serum 25OHD level above which vitamin D stores are considered adequate vary widely, from 25 nmol/L to 100 nmol/L.[3] For this report, we have adopted the widely used but arbitrary definitions of vitamin D insufficiency as serum 25OHD <50 nmol/L and vitamin D deficiency <25 nmol/L.[2]

The major biological determinant of 25OHD levels is ultraviolet B (UV-B) exposure.[4,5] In New Zealand and other countries distant from the equator, there is seasonal variation of UV-B levels due to the lower angle of the sun and greater cloud cover in winter months.[4,6]

More clothes are also worn in winter thereby reducing skin exposure to UV-B. As a result of this seasonal variation in UV-B, there is seasonal variation in 25OHD levels, such that levels are highest in late summer and early autumn and lowest in late winter and early spring.[1,4–6]

Such seasonal variation in 25OHD levels means that individuals could have adequate 25OHD levels in the summer and autumn months yet have suboptimal levels in winter and spring.

Previously, we reported that seasonal variation of 25OHD has a significant impact on the diagnosis of vitamin D insufficiency.[7] In cross-sectional studies, 49% of healthy older women and 9% of healthy middle-aged and older men were vitamin D insufficient in the month of measurement. However, much higher proportions (73% of women and 39% of men) were predicted to be vitamin D insufficient in late winter/early spring based on expected seasonal variation.

Using these data, we estimated that the 25OHD level required to ensure vitamin D sufficiency throughout the year varied by season and, in summer, was at least 70–90 nmol/L in men and 60–70 nmol/L in women.

In this report, we set out to validate these previous findings in a much larger cross-sectional sample of 25OHD measurements, to determine the minimum summertime 25OHD levels needed to ensure year-round vitamin D sufficiency in adults in Auckland and whether these levels vary by age, gender, or ethnicity, and to determine whether sine curves derived from cross-sectional data accurately predict future measurements of 25OHD.

Methods

Study subjects

We used all measurements of serum 25OHD between 1 January 2002 and 30 September 2003 at Labplus, Auckland City Hospital, in which date of birth and gender were available (98.4% of samples). 21,987 adults aged >18 years (17,265 women and 4722 men) had at least one measurement of 25OHD available for cross-sectional analysis. Where there was more than one 25OHD measurement during this period, the first measurement chronologically was included in the cross-sectional analysis.

3146 individuals had more than one measurement of 25OHD available for longitudinal analysis. Ethnicity data were obtained from the National Health Index (NHI) database using the NHI number for each sample recorded in the Labplus database. Ethnicity data were available for 13,817 individuals.

25-hydroxyvitamin D assay

Serum 25-hydroxyvitamin D was measured in duplicate using the Diasorin radioimmunoassay. Labplus takes part in, and meets the performance targets for, the Vitamin D External Quality Assessment Scheme (DEQAS).[8] The interassay CV for the Diasorin assay was 7.6% at 46 nmol/L.

Statistical analysis

Cross-sectional analysis—The methods have previously been described in detail.[7] In brief, 25OHD levels were plotted against the day of the year the blood sample was taken and a sine curve fitted. We assumed that 25OHD levels throughout the year for each individual would follow a similar sine curve to the population.

By solving the population sine curve equation for each individual, we were able to predict the 25OHD nadir for each individual, and by solving the population equation for each month, we were able to predict the 25OHD level required to ensure year round vitamin D sufficiency.

We performed these analyses with the cohort divided by gender and age, and then repeated the analyses dividing the cohort by ethnicity and age. We used analysis of variance (ANOVA) to compare the baseline, amplitude and phase shift coefficients of the sine curves between genders, age groups, and ethnic groups.

Longitudinal analysis—We sought to determine whether the sine curves from the population cross-sectional analysis would predict repeated 25OHD levels for an individual. Because we did not have access to medication history and individuals who had more than one measurement of 25OHD were more likely to have started or stopped vitamin D supplementation, we restricted the analysis to those individuals with baseline 25OHD >50 nmol/L and <100 nmol/L whose change from baseline was <1.5 times the population seasonal excursion. Such individuals were unlikely to have started or stopped vitamin D supplements between measurements. 1018 individuals met these criteria.

For each individual, we used the population sine curve equation to predict later 25OHD results based on the baseline 25OHD and the dates of the measurements. We then compared these predicted results to the measured 25OHD.

The equation for each sine curve was: 25OHD = baseline + amplitude * sine (angular frequency * day of year + phase shift). The amplitude of the sine curve is the maximal deviation from the baseline [(peak value - trough value)/2]; the angular frequency is $2*\pi$/period ($2*\pi/365$); and the phase shift is the amount of translation along the x-axis.

Curve fitting and other statistical calculations were carried out using the SAS software package (SAS Institute, Cary, NC version 9.1). All tests were two-tailed and statistical significance was set at $p<0.05$.

Results

The age and gender distribution of the population is shown in Table 1. There were no differences in the mean 25OHD levels between men and women in any of the age groups after adjusting for the month of the year by ANOVA ($p>0.14$). Therefore, while we fitted sine curves separately for men and women, for ease of interpretation we have presented the results with data for men and women pooled.

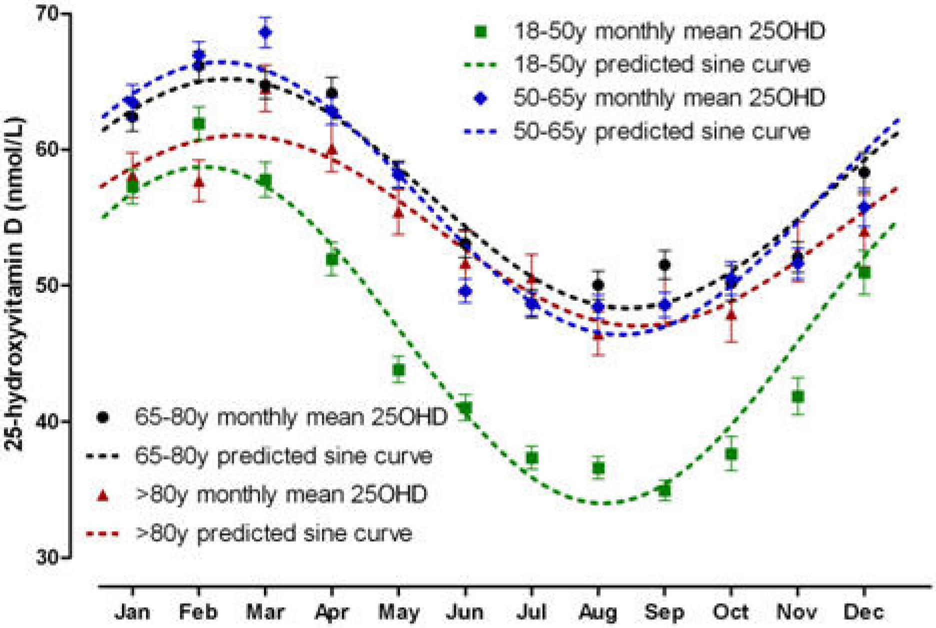
Table 1. Age and gender distribution of the study populations

Age	Female	Male	Total
18–50 years	4788	1708	6496
50–65 years	5109	1218	6327
65–80 years	4654	1127	5781
>80 years	2714	669	3383
Total	**17265**	**4722**	**21987**

Figure 1 shows the sine curves fitted for each age group together with the mean monthly 25OHD levels. There was excellent agreement between the fitted sine curve and the mean monthly 25OHD levels in all age and gender groups.

Table 2 shows the parameters of the fitted sine curves for each age group by gender. The amplitude of the sine curves tended to be higher in men than women but the difference was only statistically significant for people aged 50 to 65 years ($p=0.0061$). There were no significant differences between genders in any other parameter for any age group. There were significant differences between the age ranges for the baseline and amplitude parameters ($p<0.01$ for each parameter).

Figure 1. Sine curve of best fit for 25-hydroxyvitamin D (25OHD) versus day of the year by age with measured mean monthly 25OHD for comparison



Note: The error bars represent the standard error of the mean.

Table 2. Parameters of the fitted sine curves for 25-hydroxyvitamin D versus day of year by age and gender

Age	Female			Male		
	Baseline	Amplitude	Phase	Baseline	Amplitude	Phase
18–50 years	46.4	12.0	0.7	46.4	13.5	0.8
50–65 years	56.7	9.5	0.6	55.3	12.2	0.7
65–80 years	56.7	8.5	0.5	57.1	8.3	0.8
>80 years	54.2	6.4	0.4	53.6	9.1	0.5

The baseline represents the mean seasonally adjusted 25OHD level, the amplitude the amount of seasonal excursion from the baseline, and the phase the timing of the peak 25OHD levels (a phase value of 0 means the peak 25OHD levels occurs on the 1st April and with each 0.1 unit increase in the phase value, the peak occurs 5-6 days earlier in the year).

From the derived sine curves, 63% of people had a 25OHD nadir <50 nmol/L consistent with vitamin D insufficiency, and 25% had a 25OHD nadir <25 nmol/L consistent with vitamin D deficiency.

In comparison, the observed prevalence of vitamin D insufficiency at the time of sampling was 48%, ranging from 30–35% between January and March to 61–63% between July and September. The observed prevalence of vitamin D deficiency was

15%, ranging from 7–8% between January and March and 21–23% between July and September.

From the sine curves, we determined the minimum 25OHD level for each month required to ensure that the 25OHD level was maintained >50 nmol/L throughout the year (Table 3). In summer, this value was at least 60–75 nmol/L, and tended to be higher in men than women, and to decrease with age.

Table 3. The minimum 25-hydroxyvitamin D level (nmol/L) required to have a predicted 25-hydroxyvitamin D nadir >50 nmol/L—by month of measurement, age, and gender

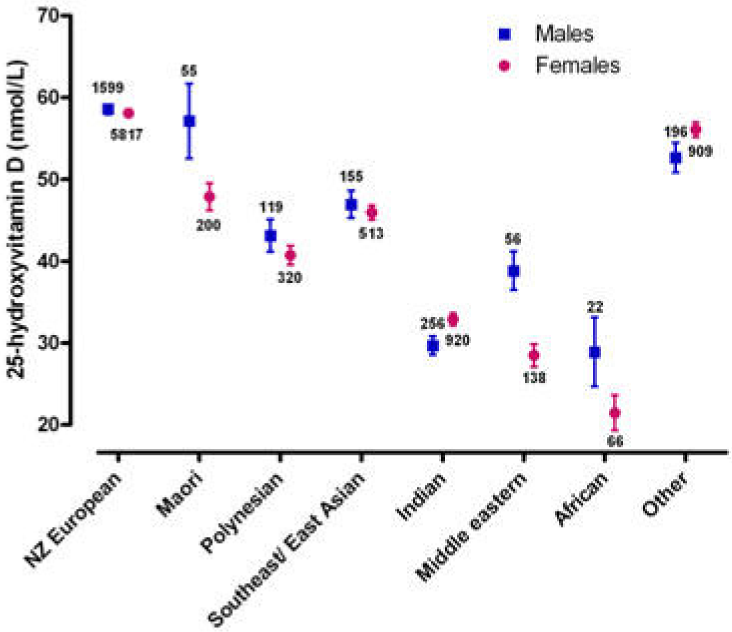
Age	**18–50 years**		**50–65 years**		**65–80 years**		**> 80 years**	
Month	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**
January	70	73	65	70	63	64	59	64
February	73	76	68	74	66	66	62	67
March	71	73	68	72	66	64	62	67
April	66	67	64	66	63	61	60	64
May	60	60	59	60	59	57	57	59
June	54	54	55	54	55	53	54	55
July	51	50	51	51	51	50	51	51
August	50	50	50	50	50	50	50	50
September	51	51	50	51	50	50	50	50
October	53	54	52	53	51	52	51	51
November	58	60	55	58	54	56	53	55
December	64	67	60	65	58	60	56	59

We repeated these analyses on the data for which ethnicity information was available. Figure 2 shows the baseline values of the fitted sine curves for each of the ethnic groups by gender. These values represent the seasonally-adjusted mean 25OHD level. People of Indian, African, and Middle Eastern descent had markedly low 25OHD levels.

To ensure suitable group sizes, we restricted all further analyses to the four broad ethnic groups for which more than 500 samples were available and subdivided the groups into only two age groups (18–50 years and >50 years). There were insufficient samples to permit subdividing the groups by gender, however there were no significant differences in monthly mean 25OHD levels between genders for each ethnic group (p>0.07).

Figure 3 shows the sine curves fitted for each ethnic group by age range. There was excellent agreement between the fitted sine curves and the mean monthly 25OHD levels in all age and ethnic groups (data not shown).

Figure 2. Mean 25-hydroxyvitamin D (nmol/L) by gender for ethnicity categories recorded in the NHI database.



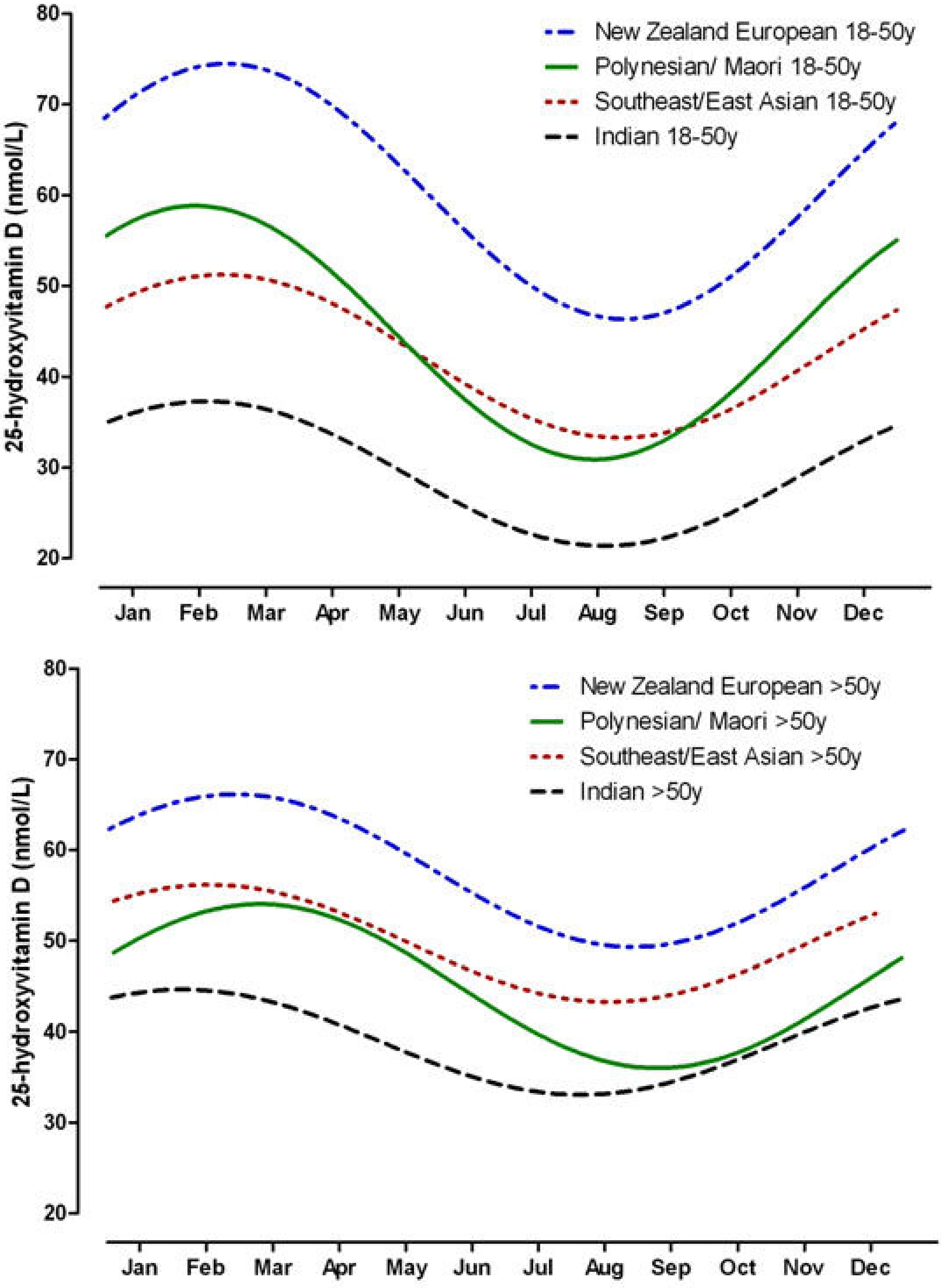
The error bars represent the standard error of the mean. The numbers above or below the points are the number of measurements.

Table 4 shows the parameters of the fitted sine curves for each ethnic group by age group. The baseline of the sine curves varied with ethnicity (p<0.001), and the amplitudes with age (p=0.05), but there was no significant differences in any of the other parameters with age or ethnic group.

Table 4. Parameters of the fitted sine curves of 25-hydroxyvitamin D by age and ethnicity

Ethnicity	Age	Baseline	Amplitude	Phase
Southeast/East Asian	18–50 years	42.3	9.0	0.6
	>50 years	49.7	6.4	0.8
New Zealand European	18–50 years	60.4	14.0	0.6
	>50 years	57.7	8.4	0.6
Polynesian/Māori	18–50 years	44.9	14.0	0.8
	>50 years	45.0	9.0	0.4
Indian	18–50 years	29.3	8.0	0.7
	>50 years	38.9	5.8	1.0

Figure 3. Sine curve of best fit for 25-hydroxyvitamin D versus day of year by ethnicity



The baseline represents the mean seasonally adjusted 25OHD level, the amplitude the amount of seasonal excursion from the baseline, and the phase the timing of the peak

25OHD levels (a phase value of 0 means the peak 25OHD levels occurs on the 1st April and with each 0.1 unit increase in the phase value, the peak occurs 5–6 days earlier in the year).

Table 5 shows the minimum 25OHD levels in each month required to ensure that the 25OHD level was maintained >50 nmol/L throughout the year for the different ethnic groups. These values were between 60–80 nmol/L in the summer months.

Table 5. The minimum 25-hydroxyvitamin D level (nmol/L) required to have a predicted 25-hydroxyvitamin D nadir >50 nmol/L—by month of measurement, age, and ethnicity

Age	Southeast/East Asian		New Zealand European		Polynesian/Māori		Indian	
	18–50 years	>50 years	18–50 years	>50 years	18–50 years	>50 years	18–50 years	>50 years
January	64	61	72	63	74	62	63	61
February	67	63	76	66	77	66	65	62
March	66	61	76	66	74	67	64	59
April	63	58	71	63	68	65	61	56
May	58	55	64	58	60	61	56	53
June	54	52	57	54	54	56	53	51
July	51	50	52	51	50	52	50	50
August	50	50	50	50	51	50	50	51
September	50	50	50	50	51	50	50	51
October	52	52	52	51	54	51	52	53
November	55	55	58	55	61	53	56	55
December	60	58	65	59	68	58	60	58

Finally, we assessed whether the sine curves derived from the cross-sectional analysis of the entire population could predict repeated 25OHD levels for an individual. 1018 individuals had more than one 25OHD measurement (total 1487 measurements, median 2, range 2–18) and were unlikely to have started or stopped vitamin D supplementation between measurements. For the repeated measurements, the mean (SD) measured 25OHD level was 67 (17) nmol/L and the mean predicted 25OHD level was 65 (15) nmol/L, resulting in a difference of 2.0 (13) nmol/L.

The differences between the measured and predicted level for each age group were 1.7 (16) nmol/L, 1.2 (14) nmol/L, 2.4 (12) nmol/L, and 2.4 (12) nmol/L for 18–50, 50–65, 65–80, and >80 years respectively. The mean differences between measured and predicted levels were similar when the results were grouped by the month of the year of the measured sample (range -3.7 to 4.9 nmol/L) and when grouped by the number of months between the measured and predicted levels (range -1.4 to 5.7 nmol/L).

Overall, 74% of predicted 25OHD levels were within ± 10 nmol/L of the measured 25OHD level, and there was a 95% probability that the measured 25OHD level would be in the range of the predicted level ±26 nmol/L. By comparison, for a single 25OHD measurement, there is a 95% probability that the true level lies within approximately 10% of the measured value, or ±6.5 nmol/L at a 25OHD level of 65 nmol/L.

Discussion

In this very large sample of 25OHD measurements, we have confirmed our previous findings that seasonal variation in 25OHD substantially impacts upon the diagnosis of vitamin D sufficiency.[7] 48% of individuals (range: 30-63%) had 25OHD levels <50 nmol/L in the month of measurement, but based on expected seasonal variation, a much higher proportion (63%) were predicted to have vitamin D insufficiency in late winter/early spring. Thus a substantial proportion of individuals tested were predicted to have suboptimal 25OHD levels in the winter and spring months despite having apparently adequate levels at the time of testing.

Using these data, a 25OHD level in late summer to early autumn of 60–75 nmol/L is required to ensure vitamin D sufficiency throughout the year, and this value tends to be higher in men than women, and to decrease with age.

There were significant differences in 25OHD levels between the ethnic groups, as expected, with New Zealand Europeans having higher levels than people of Māori, Polynesian, or Southeast/East Asian descent; with people of Indian, Middle Eastern, or African descent having the lowest levels. The mean seasonally-adjusted 25OHD levels for the latter three groups were very low, identifying people of these ethnic groups as being at high risk for complications of vitamin D deficiency. A low threshold for vitamin D supplementation in these groups is warranted.

However, there were no significant differences between ethnic groups in the amount of seasonal variation of 25OHD, and in all groups older adults had less seasonal variation of 25OHD than younger adults. A late summer or early autumn 25OHD level of 60–80 nmol/L is required to ensure vitamin D sufficiency throughout the year, consistent with the results from the entire cohort.

Using the equations from the population sine curve together with one measurement from an individual accurately predicted future 25OHD results at a cohort level, with the mean predicted level for an individual differing by only 2–3 nmol/L from the mean measured level. The results were similar regardless of the time of the year the samples were drawn and the duration of time between the samples. However, this approach is associated with less precision at an individual level: the precision of a predicted 25OHD level was about ¼ of the precision of a single laboratory measurement of 25OHD, although the majority of the predicted measurements were within 10 nmol/L of the measured level.

The precision of this approach might potentially be improved if information regarding usage of vitamin D supplementation was known.

The monthly thresholds for the diagnosis of vitamin D sufficiency depend primarily on the amount of seasonal variation of 25OHD and the threshold for vitamin D sufficiency adopted.

The amount of seasonal variation of 25OHD is determined by the latitude, the climate, and lifestyle. At higher latitudes, lower angles of incidence of incoming solar radiation during winter result in UV rays travelling a greater distance through the atmosphere and therefore increased atmospheric absorption of UV radiation. Increased cloud cover in winter may also cause increased atmospheric absorption of

UV radiation. In addition, more clothes are worn in winter leading to reduced exposure of the skin to UV-B.

In this study, the 25OHD measurements were obtained from people living in northern New Zealand. However, the seasonal change in 25OHD was similar to a recent study from Christchurch[6] and a New Zealand population-based study[1] suggesting that these findings are broadly applicable across the whole of New Zealand to a wide range of ethnic groups. However, the seasonal changes in 25OHD were greater in our previous studies of healthy volunteers[4,5,7] than in this study, suggesting that such individuals might require higher summertime 25OHD levels than we report here. For example, in those studies older women (mean age 74 years) required 25OHD of at least 60–70 nmol/L and middle-aged and older men (mean age 57 years) required 25OHD of 70–90 nmol/L to ensure year-round 25OHD levels >50 nmol/L.

The differences in the monthly thresholds for vitamin D sufficiency between men and women and across different age groups arise from differences in the amount of seasonal variation of 25OHD which in turn are due to differences in the amount of UV-B exposure.

The differences in the thresholds were generally small and are most likely related to differences between the groups in behavioural and cultural factors associated with sunlight exposure, physical activity, and skin protective practices.[5] 25OHD levels may also decline with age because photolysis of steroid precursors in the skin by UV-B is less efficient in the elderly than in younger people.[9]

There is no universally accepted definition of vitamin D sufficiency, with the most commonly recommended thresholds between 50–80 nmol/L.[3] Such thresholds are usually stated without reference to the season of the year, but presumably refer to the lowest 25OHD level during the year.

Although we have used a threshold of 50 nmol/L for vitamin D sufficiency, our findings can be readily applied to other thresholds by adding the difference between any selected level for vitamin D sufficiency and 50 nmol/L to the values in Table 3 or Table 5. For example, to maintain 25OHD levels greater than 80 nmol/L year-round, individuals would need levels >90–105 nmol/L in the summer months.

It is well recognised that vitamin D deficiency causes musculoskeletal effects including myopathy, falls, osteopenia, osteomalacia, and fractures.[2] Recent observational evidence has suggested that vitamin D deficiency is associated with increased occurrence of a number of chronic medical conditions including ischaemic heart disease, hypertension, autoimmune diseases (such as multiple sclerosis and Type 1 diabetes), chronic lung diseases, and a variety of cancers.[10] However, currently there is little evidence from randomised controlled trials that vitamin D supplementation can prevent development or progression of such diseases.

There are limitations to our study, particularly related to selection bias and the lack of information regarding vitamin D supplements. The study was restricted to individuals who had a measurement of 25OHD. It is likely that such individuals were at higher risk of vitamin D deficiency and therefore had lower 25OHD levels and different amounts of sunshine exposure to the population.

Selection bias might also account for the lower-than-expected 25OHD levels observed in the young adults and the lack of a reduction in 25OHD levels with age that is commonly reported. It is also possible that some individuals had requested a measurement of 25OHD because they were more health conscious. Such individuals may have had higher 25OHD levels than the population.

We did not have any knowledge of the use of vitamin D supplements which would be expected to reduce the amount of seasonal excursion of 25OHD. However, the 25OHD levels and amount of seasonal excursion of 25OHD were broadly similar to those in the New Zealand population-based survey,[1] suggesting that any impact on our findings is small.

In summary, seasonal variation of 25OHD levels significantly impacts on the thresholds for diagnosis of vitamin D sufficiency. In northern New Zealand, 25OHD levels of at least 60–75 nmol/L in late summer to early autumn are required to ensure year round 25OHD levels of >50 nmol/L. This threshold tends to be slightly lower for women than for men, to vary with ethnicity, and to decrease with age, but is probably broadly applicable across New Zealand.

People of Indian, Middle Eastern, and African descent are at particular risk of vitamin D insufficiency. It is important that clinicians take into account the season of sampling when determining whether a patient is at risk of vitamin D insufficiency during the year.

Competing interests: None known.

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