ABSTRACT: In this population-based study, seasonal periodicity was seen with reduced serum vitamin D, increased serum PTH, and increased bone resorption in winter. This was associated with an increased proportion of falls resulting in fracture and an increased risk of wrist and hip fractures.

Introduction: In a population of women who reside in a temperate climate and do not generally receive dietary vitamin D supplementation, we investigated whether seasonal vitamin D insufficiency is associated with increased risk of fracture.

Materials and Methods: An observational, cross-sectional, population-based study set in southeastern Australia (latitude 38–39° S). Participants were drawn from a well-defined community of 27,203 women ≥55 years old: 287 randomly selected from electoral rolls, 1635 with incident fractures, and 1358 presenting to a university hospital with falls. The main outcome measures were annual periodicities of ultraviolet radiation, serum 25-hydroxyvitamin D [25(OH)D], serum parathyroid hormone (PTH), serum C-telopeptide (CTx), BMD, falls, and fractures.

Results: Cyclic variations in serum 25(OH)D lagged 1 month behind ultraviolet radiation, peaking in summer and dipping in winter (p < 0.001). Periodicity of serum PTH was the inverse of serum 25(OH)D, with a phase shift delay of 1 month (p = 0.004). Peak serum CTx lagged peak serum PTH by 1–2 months. In late winter, a greater proportion of falls resulted in fracture (p < 0.001). Seasonal periodicity in 439 hip and 307 wrist fractures also followed a simple harmonic model (p = 0.078 and 0.002, respectively), peaking 1.5–3 months after the trough in 25(OH)D.

Conclusions: A fall in 25(OH)D in winter is accompanied by increases in (1) PTH levels, (2) bone resorption, (3) the proportion of falls resulting in fracture, and (4) the frequency of hip and wrist fracture. Whether vitamin D supplementation in winter can reduce the population burden of fractures requires further investigation.

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Key words: vitamin D, parathyroid hormone, bone turnover markers, seasonality, population studies

INTRODUCTION

Increased risk of fracture caused by vitamin D and calcium deficiency is well documented among the institutionalized elderly,(1,2) but little is known about the role of mild vitamin D insufficiency in the pathogenesis of falls and fractures in the ambulant community from which most fractures arise. Vitamin D deficiency increases postural instability through increased body sway and muscle weakness and reduced ability to counteract falls.(3–5) Vitamin D deficiency is also associated with secondary hyperparathyroidism, increased bone remodeling, bone loss, and structural damage such as increased intracortical porosity, cortical thinning and porosity, and with severe deficiency states, impaired bone mineralization. (6–8) Thus, vitamin D deficiency predisposes to fracture through two independent pathways: increased likelihood of falling and increased bone fragility.

Although many studies have demonstrated seasonal changes in vitamin D levels,(9–15) there have been no population-based prospective studies examining the relationship between circulating 25-hydroxyvitamin D [25(OH)D] and secondary hyperparathyroidism, falls, and fractures. We assessed seasonal variations in serum 25(OH)D and parathyroid hormone (PTH) in conjunction with numbers of falls and fractures to test the hypothesis that decreased serum 25(OH)D in winter will result in secondary hyperparathyroidism and increased frequency of falls, leading to a higher incidence of fractures.

MATERIALS AND METHODS

Subjects

Study protocols were approved by the Barwon Health Research and Ethics Advisory Committee. The study is set in the Barwon Statistical Division (latitude 38–39° S) surrounding the regional city of Geelong in southeastern Aus-
tralia. The demographics for the region have been well characterized.\textsuperscript{16,17} All biological data were collected from a resident population of 27,203 women ≥55 years of age.

Random sample

An age-stratified sample of women was drawn at random from Commonwealth electoral rolls during the period 1994–1997, with an acceptance rate of 77.1%.\textsuperscript{17,18} After an overnight fast, serum samples were obtained from 287 women (median age, 72.8 years; interquartile range [IQR], 64.6–81.6; mean weight, 66.1 ± 13.0 kg) who were free from exposure to drugs and diseases known to influence calcium metabolism. Ninety-nine percent of the subjects were white, and none were shrouded for religious reasons. Self-reported details of lifestyle and medication use were documented by questionnaire at interview. Written, informed consent was obtained from all participants.

Falls presentations

During the period of 1995–1997, all women in the region ≥55 years old and presenting with falls to the Emergency Department at the Geelong Hospital were identified from the Sanderson Pickware database, which records demographic and triage details of all patients. This is the sole Emergency Department in the region, with a patient throughput of 33,000/year. Fractures resulting from falls were confirmed radiologically. A total of 1358 women attended the Emergency Department with falls. A total of 1501 falls presentations were recorded (mean age, 76.4 ± 10.4 years).

Fracture cases

Fracture cases from all causes were prospectively identified during the 3-year period from 17 February 1994 to 16 February 1997 from radiology reports for women ≥55 years old residing in the study region\textsuperscript{19} using a previously validated method for ascertaining incident fractures.\textsuperscript{20} The date of fracture was taken as the initial date of radiological diagnosis. A total of 1635 women attended the Emergency Department with fractures. A total of 1501 falls presentations were recorded (mean age, 76.4 ± 10.4 years).

Biochemical assays and bone densitometry

Blood samples and measurements of BMD were obtained concurrently from women in the random sample, not in the fracture cases. Samples were stored at −80°C for random batch analyses. Serum concentration of 25(OH)D was assayed using an equilibrium radioimmunoassay after extraction with acetonitrile (Incstar, Stillwater, MN, USA). The intra- and interassay precisions were 6% and 15%, respectively. Serum intact PTH was determined by a chemiluminescent enzyme assay (Immulite; Diagnostic Products Corp., Los Angeles, CA, USA) with intra- and interassay precision of 5%. The serum bone resorption marker, C-telopeptide (CTX), was measured using an electrochemiluminesence immunoassay (Crosslips; Roche Diagnostics, Mannheim, Germany), with an interassay precision of 8.5%. Fasting reduces the circadian variability to 10%.\textsuperscript{21} Serum bone alkaline phosphatase (bALP), a marker of bone formation, was calculated from total alkaline phosphatase (tALP) measured with a colorimetric assay (p-nitrophenyl phosphate with AMP buffer; Roche), after bALP precipitation using wheat germ lectin (Roche) as recommended by the manufacturer. The interassay precision was 11.3%. Serum CTX and bALP were determined in 242 subjects. Samples with bone remodeling marker concentrations below the lower limits of detection (10 pg/ml for CTX and 5 U/liter for bALP) were assigned these values. Omission of these samples (two CTX and three bALP) from the data set did not alter the results, so they were retained in the analysis.

DXA was performed using a Lunar DPX-L densitometer and analyzed with Lunar DPX-L software version 1.31. BMD (g/cm²) was measured at the spine in the posterior-anterior projection (L₂-L₄), proximal femur, whole body, ultradistal (UD) forearm, and mid-forearm sites. In vivo short-term precision for BMD was 0.6% for spine, 1.6% for the femoral neck, 2.1% for Ward’s triangle, 1.6% for the trochanter, 0.4% for the whole body, 2.1% for ultradistal forearm, and 1.1% for mid-forearm.

Ultraviolet radiation, rainfall, and temperature

Mean monthly biologically effective solar radiation data (1994–1997) were obtained from the Australian Radiation Protection and Nuclear Safety Agency. Solar ultraviolet (UV) radiation spectral measurements were recorded at the nearest facility (Yallambie, latitude 38° S) with an installed spectroradiometer incorporating a Spex 1680B double grating monochromator with an overall calibration accuracy of ±10%.\textsuperscript{22} Data were expressed in minimal erythemal doses. Rainfall and minimum daily temperatures for the Geelong region (1994–1997) were obtained from the Bureau of Meteorology.

Statistics

Using the Edward’s model,\textsuperscript{23} a method for recognizing and estimating cyclic trends, we determined the seasonal periodicity of the data. By fitting a sine curve to the data points, we estimated the amplitude of the peak value, the mesor (midline-estimating statistic of rhythm) or annual mean, and the amplitude of the seasonal variation. For continuous data (serum 25(OH)D, PTH normalized by natural log transformation, BMD, and the markers of bone turnover—CTX and bALP), regression analysis was used to test the significance of periodicity. For count data (frequency of falls and fractures), a χ² test was used to distinguish events based on the Edward’s model after standardizing month lengths to 30 days.\textsuperscript{23} The difference in proportion of falls resulting in fracture for each 6-month period, defined by the simple harmonic model developed above, was tested using a χ² test. Pearson’s correlation coefficients were determined for analysis of associations between BMD or serum bone markers and serum 25(OH)D and PTH. Analyses were performed using the Minitab software package, release 12 (Minitab, State College, PA, USA).
RESULTS

The nadir in UV radiation occurred in mid-winter (July) after the peak 6 months earlier in mid-summer (January). Serum 25(OH)D followed a similar periodicity, with a phase shift delay of 1 month (Fig. 1). Both measurements followed a simple harmonic curve ($p < 0.001$). The model for serum 25(OH)D predicted a mean minimum of 50 nM in winter and a mean maximum of 78 nM in summer. During the 2-month period surrounding the nadir of serum 25(OH)D, 17.6% of the subjects had serum 25(OH)D levels below 28 nM, and 60.3% were below 50 nM. Seasonal variation in serum PTH was the inverse of serum 25(OH)D ($r = -0.3$, $p < 0.001$). The maximum of the sinusoidal curve for serum PTH occurred in late winter and the minimum in late summer, with a range of 6.0–4.6 pM (lnPTH ranged from 1.79 to 1.53) and a phase shift delay of 1 month after changes in serum 25(OH)D ($p = 0.004$; Fig. 1). Seasonal periodicity in serum CTx followed 1–2 months after changes in PTH ($p = 0.023$), with the sinusoidal curve for CTx peaking in late winter and indicating a range of 374.5–463.5 pg/ml (Fig. 1). The mesor and amplitude at the peak and nadir for each sinusoidal curve are listed for serum 25(OH)D, lnPTH, and CTx in Table 1. The periodicity remained significant after adjusting serum 25(OH)D, lnPTH, and CTx for age ($p < 0.001$, $p = 0.057$, and $p = 0.044$, respectively). There was a positive correlation between serum CTx and PTH ($r = 0.15$, $p = 0.01$). No seasonal periodicity was detected for serum bALP or any significant correlation with PTH or 25(OH)D.

An annual periodicity was observed for rainfall, with the maximum recorded in late winter ($p = 0.07$), and for mean ambient lowest temperatures, with a nadir in winter, which was 1 month after lowest levels of UV radiation ($p = 0.002$).

During the 3 years, 1358 patients presented to the Emergency Department after 1501 falls that resulted in 946 fractures. There was a peak in falls frequency in winter and a nadir in summer. This seasonal periodicity did not reach statistical significance ($p = 0.365$). However, the seasonal variation in the proportion of falls resulting in fracture was significant, with the proportion occurring 3 months on either side of the peak in winter (67.5%; 95% CI, 64.2–70.8) exceeding the proportion during the 3 months on either side of the nadir in summer (58.2%; 95% CI, 54.6–61.8; Fig. 2; $p < 0.001$). The peak proportion occurred between the trough in serum 25(OH)D and the peak in serum PTH (Fig. 3).

There were 439 hip and 307 wrist fractures during the 3-year ascertainment. Monthly variations in these fractures were described by simple harmonic models ($p = 0.078$ and $p = 0.002$, respectively), with highest frequencies in late winter and lowest in late summer (Fig. 2). Peaks in fracture frequency followed 0.5–2 months after peak serum PTH and 1.5–3 months after the nadir in serum 25(OH)D (Fig. 3). In contrast, at sites generally not associated with bone fragility (face, skull, clavicle, scapula, carpal bones, hand, finger, patella, foot, and toe, collectively, $n = 222$), there were no excess fractures in winter compared with summer ($p = 0.476$).

Seasonal periodicity was observed in unadjusted BMD at the UD forearm and mid-forearm sites ($p = 0.028$ and $p = 0.078$).

### Table 1. Mesor and Amplitude at the Peak and Nadir for Each Sinusoidal Curve Representing the Seasonal Periodicity for Serum Concentrations for 25(OH)D, PTH, and CTx

<table>
<thead>
<tr>
<th></th>
<th>Mesor</th>
<th>Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/liter)</td>
<td>63.6</td>
<td>14.0</td>
</tr>
<tr>
<td>PTH (pmol/liter), log transformed</td>
<td>1.66</td>
<td>0.13</td>
</tr>
<tr>
<td>CTx (pg/ml)</td>
<td>419.0</td>
<td>44.5</td>
</tr>
</tbody>
</table>

FIG. 1. Seasonal variations in serum 25(OH)D, PTH, and CTx. The fitted sine curve is the continuous line, and 95% CIs about the regression curves are shown as discontinuous lines.
and there were trends at the whole body and total hip (\( p = 0.123 \) and \( p = 0.169 \), respectively). No association was observed at the spine. Significance was lost at all sites after adjusting for age. Troughs in BMD occurred in late winter, after 0.5–2.5 months after peak PTH and within 1 month of peak CTx. There was a negative correlation between BMD and serum PTH that achieved significance at the UD forearm and mid-forearm sites (\( r = -0.17; p = 0.002 \)) and a consistent positive correlation with 25(OH)D that failed to reach significance.

**DISCUSSION**

This prospective study was based in the Barwon Statistical Division, a mixed urban and rural community of 218,000 inhabitants with well-defined demographics, which were demonstrated to be representative of the Australian community.\(^{16,17} \) The region has only two radiological practice groups, so fracture ascertainment is virtually complete,\(^ {20} \) and most acute cases of serious falls and fractures are seen in the region’s sole university teaching hospital.

Using a cross-sectional analysis, we report annual periodicities in circulating 25(OH)D, PTH, and CTx, and we confirm previous reports of an inverse relationship between serum 25(OH)D and PTH.\(^ {24–26} \) We also confirm the well-documented reports of seasonal variation in distal forearm and proximal femur fractures.\(^ {27–33} \)

An increase in the incidence of falls during winter has been reported for elderly women in New Zealand, with the effect attributed to the cold.\(^ {34} \) Our data suggest more presentations from falls to the Emergency Department in winter when temperatures and serum 25(OH)D concentrations were relatively low and rainfall relatively high. While not capturing all the falls in the region, patients presenting to the Emergency Department represent the more serious falls. Sixty-three percent of the falls presentations resulted in fracture, which is several-fold greater than the proportion of falls resulting in fracture reported for the elderly from the general population.\(^ {35} \) These results support the hypothesis that, during winter, there is an increased frequency of falls.

In many studies, the increased incidence of fractures during winter was attributed to increased numbers of falls occurring because of the extreme conditions of ice and snow.\(^ {33,36–39} \) However, residual effects of seasonal variations in proximal femur and distal forearm fractures remain after adjusting for weather conditions.\(^ {31,40,41} \) Furthermore, seasonal changes in proximal femur fracture rates have been reported in other temperate climates including Auckland,
New Zealand (latitude 36° S), (32) the state of New South Wales in Australia (latitude 29–37° S) (30) and Hong Kong, (32,42) where, despite the subtropical climate, there are seasonal differences in serum 25(OH)D levels. (43)

Thus, seasonality of fractures is multifactorial, with poor weather conditions contributing. (30,31,40,42) The periodicity in circulating vitamin D, PTH, and falls is likely to be an important component to the seasonality in fracture risk. During winter, the relatively low serum 25(OH)D induces a higher serum PTH, which in turn increases bone remodeling rate and bone fragility. (44) Seasonal periodicity in BMD or BMC has been reported in some, (45–49) but not all, (50,51) studies. Our data support the hypothesis of reduced BMD in late winter; however, seasonal periodicity failed to reach significance.

During winter, a greater proportion of falls presenting to the Emergency Department resulted in fracture, which could be because of the presence of more fragile bone, increased severity of falls, or both of these factors. While it is well established that vitamin D deficiency decreases proximal muscle strength and increases the risk of falls; (3–5) we are not aware of any data linking this with increased severity of falls. Nevertheless, it is reasonable to assume that the impact velocity will be increased or the characteristics of falls altered in the presence of proximal muscle weakness. Although the Barwon Statistical Division does not experience ice or snow, falls severity could have been increased by rain or wind. However, only 28% of falls-related hip fracture occur outdoors in this region. (19)

There is evidence that hyperparathyroidism secondary to vitamin D deficiency/insufficiency is associated with increased bone fragility. A correlation between serum PTH and higher activation frequency, bone resorption, and formation has previously been reported. (52) Increased bone remodeling is associated with higher fracture risk. (53,54) and there is evidence for seasonal variation in biochemical measures of bone remodeling in some, (53,55–57) but not all, (51,58) studies. We have demonstrated a significant periodicity in a serum marker of bone resorption but not bone formation. This, in conjunction with the correlation of the bone resorption marker with serum PTH, supports the hypothesis that increased rates of bone remodeling are associated with higher serum PTH. In histological specimens from proximal femur fracture patients, changes in the severity of osteomalacia have been shown to parallel seasonal changes in fracture rates. (59) Therefore, it is probable that low wintertime vitamin D concentrations reduce bone strength through increased bone turnover and impaired mineralization, in addition to increasing the risk of falling. All these factors make it likely that vitamin D deficiency is responsible for a proportion of the fractures in the community. Supplementation with vitamin D may therefore prevent fractures, not only in the institutionalized elderly, but also in the ambulant healthy population. Vitamin D supplementation has been reported to prevent low vitamin D levels developing during winter, (48) and in randomized controlled trials, vitamin D plus calcium reduced the risk of nonvertebral fractures in elderly people living in institutions or at home. (1,2,60) There is evidence that vitamin D alone reduces fracture rates of the upper limb, (61) and at any site, (62) but this is not supported in all studies. (63)

We have previously reported that vitamin D insufficiency is common in this temperate climate population, with 42% of women having serum 25(OH)D less than 50 nM and 10% of women having serum 25(OH)D less than 28 nM; the proportion increases to 51% and 14%, respectively, during winter. (15) Dietary vitamin D intakes are low, and the only food generally fortified with vitamin D is margarine. The major source of vitamin D is casual exposure to sunlight, and dietary sources are significant during the winter months only. Vitamin D supplements are regularly used by only 9% of the women, presumably because of the general misconception that, in our sunny climate, sunlight exposure is sufficient to maintain serum levels. Systematic and population-based studies of altering vitamin D intake in the community by supplementation are feasible. (62) Our data suggest that if the lower fracture rate observed during summer was maintained throughout the year, the number of hip and wrist fractures in women could be reduced by 16% and 30%, respectively, with substantial implications for public health programs.

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