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Clinical Research Article

Longitudinal Stability of Vitamin D Status and Its Association With Bone Mineral Density in Middle-aged Australians

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Abstract

Context: The skeletal effects of vitamin D remain controversial and it is uncertain whether variation in serum 25-hydroxyvitamin D (25OHD) levels over time influences bone mineral density (BMD).

Objective: We evaluated longitudinal stability of serum 25OHD and associations with changes in BMD in participants aged 46-70 years at baseline.

Methods: We studied 3698 Busselton Healthy Ageing Study participants (2040 female) with serum 25OHD and dual-energy x-ray absorptiometry (DXA) BMD assessments at baseline and at ~6 years follow-up. Restricted cubic splines were used to evaluate associations between changes in 25OHD and BMD.

Results: Mean season-corrected serum 25OHD was 81.3 ± 22.7 and 78.8 ± 23.1 nmol/L at baseline and 6 years, respectively, and showed moderate correlation (intraclass correlation coefficient: 0.724). Significant predictors of change in 25OHD concentration (Δ25OHD) included baseline 25OHD, change in body mass index and vitamin D supplementation at follow-up. Greater decline in serum 25OHD over time was associated with significantly greater reduction in BMD at total hip and femoral neck, but the magnitude of the differences was small (estimated differences 0.004 g/cm^2 and 0.005-0.007 g/cm^2, respectively, for lowest quartile of Δ25OHD compared with higher quartiles, adjusted for sex, baseline BMD, 25OHD, and demographics). No significant associations between Δ25OHD and lumbar spine BMD were observed. Increase in 25OHD levels was not associated with change in BMD.

Conclusions: In this predominantly vitamin D–replete middle-aged cohort, serum 25OHD showed moderate longitudinal stability. Declining serum 25OHD over time was associated with greater reduction in BMD at the total hip and femoral neck.

Key Words: 25-hydroxyvitamin D, longitudinal stability, bone mineral density, middle-aged adults, Busselton Healthy Ageing Study

Abbreviations: 25OHD, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; IPAQ, International Physical Activity Questionnaire; SEM, standard error of the mean.

Despite extensive research, the skeletal effects of insufficient vitamin D status in adults are still controversial. Observational studies, based on a single 25-hydroxyvitamin D (25OHD) measurement at baseline, have shown that circulating 25OHD levels are positively associated with bone mineral density (BMD) in middle to older aged adults, up to a threshold level (which in different studies varies widely from 50 nmol/L [1-3] to 90-100 nmol/L [4, 5]), above which the relationship is attenuated or plateaus. By contrast, in Mendelian randomization studies (designed to minimize bias from confounding), genetically higher 25OHD status has not been positively associated with BMD [6, 7]. However, the Mendelian randomization studies have important limitations: only linear relationships between predictor and outcome variables were evaluated to date, and the single nucleotide polymorphisms (SNPs) included explain only a small percentage of the variance in 25OHD levels (2%-10%) [8]. Randomized controlled trials of vitamin D supplementation, recruiting mostly vitamin D–replete participants, have shown minimal effect on bone mass [9]. The recent VITAL study of 2000 IU/day supplementation showed no significant effect overall on changes in dual-energy x-ray absorptiometry (DXA)-assessed BMD after 2 years in a subcohort of 771 participants (men ≥ 50 and women ≥ 55 years of age), although

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improved spine BMD (0.75%) and reduced bone loss (0.56%) at total hip were observed in a subgroup with low free 25OHD concentration at baseline (<14.2 pmol/L) [10]. Furthermore, no significant reduction in fracture risk was observed over 5.3 years follow-up in the whole cohort of 25 871 participants [11]. The ViDA trial of 100 000 IU/month supplementation in 452 older adults showed ~0.5% reduction in bone loss at total hip and femoral neck after 2 years, and in a subgroup of participants with baseline serum 25OHD ≤ 30 nmol/L (n = 46), greater reduction in bone loss of 2% was observed at the spine and femoral neck [12].

Vitamin D status in individuals is not necessarily stable over time. Only a few studies have evaluated repeated measures of 25OHD in individuals at intervals ranging from 1 to 14 years. The results show moderate stability, with higher correlation coefficients for short intervals of 1 to 3 years (0.66-0.75) [13, 14] than for longer intervals such as 5 years (0.61) or 14 years (0.39-0.52) [15, 16]. There have been no previous studies examining changes in vitamin D status and changes in BMD in large cohorts, and the influence of variation in serum 25OHD levels over time on age-related bone loss is uncertain. Therefore, studies with more than one measure of 25OHD will advance understanding of the relationship between vitamin D and skeletal health, and also aid interpretation of previous studies in which 25OHD was measured only at baseline.

In the Busselton Healthy Ageing Study, a cohort study of a representative middle-aged population in Western Australia, serum 25OHD and DXA BMD were assessed in participants at baseline and after ~6 years. The aims of this study were to evaluate: (1) stability of vitamin D status over a 6-year period; (2) predictors of change in serum 25OHD; and (3) associations between changes in 25OHD and changes in BMD.

Methods

Participants

The Busselton Healthy Ageing Study is a prospective study of noninstitutionalized "baby boomers" (born from 1946 to 1964) living in the Shire of Busselton, a coastal town in the southwest of Western Australia (latitude −33.6°) with a predominantly White population. The rationale and design of the study have been detailed previously [17]. All eligible residents listed on the electoral roll, for which registration is compulsory in Australia, have been invited to participate. Phase 1 of the study (baseline survey) was conducted between May 2010 and December 2015, with 5107 participants recruited (comprising ~80% of those eligible). The follow-up (at 6 years) survey was conducted between March 2016 and January 2022 with 3888 of the original cohort (76%) attending. After excluding participants with missing data and outliers (17 without baseline 25OHD, 8 without baseline BMD, 149 without 25OHD at 6 years, 7 without BMD at 6 years, and 9 with baseline serum 25OHD > 200 nmol/L), 3698 participants (2040 females) with DXA and 25OHD measures at both time points were included in this analysis. The study received ethics approval from the University of Western Australia Human Research Ethics Committee (Number RA/4/1/2203) and written informed consent was obtained from each participant.

DXA Scans

At both time points, BMD (g/cm^2) of anterior-posterior lumbar spine (L1-L4), femoral neck and total hip were measured by DXA scanning using a GE Lunar Prodigy Pro densitometer (Madison, WI, USA). Scans were analyzed using enCORE Version 16 software (GE Health), with the "copy" feature used to analyze follow-up scans; manual inspection of regions of interest and adjustment where necessary were made by 2 independent reviewers (K.Z. and M.H.) [18]. The DXA machine had annual servicing and calibration according to manufacturer's specifications. Calibration using a phantom was performed prior to each scanning session and the quality assurance plot showed no obvious shift in the phantom BMD values over the study period (coefficient of variation = 0.30%). The precision error was < 2.0% for each measured site at standard speed based on repeated scans in a random sample of 30 subjects.

Serum 25OHD Assessment

Fasting blood samples were collected at baseline and 6 years, and serum 25OHD was measured using the ARCHITECT 25-OH Vitamin D immunoassay (Abbott, Cat# 5P02, RRID: AB_2924942; Abbott Laboratories, Abbott Park, Illinois, USA). The inter-assay coefficient of variation was 4.0% at 57.5 nmol/L and 2.6% at 178.3 nmol/L. A total of 117 samples from baseline (randomly selected within 3 strata of 25OHD) were also assayed using isotope-dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS) according to published methodology [19]. Both assays are accredited by the National Association of Testing Authorities, Australia (NATA), use calibrators aligned to reference material NIST 972 and are included in the Vitamin D External Quality Assessment Scheme (DEQAS). There was a strong correlation between the 2 techniques ($r^2 = 0.88$) [18], with a tendency for the immunoassay to overestimate 25OHD at higher concentrations (for immunoassay 25OHD ≤110 nmol/L (N = 3267), difference in mean value 3.4 (95% CI 0.9, 6.0) nmol/L; for immunoassay 25OHD >110 nmol/L (N = 431), difference in mean value 30.8 (95% CI 23.3, 38.3) nmol/L). For each time point, we calculated season-corrected serum 25OHD values using a sinusoidal model fitted to serum 25OHD level, with week of attendance as the predictor variable. Residual values were added to the mean serum 25OHD level to obtain predicted mean annual values for each participant [20, 21].

Other Assessments

At baseline and 6 years, standing height and body weight were measured using standard anthropometric techniques with the participants lightly clothed and shoeless. Body mass index (BMI) was calculated as weight (kg)/height (m)^2. Data on health history, medication use, alcohol consumption and smoking habit (current, never or previous smokers) were collected using a questionnaire [17]. Physical activity level was assessed using the International Physical Activity Questionnaire (IPAQ), and categorized as low, medium and high according to the IPAQ scoring protocol [22].

Data Analysis

Variables are presented as means ± SD for summary statistics, means (95% CI) or means ± standard error of mean (SEM) for estimated (adjusted) values unless otherwise stated. Comparisons of characteristics between males and females were made by Student *t* test and chi-squared test. Stability of both raw values of 25OHD and season-corrected 25OHD

Table 1. Characteristics of participants

	All (n = 3698)	Male (n = 1658)	Female (n = 2040)	*P* value[a]
White, %	99.1	99.3	98.9	0.261
Measurement interval, years	6.2 ± 0.9	6.2 ± 0.9	6.2 ± 0.9	0.977
Baseline characteristics				
Age, years	57.9 ± 5.7	58.1 ± 5.8	57.8 ± 5.7	0.122
Measured serum 25OHD, nmol/L	81.6 ± 24.4	85.1 ± 24.4	78.7 ± 23.9	<0.001
Season-corrected serum 25OHD, nmol/L	81.4 ± 23.1	85.1 ± 22.8	78.4 ± 23.0	<0.001
Body mass index, kg/m^2	28.0 ± 4.8	28.4 ± 4.1	27.7 ± 5.4	<0.001
Physical activity, % Low				
Low	22.7	19.7	25.0	<0.001
Medium	31.9	26.4	36.5	
High	45.4	53.9	38.5	
Smoking current, %	7.8	8.4	7.3	0.180
Alcohol intake, %				
0-7 glasses/week	50.1	33.5	63.5	<0.001
7-14 glasses/week	22.3	20.4	23.9	
>14 glasses/week	27.6	46.1	12.6	
Osteoporosis medication, %	0.5	0.1	0.9	<0.001
Avoidance of dairy products, %	1.6	1.1	2.1	0.030
Vitamin D supplement, %				
Baseline	11.7	5.6	16.6	<0.001
6 years	12.8	5.0	19.2	<0.001
Change over 6 years				
ΔMeasured serum 25OHD, nmol/L	−3.0 ± 24.2	−6.3 ± 22.5	−0.4 ± 25.2	<0.001
ΔSeason-corrected serum 25OHD, nmol/L	−2.9 ± 21.7	−6.5 ± 18.8	−0.1 ± 23.3	<0.001
ΔBody mass index, kg/m^2	0.3 ± 2.0	0.2 ± 1.7	0.4 ± 2.2	0.034

Values are mean ± SD unless otherwise stated. Abbreviation: 25OHD, 25-hydroxyvitamin D.
[a]*P* values obtained using Student *t* test or chi-square test for comparisons between males and females.

were assessed using Pearson correlation coefficient [23] and intraclass correlation coefficient. Linear regression analysis was used to evaluate predictors of change in season-corrected serum 25OHD over 6 years, with sex, baseline season-corrected 25OHD, age, BMI, and lifestyle factors, change in BMI from baseline to follow-up, and vitamin D supplementation at baseline and 6 years as independent variables; the semi-partial R^2 for each predictor variable was calculated to estimate the proportion of the variance associated uniquely with each predictor.

Restricted cubic spline modeling, which allows the assessment of whether the relationship is nonlinear, was used to evaluate relationships between baseline serum 25OHD and BMD as well as between changes in serum 25OHD (Δ25OHD, 6 years − baseline) and changes in BMD (ΔBMD, 6 years − baseline) using R package "rms" with 3 knots (10th, 50th and 90th percentile) [24]. Covariates adjusted for in the models included sex, race, and baseline age, BMI, physical activity, smoking, alcohol intake, osteoporosis medication, vitamin D supplement, and dairy avoidance; models for ΔBMD additionally adjusted for measurement interval, baseline BMD, and baseline 25OHD. For lumbar spine BMD, as there was significant interaction between 25OHD variables and sex, male and female participants were analyzed separately. The least square means of baseline BMD or ΔBMD of each site with 95% CIs at mid-quartile levels of each of baseline 25OHD or Δ25OHD were estimated, and comparisons between mid-quartile means were made.

In addition, we analyzed by categories of vitamin D status. We defined low vitamin D status as 25OHD below 50 nmol/L, based on the sufficient level recommended by the U.S. Institute of Medicine Committee (50 nmol/L) [25], high vitamin D status as reaching the sufficient level recommended by the Endocrine Society (75 nmol/L) [26], and medium level as between 50 and 74.9 nmol/L. Tracking patterns were defined as: low (25OHD <50 nmol/L at both time points, n = 99); decreasing (moved to a lower category over time, n = 796); increasing (moved to a higher category over time, n = 538); medium (25OHD 50-74.9 nmol/L at both time points, n = 750); and high vitamin D status (25OHD ≥75 nmol/L at both time points, n = 1515). Comparisons between the 5 tracking pattern groups with regard to change in BMD over the 6 years were performed using a general linear model, adjusted for sex, race, measurement interval and baseline BMD, age, BMI, physical activity, smoking, alcohol intake, osteoporosis medication, dairy avoidance, and vitamin D supplement use. Statistical significance level was set at $P < 0.05$ (two-tailed). All analyses were performed using IBM SPSS (version 27, IBM, Chicago, IL, USA) and R (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria) [27].

Table 2. Pearson's correlation coefficients and intraclass correlation coefficients for raw and season-corrected serum 25-hydroxyvitamin D values at baseline and 6 years

	Pearson's correlation coefficient		Intraclass correlation coefficient (95% CI)	
	Raw values	Season-corrected values	Raw values	Season-corrected values
All (n = 3698)	0.505	0.567	0.671 (0.649, 0.692)	0.724 (0.705, 0.741)
Sex				
Male (n = 1658)	0.552	0.648	0.710 (0.681, 0.737)	0.786 (0.765, 0.806)
Female (n = 2040)	0.477	0.520	0.645 (0.613, 0.675)	0.683 (0.655, 0.710)
BMI category				
<30 kg/m^2 (n = 2633)	0.496	0.563	0.663 (0.637, 0.688)	0.720 (0.698, 0.741)
≥30 kg/m^2 (n = 1065)	0.480	0.526	0.648 (0.603, 0.688)	0.688 (0.648, 0.723)
Age group				
<55 years (n = 1261)	0.521	0.576	0.685 (0.648, 0.718)	0.731 (0.700, 0.759)
≥55 years (n = 2437)	0.496	0.563	0.663 (0.636, 0.689)	0.720 (0.697, 0.741)
Vitamin D supplementation at either time point				
No (n = 2935)	0.566	0.644	0.723 (0.702, 0.742)	0.784 (0.767, 0.799)
Yes (n = 763)	0.350	0.385	0.517 (0.444, 0.581)	0.555 (0.487, 0.614)

Abbreviation: BMI, body mass index.

Table 3. Predictors of change in season-corrected serum 25-hydroxyvitamin D levels over 6 years

	Regression coefficients (95% CI)	*P* value	R^{2a}
Season-correct serum 25OHD at baseline, nmol/L	−0.423 (−0.449, −0.396)	<0.001	0.180
Sex—male	−2.021 (−3.352, −0.691)	0.003	0.002
Caucasian	−0.194 (−6.341, 5.953)	0.951	—
Baseline age, years	0.039 (−0.064, 0.143)	0.457	—
Baseline BMI, kg/m^2	−0.295 (−0.423, −0.167)	<0.001	0.004
Change in BMI over 6 years, kg/m^2	−1.256 (−1.558, −0.954)	<0.001	0.013
Current smoking	−1.110 (−3.315, 1.096)	0.324	—
Alcohol intake 7-14 glasses/week	0.424 (−1.081, 1.928)	0.581	—
Alcohol intake ≥14 glasses/week	1.892 (0.382, 3.402)	0.014	0.001
Moderate level of physical activity	−0.590 (−2.207, 1.027)	0.474	—
High level of physical activity	−0.058 (−1.603, 1.487)	0.941	—
Vitamin D supplement use at baseline	−0.643 (−2.541, 1.255)	0.507	—
Vitamin D supplement use at 6 years	17.276 (15.440, 19.112)	<0.001	0.065
Total variance explained			0.300

Abbreviation: 25OHD, 25-hydroxyvitamin D; BMI, body mass index.
[a]R^2 for individual predictor variable refers to semi-partial R^2, which is the proportion of the variance associated uniquely with the predictor.

Results

In total, 3698 participants (2040 female) were included in the analysis. Compared with members of the original cohort not included in this analysis (n = 1409), those included were not significantly different in baseline age (57.9 ± 5.7 vs 58.2 ± 6.0 years, $P = 0.145$), season-corrected 25OHD (81.4 ± 23.1 vs 80.6 ± 28.0 nmol/L, $P = 0.355$), or proportion female (55.2% vs 54.0%, $P = 0.458$), but had slightly lower BMI (28.0 ± 4.8 vs 28.6 ± 5.2 kg/m^2, $P < 0.001$).

Table 1 shows participant characteristics. The mean measurement interval between baseline and follow-up was 6.2 ± 0.9 years, and mean serum season-corrected 25OHD decreased in male participants from baseline to 6 years (85.1 ± 22.8 vs 78.6 ± 21.9 nmol/L, $P < 0.001$) but did not change significantly in female participants (78.4 ± 23.0 vs 78.5 ± 24.6 nmol/L, $P = 0.868$). At baseline, 16.6% women and 5.6% men were taking vitamin D supplement, and at 6 years, 19.2% and 5.0%, respectively.

Serum 25OHD at baseline and 6 years showed moderate correlation, with Pearson correlation coefficient 0.505 for the raw values and 0.567 for season-corrected 25OHD, and the corresponding intraclass correlation coefficients of 0.671 and 0.724, respectively. The correlation was stronger in male than female participants, stronger in those not taking vitamin D supplementation at either visit than those who took supplements, slightly stronger for those with BMI

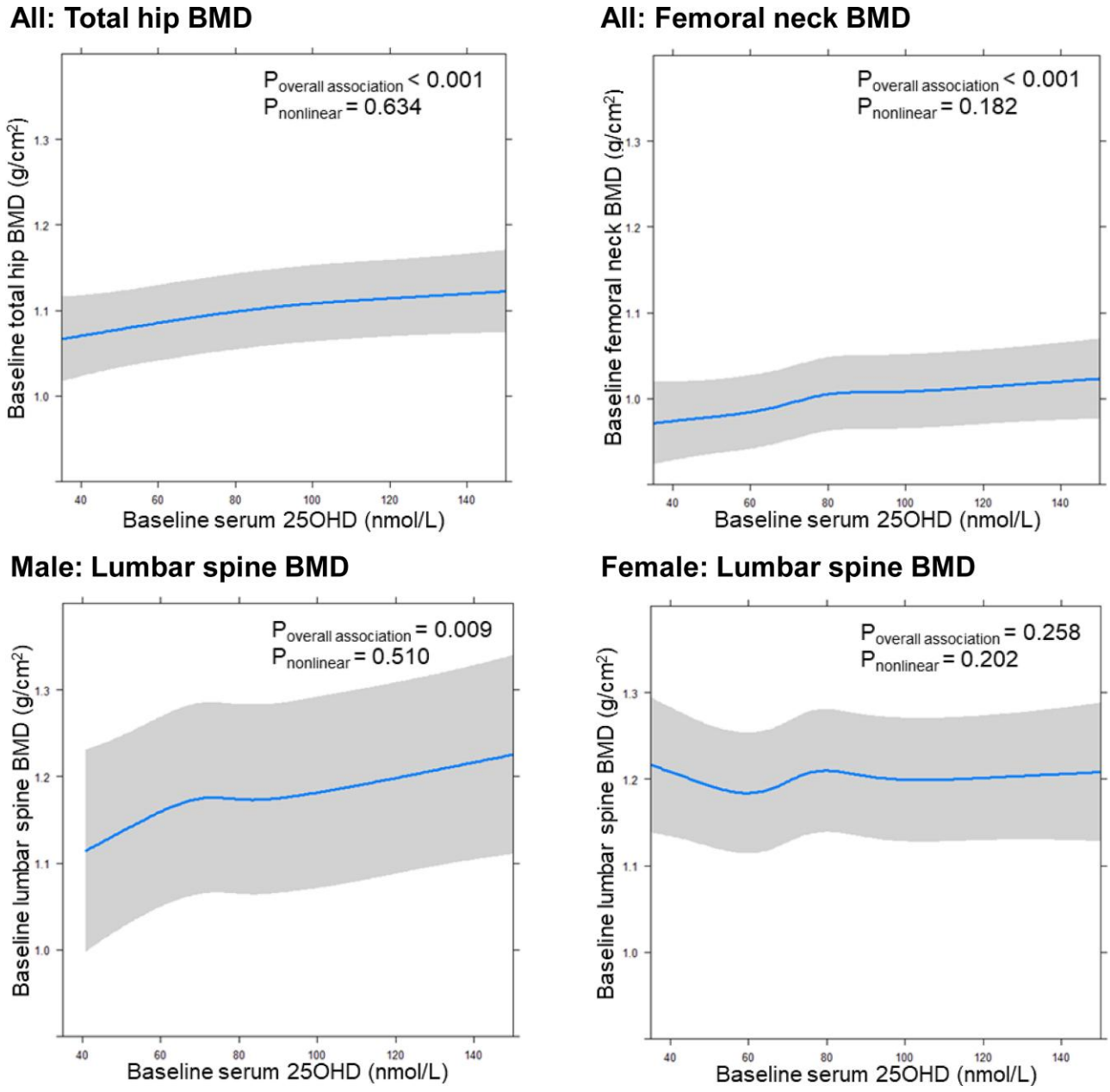

Figure 1. Associations between baseline serum 25-hydroxyvitamin D (25OHD) levels and bone mineral density (BMD) based on fitted restricted cubic spline regression with 3 knots, adjusted for sex, race, and baseline age, BMI, physical activity, smoking, alcohol intake, osteoporosis medication, vitamin D supplement, and dairy avoidance. Analysis for lumbar spine BMD was made for male and female participants separately due to significant interaction with sex. Gray shadow represents 95% CI.

< 30 kg/m^2 compared with BMI ≥ 30 kg/m^2, but was similar for those aged < 55 or ≥ 55 years (Table 2). Linear regression models showed that baseline 25OHD, male sex, baseline BMI, and change in BMI were negatively associated with change in 25OHD concentration over 6 years, whereas vitamin D supplement use at 6 years was associated with increasing 25OHD. Baseline 25OHD uniquely accounted for 18.0% of the variation, whereas vitamin D supplement use at 6 years accounted for 6.5% (Table 3). Variables examined in the model accounted for only 30% of the variation in Δ25OHD.

At baseline, restricted cubic spline analyses showed significant positive associations between serum 25OHD and BMD of total hip and femoral neck in all participants and with lumbar spine BMD in males (Fig. 1). When participants were divided into quartiles of baseline 25OHD, estimated total hip and femoral neck BMD were significantly higher in Q3 and Q4 compared with Q1 (by 0.019-0.028 g/cm^2) and with Q2 (by 0.008-0.017 g/cm^2); participants in Q2 also had significantly higher total hip and femoral neck BMD than those in Q1 (by 0.011-0.015 g/cm^2). In males, estimated lumbar spine BMD was significantly higher for the highest quartile of baseline 25OHD compared with the lowest quartile (by 0.029 g/cm^2) (Table 4).

For changes over 6 years, restricted cubic spline analyses showed that greater decline in vitamin D status over time was associated with greater bone loss at total hip and femoral neck in all participants, whereas increase in 25OHD levels did

Table 4. Estimated mean baseline bone mineral density at mid-quartile levels of baseline serum 25-hydroxyvitamin D

	Q1	Q2	Q3	Q4
All (n = 3698), mid-quartile 25OHD	56.6 nmol/L	72.1 nmol/L	86.0 nmol/L	107.6 nmol/L
Total hip BMD, g/cm^2	1.083 (1.038, 1.127)	1.094 (1.050, 1.138)[a]	1.102 (1.057, 1.146)[a,b]	1.111 (1.066, 1.155)[a,b]
Femoral neck BMD, g/cm^2	0.982 (0.939, 1.025)	0.997 (0.954, 1.039)[a]	1.007 (0.964, 1.050)[a,b]	1.010 (0.966, 1.053)[a,b]
Male (n = 1658), mid-quartile 25OHD	60.8 nmol/L	76.0 nmol/L	89.4 nmol/L	110.8 nmol/L
Lumbar spine BMD, g/cm^2	1.161 (1.051, 1.271)	1.175 (1.066, 1.285)	1.175 (1.065, 1.284)	1.190 (1.079, 1.301)[a]
Female (n = 2040), mid-quartile 25OHD	53.9 nmol/L	69.2 nmol/L	83.1 nmol/L	103.7 nmol/L
Lumbar spine BMD, g/cm^2	1.187 (1.118, 1.257)	1.196 (1.126, 1.265)	1.209 (1.138, 1.280)	1.199 (1.128, 1.271)

Values are restricted cubic spline estimated least square mean (95% CI) for the mid-quartile levels of baseline serum 25-hydroxyvitamin D (25OHD); analysis for lumbar spine BMD was made for males and females separately due to significant interaction with sex.
Abbreviations: 25OHD, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, body mass index.
[a]$P < 0.05$ vs Q1.
[b]$P < 0.05$ vs Q2, adjusted for sex, race, and baseline age, BMI, physical activity, smoking, alcohol intake, osteoporosis medication, vitamin D supplement, and dairy avoidance.

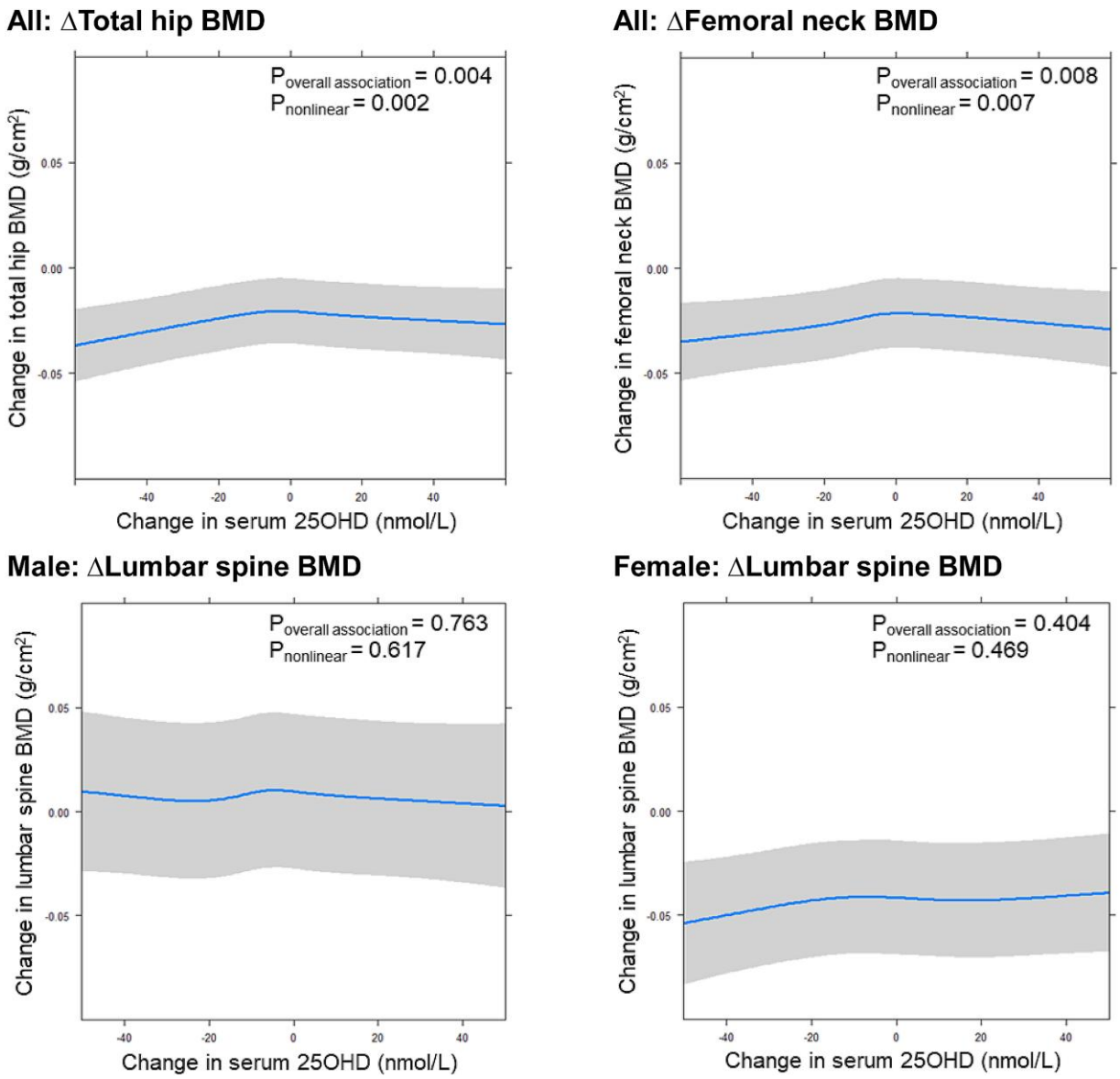

Figure 2. Associations between changes in serum 25-hydroxyvitamin D (25OHD) levels and changes in bone mineral density (BMD) over 6 years based on fitted restricted cubic spline regression with 3 knots, adjusted for sex, race, measurement interval and baseline BMD, 25OHD, age, BMI, physical activity, smoking, alcohol intake, osteoporosis medication, vitamin D supplement, and dairy avoidance. Analysis for lumbar spine BMD was made for males and females separately due to significant interaction with sex. Gray shadow represents 95% CI.

Table 5. Estimated mean changes in bone mineral density over 6 years at mid-quartile levels of changes in serum 25-hydroxyvitamin D

	Q1	Q2	Q3	Q4
All (n = 3698), mid-quartile Δ25OHD	−23.7 nmol/L	−9.2 nmol/L	1.4 nmol/L	18.6 nmol/L
ΔTotal hip BMD, g/cm^2	−0.025 (−0.041, −0.010)	−0.021 (−0.037, −0.006)[a]	−0.021 (−0.036, −0.005)[a]	−0.023 (−0.039, −0.007)
ΔFemoral neck BMD, g/cm^2	−0.028 (−0.045, −0.011)	−0.023 (−0.040, −0.007)[a]	−0.021 (−0.038, −0.005)[a]	−0.023 (−0.040, −0.006)[a]
Male (n = 1658), mid-quartile Δ25OHD	−25.4 nmol/L	−11.0 nmol/L	−1.8 nmol/L	11.6 nmol/L
ΔLumbar spine BMD, g/cm^2	0.005 (−0.032, 0.042)	0.008 (−0.029, 0.046)	0.010 (−0.027, 0.047)	0.007 (−0.030, 0.045)
Female (n = 2040), mid-quartile Δ25OHD	−22.3 nmol/L	−7.0 nmol/L	4.7 nmol/L	23.7 nmol/L
ΔLumbar spine BMD, g/cm^2	−0.044 (−0.071, −0.016)	−0.041 (−0.068, −0.014)	−0.042 (−0.069, −0.015)	−0.043 (−0.070, −0.015)

Values are restricted cubic spline estimated least square mean (95% CI) for the mid-quartile levels of change in serum 25-hydroxyvitamin D over 6 years (Δ25OHD); analysis for lumbar spine BMD was made for males and females separately due to significant interaction with sex.
Abbreviations: 25OHD, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, body mass index.
[a]$P < 0.05$ vs Q1, adjusted for sex, race, measurement interval, and baseline BMD, 25OHD, age, BMI, physical activity, smoking, alcohol intake, osteoporosis medication, vitamin D supplement, and dairy avoidance.

not appear to be associated with change in BMD (Fig. 2). When analyzed by quartiles of Δ25OHD, participants in Q1 (with the greatest reduction in serum 25OHD) had significantly greater loss of BMD at the total hip (by 0.004 g/cm^2) compared with Q2 and Q3 and femoral neck (by 0.005-0.007 g/cm^2) compared with the 3 higher quartiles (Table 5). No significant associations with Δlumbar spine were observed (Fig. 2 and Table 5).

Analyses by vitamin D status tracking pattern groups showed that participants in the decreasing vitamin D status group had significantly greater decline in femoral neck BMD compared with the increasing, medium, and consistently high vitamin D status tracking patterns (by 0.004-0.006 g/cm^2) after adjustment for covariates (sex, measurement interval, and baseline BMD, age, BMI, vitamin D supplementation and lifestyle factors) (Table 6). No significant associations with Δlumbar spine or Δtotal hip BMD were observed (Table 6).

Discussion

In this community-based, middle-aged, predominantly vitamin D–replete Australian cohort, serum 25OHD measured 6 years apart showed moderate correlation, with an intraclass correlation coefficient of 0.724. At baseline, 25OHD positively associated with BMD of total hip and femoral neck in the cohort as a whole and with lumbar spine BMD in males. Over 6 years, greater decline in serum 25OHD was associated with higher bone loss at total hip and femoral neck, whereas increasing 25OHD levels were not associated with changes in BMD. To our knowledge, this is the first published study to examine relationships between 25OHD and BMD measured on more than one occasion in a large cohort.

With regard to longitudinal stability of 25OHD status, the correlation coefficients observed in the present study of samples collected ~6 years apart (0.51 raw values, 0.57 seasonally-adjusted) are slightly lower than previously reported for samples collected 1 to 3 years apart (0.66-0.75) [13, 14] or at a 5-year interval (0.61) [15], but higher than reported for samples collected 14 years apart (0.39 for all participants, 0.52 for samples collected in the same season) [16], suggesting variability in circulating 25OHD concentrations is greater over longer intervals.

Significant predictors of change in serum 25OHD concentration over 6 years included baseline 25OHD, sex, baseline and change in BMI, and vitamin D supplement use at 6 years. With regard to BMI, there is a well-established inverse relationship between serum 25OHD and body weight and BMI, thought to be due to volume dilution and/or sequestration effect [28, 29]; in the present study, change in BMI uniquely explained more of the variation of change in 25OHD than baseline BMI. Vitamin D supplement at follow-up, but not at baseline, was a significant predictor of the change in 25OHD levels over 6 years, associated with 17 nmol/L increase in 25OHD concentration and uniquely explained 6.5% of the variation. The greater variability observed in women could reflect their greater use of vitamin D supplements than men. These results extend knowledge regarding the longitudinal stability of vitamin D status and may assist interpretation of previous studies in which 25OHD was measured only at baseline.

The positive relationship at baseline between serum 25OHD and BMD in this middle-aged cohort is consistent

Table 6. Estimated mean changes in bone mineral density over 6 years by vitamin D status tracking groups

	Low n = 99	Decreasing n = 796	Increasing n = 538	Medium n = 750	High n = 1515	*P* value
ΔTotal hip BMD, g/cm^2	−0.027 ± 0.004	−0.032 ± 0.002	−0.028 ± 0.002	−0.028 ± 0.002	−0.027 ± 0.001	0.076
ΔFemoral neck BMD, g/cm^2	−0.037 ± 0.005	−0.036 ± 0.002[a,b,c]	−0.031 ± 0.002	−0.030 ± 0.002	−0.032 ± 0.001	0.052
ΔLumbar spine BMD, g/cm^2	0.001 ± 0.006	−0.005 ± 0.002	−0.006 ± 0.003	−0.004 ± 0.002	−0.004 ± 0.002	0.883

Values are estimated mean ± SEM. Vitamin D status tracking groups defined as: low (both serum 25-hydroxyvitamin D measures <50 nmol/L), decreasing (moved to lower category), increasing (moved to higher category), medium (both measures 50-74.9 nmol/L) or high (both measures ≥75 nmol/L).
Abbreviation: BMD, bone mineral density.
[a] $P < 0.05$ vs Increasing.
[b] $P < 0.05$ vs Medium.
[c] $P < 0.05$ vs High, general linear model adjusted for sex, race, measurement interval, and baseline BMD, age, BMI, physical activity, smoking, alcohol intake, osteoporosis medication, vitamin D supplement, and dairy avoidance.

with positive associations reported in cross-sectional studies of other age groups and populations [1-5]. In the longitudinal analysis, greater decline in 25OHD levels over a 6-year period was associated with greater reductions in BMD at total hip and femoral neck but not lumbar spine. This could reflect a causal relationship in which low 25OHD results in increased bone turnover and reduced mineralization [30, 31] (which is reported to occur preferentially in cortical bone over trabecular bone [32]), or it could reflect other factors, such as poorer general health, affecting both 25OHD and BMD through other mechanisms. The magnitude of the observed BMD differences between quartiles of Δ25OHD was relatively small, and in line with the modest effects observed in vitamin D supplementation studies [9, 12]. Increasing 25OHD levels were not associated with changes in BMD, probably reflecting the vitamin D–replete status of the majority of participants. Overall, the data are consistent with the view that in vitamin D–replete individuals, variation in vitamin D status has a relatively minor influence on BMD.

Strengths of our study include the large sample size, community-based design, and the measurements of serum 25OHD and BMD at 2 time points to allow longitudinal analysis of vitamin D status and associations with bone loss in this representative population. The study also has limitations. First, it is observational in nature and although important confounding variables, including lifestyle factors, were accounted for in the restricted cubic spline analyses, it remains possible that the greater bone loss observed with greater decline in 25OHD was due to uncontrolled or residual confounding, as those with higher vitamin D status are more likely to be physically active and have healthier lifestyles [33], whereas inactivity is associated to both reduced vitamin D status and greater bone loss. Second, most participants were White, therefore generalizing the study findings to other ethnic groups should be exercised with caution. Third, the small number of participants with serum 25OHD below 50 nmol/L at either visit limited the statistical power of the analysis by vitamin D status tracking pattern groups and made it impractical to study the impact of overt vitamin D deficiency.

In conclusion, in this middle-aged, community-based cohort, serum 25OHD measured 6 years apart showed moderate longitudinal stability. Participants with greater decrease in circulating 25OHD levels had significantly greater decline in BMD than those with stable or increasing levels, but the magnitude of the BMD changes was small. We conclude that the impact of variation in vitamin D status on BMD in this predominantly vitamin D–replete population is relatively minor.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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