Vitamin D Status and 5-Year Changes in Periodontal Disease Measures Among Postmenopausal Women: The Buffalo OsteoPerio Study

Amy E. Millen,* Christopher A. Andrews,† Michael J. LaMonte,* Kathleen M. Hovey,* Mya Swanson,* Robert J. Genco,‡ and Jean Wactawski-Wende*

Background: Vitamin D is hypothesized to prevent periodontal disease progression through its immune-modulating properties and its role in maintaining systemic calcium concentrations. The authors investigated associations between plasma 25-hydroxyvitamin D [25(OH)D] (collected 1997 to 2000) and the 5-year change in periodontal disease measures from baseline (1997 to 2000) to follow-up (2002 to 2005) among 655 postmenopausal women in a Women's Health Initiative Observational Study ancillary study. Exploratory analyses were conducted in 628 women who also had 25(OH)D measures at follow-up.

Methods: Four continuous measures of the 5-year change in periodontal disease were assessed using alveolar crest height (ACH), clinical attachment level (CAL), probing depth (PD), and percentage of gingival sites that bled on assessment. Linear regression was used to estimate β-coefficients, standard errors, and *P* values corresponding to change in periodontal disease (a 1-mm change in ACH, CAL, or PD or a 1-unit change in the percentage of gingival sites that bled) for a 10-nmol/L difference in 25(OH)D. Models were adjusted for age, education, dental visit frequency, smoking, diabetes status, current medications affecting bone health, baseline measures of periodontal disease, body mass index, and recreational physical activity.

Results: No statistically significant associations were observed between baseline 25(OH)D and change in periodontal disease measures, overall or in a subset (n = 442) of women with stable 25(OH)D concentrations [25(OH)D change <20 nmol/L from baseline to follow-up]. Results also did not vary significantly in analyses that were stratified by baseline periodontal disease status.

Conclusions: No association between baseline 25(OH)D and the subsequent 5-year change in periodontal disease measures was observed. Vitamin D status may not influence periodontal disease progression. More studies are needed to confirm these results. *J Periodontol 2014;85:1321-1332.*

KEY WORDS

Alveolar bone loss; epidemiology; periodontal diseases; postmenopause; vitamin D; women.

* Department of Epidemiology and Environmental Health, School of Public Health and Health Professions, University at Buffalo, The State University of New York, Buffalo, NY.
† Department of Ophthalmology and Visual Sciences, University of Michigan Medical School, Ann Arbor, MI.
‡ Department of Oral Biology, School of Dental Medicine, University at Buffalo.

doi: 10.1902/jop.2014.130686

Periodontal disease is a common chronic, inflammatory disease of aging which, if not controlled, can lead to tooth loss. It is estimated that 8.7%, 30.0%, and 8.5% of the United States population aged >30 years[1] have mild, moderate, and severe disease, respectively, based on a full-mouth periodontal examination and the current Centers for Disease Control and Prevention and the American Academy of Periodontology (CDC/AAP) definition.[2] Among those aged 50 to 64 and ≥65 years, prevalence of any periodontal disease is estimated to be even higher, approximately 57% and 70% of the population, respectively.[1] Modifiable factors that reduce development and progression of periodontal disease are of interest to the general public and to dental professionals who want to reduce the burden of tooth loss.

Vitamin D status has been hypothesized to prevent and reduce the progression of periodontal disease.[3] In the last decade, research has focused on vitamin D as a potential anti-inflammatory[4] and antimicrobial agent.[5] Vitamin D is also essential in maintaining bone health and mineralization,[6] presumably inclusive of alveolar bone. In a cross-sectional analysis using data from postmenopausal women enrolled in the Buffalo Osteoporosis and Periodontal Disease (OsteoPerio) Study, an ancillary study of the Women's Health Initiative Observational Study, the present authors previously showed that vitamin D status, assessed with plasma concentrations of 25-hydroxyvitamin D [25(OH)D], was associated with clinical measures of oral health.[7] Women with 25 (OH)D concentrations ≥50 nmol/L compared to <50 nmol/L had reduced odds of gingival bleeding (a measure of gingival inflammation) and reduced odds of moderate-to-severe periodontitis, assessed using the CDC/AAP definition. However, vitamin D status was not significantly associated with radiographic measures of alveolar crest height (ACH), which tend to reflect the chronic phase of destructive periodontitis.

Most,[7-12] although not all,[13] previous cross-sectional and case-control studies have supported vitamin D status as a potential modifiable risk factor for periodontal disease. Few studies[14-18] have examined associations between vitamin D status and periodontal disease measures taken over time. Garcia et al.[14] conducted a 1-year study of 51 patients with moderate-to-severe chronic periodontal disease attending a periodontal disease maintenance program. Patients who reported baseline use of calcium and vitamin D supplements compared with non-users had less periodontal disease (considering collectively a number of clinical measures) at baseline, 6 months, and 12 months, although results were not statistically significant at 12 months. In a larger epidemiologic study of 550 men, Krall[15] found no association between self-reported baseline intake of vitamin D from foods and supplements and 7-year progression in alveolar bone loss. In another study of 562 men, Alshouibi et al.[17] used a repeated-measures cross-sectional design to examine associations between vitamin D intake and periodontal disease collected one to four times from 1986 to 1998. Vitamin D intake was associated with lower odds of moderate-to-severe periodontal disease. However, these studies did not consider sunlight exposure as a source of vitamin D when assessing vitamin D status. Vitamin D can be synthesized in the skin on exposure to ultraviolet B radiation.[19] A recently published study by Jimenez et al.[18] showed that higher predicted 25(OH)D concentrations were associated with a lower incidence of self-reported tooth loss in a 20-year prospective cohort of men. That study was limited by its use of a predictor score, instead of direct measures of the biomarker 25(OH)D, to assess vitamin D status. Jimenez et al.[18] also relied on self-reported incident periodontal disease outcomes instead of clinical measures of disease progression. One randomized clinical trial of 145 men and women, nested within a larger clinical trial of bone loss of the hip, found that supplementation of vitamin D (700 IU/day) and calcium (500 mg/day) was associated with decreased odds of tooth loss over 3 years (27% of the placebo group versus 13% of the supplemented group lost ≥1 tooth).[16] However, that trial examined supplementation with both calcium and vitamin D together, limiting the ability to differentiate if both or just one nutrient was influential.

The purpose of this paper is to build on the currently published cross-sectional analyses in the OsteoPerio Study.[7] The authors previously observed that adequate compared to inadequate or deficient vitamin D status was associated with reduced odds of periodontal disease;[7] however, temporality could not be determined from that cross-sectional study. The authors had the ability to examine prospective associations between baseline plasma 25(OH)D and the 5-year change in periodontal disease measures inclusive of clinical attachment level (CAL), probing depth (PD), ACH, and percentage of gingival sites that bled on assessment in the OsteoPerio Study, a well-defined cohort of postmenopausal women. The biomarker 25(OH)D reflects contribution from all three sources of vitamin D: diet, supplements, and sunlight. The authors hypothesized that baseline plasma 25 (OH)D concentrations would be inversely associated with changes in periodontal disease measures, reflecting progression of disease during 5 years of follow-up.

MATERIALS AND METHODS

Study Sample

As previously described,[7] 1,362 women (aged 50 to 79 years) participated in the baseline OsteoPerio Study conducted from 1997 to 2000. Of them, five women were missing data on the study baseline questionnaires. An additional 39 women were missing data on ≥1 baseline periodontal disease measure (ACH [n = 16], CAL [n = 20], PD [n = 19], gingival bleeding measures [n = 13]). Of the remaining 1,318 women, baseline plasma sample collection was implemented after the study began, and samples were available for 25(OH)D assays in 921 women. One additional woman was excluded because her baseline 25(OH)D was determined to be an extreme value (530 nmol/L). Of the remaining 920 women, 675 (73%) attended the follow-up exam (2002 to 2005) and had the follow-up periodontal disease measures needed to compute change in disease with time. Additionally, participants in this longitudinal sample were excluded if they were missing data on pertinent risk factors measured at baseline (education [n = 10] and physical activity [n = 10]). This left a final analytic sample of n = 655. All participants signed informed consent. The study protocol was approved by the University at Buffalo's Health Sciences Institutional Review Board, and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Study Visit

These 655 study participants attended a clinic visit at baseline (1997 to 2000) and follow-up 5 years later (2002 to 2005). Before the study visits, participants completed questionnaires to assess their demographic information, family and medical history, lifestyle habits including physical activity, osteoporosis risk factors, oral health history, and current medication and supplement use. At the clinic visit, questionnaires were reviewed for completeness, physical measurements were taken to assess weight and height, and participants underwent dual-energy x-ray absorptiometry[§] to determine systemic bone mineral density (BMD). BMD was used to define worst-site T score using all skeletal sites measured[20,21] for assessment of osteoporosis status: normal, T ≥–1.0; osteopenia, –2.5 > T <–1.0; osteoporosis, T ≤–2.5).

Oral Health Exam Outcome Measures at Baseline and Follow-Up

Full-mouth oral clinical examinations were conducted by trained and calibrated dental examiners using standardized protocols, the details of which were described previously.[7,20,21] Measures of ACH, PD, CAL, and gingival bleeding were assessed at baseline and follow-up and are described in detail elsewhere.[7,20-22] ACH, in millimeters, was assessed using standardized intraoral radiographs[‖] and measured mesially and distally for each tooth present (excluding third molars and canines) for a maximum of 48 sites. The average ACH of all sites measured is referred to as the whole-mouth mean ACH. Using whole-mouth mean ACH levels and self-reported tooth loss due to periodontitis, participants were grouped into three categories of periodontal disease: none, mild/moderate, or severe.[20] A constant-force electronic periodontal probing system[¶] was used to measure PD, in millimeters, on six surfaces of each tooth, and the corresponding CAL, in millimeters, was determined for each surface site assessed with the use of a manual periodontal probe.[#] Measures of whole-mouth mean PD and CAL level were computed. Women were also categorized as having none/mild, moderate, or severe periodontal disease using the CDC/AAP Working Group definition.[23] Gingival bleeding was assessed by inserting the same manual type periodontal probe approximately 2 mm into the gingival sulcus/pocket at three gingival sites per tooth, except third molars. Each site was scored either 0 (absence) or 1 (presence) for bleeding. The mean of all gingival bleeding scores was computed, representing the proportion of all sites assessed that bled in the mouth (when multiplied by 100, this represents the percentage of bleeding sites in a mouth).

Measure of 5-Year Change in Periodontal Disease

Using the periodontal disease measures taken at baseline and follow-up, the authors defined continuous measures of 5-year change in periodontal disease (follow-up value – baseline value), with positive values representing loss in alveolar bone and clinical attachment, worsening of PD with time, or a greater percentage of gingival sites that bled with time (Table 1). In every case, change was computed from all available 5-year measurements and their corresponding measurements at baseline. For ACH, the change at each site was determined using overlaid oral radiographs taken at the two time periods. Change measures from all sites were averaged, and this is referred to as the ACH side-by-side mean change. This method was developed by Hausmann et al.[24] and described as used in this study by LaMonte et al.[21] The whole-mouth mean change for CAL and PD and the change in the percentage of all gingival sites in the mouth that bled on assessment were determined by subtracting the whole-mouth mean CAL/PD or the percentage of bleeding sites at baseline from the follow-up values.

§ QDR-4500A, Hologic, Bedford, MA.
‖ Bennett HFQ 300, Bennett X-Ray, Copiague, NY.
¶ The Florida Probe System, Florida Probe, Gainesville, FL.
# Michigan O periodontal probe, Hu-Friedy, Chicago, IL.

Table 1.

Change in Periodontal Disease Measures From Baseline (1997 to 2000) to Follow-Up (2002 to 2005) Among 655 Participants in the Buffalo OsteoPerio Study Who Had Baseline Plasma 25(OH)D Concentrations and Attended the Follow-Up Exam

Periodontal Disease Change Measure	Mean	SD	Median	Minimum	Max
ACH side-by-side mean change (mm)	0.18	0.22	0.16	–0.45	2.90
CAL whole-mouth mean change (mm)	–0.17	0.50	–0.19	–1.59	3.23
PD whole-mouth mean change (mm)	–0.15	0.39	–0.15	–1.88	2.14
Change in the percentage of gingival sites that bled on assessment	–21.8	24.0	–19.6	–89.7	77.3

Change measures were calculated by subtracting baseline periodontal disease measures from follow-up periodontal disease measures, with positive values indicating a greater loss in alveolar bone, clinical attachment, or worsening of PD with time or a greater percentage of gingival sites that bled with time.

Assessment of Plasma 25-(OH)D

Blood draws were conducted on the same day as the clinical periodontal examinations. Assessment of plasma 25(OH)D at baseline and follow-up has been previously reported.[25] Assays were conducted during a consistent 4-month period using a competitive chemiluminescence immunoassay.** Using the investigators' masked, duplicate quality control samples nested in each batch, the within-pair coefficient of variation was 4.9%. Only a subset of the sample (n = 628) had 25(OH)D measures at follow-up. For both baseline and follow-up 25(OH)D measures, values were adjusted for season of blood draw using the residual method. Residuals were computed from regression of 25(OH)D on the day of the year of blood draw and added back to the sample mean as previously described.[7]

Statistical Analyses

Data were analyzed with statistical software.†† To better understand how loss to follow-up may have influenced the results, the authors compared characteristics of study participants included in the current prospective analysis (n = 655) to study participants who had baseline plasma 25(OH)D concentrations but were not included in the current analyses because they did not attend follow-up, did not have periodontal disease measures needed to compute change measures, or were missing data on pertinent covariates (n = 265). Next, in the current analytic sample of 655 women, summary measures of periodontal change variables were described (Table 1) and mean baseline plasma 25(OH)D was described according to participant characteristics and periodontal disease risk factors (Table 2). Differences in means were examined using analysis of variance, and differences among categorical variables were examined using χ^2 tests. Tests were considered statistically significant with P values of <0.05 (two-sided).

Scatter plots of periodontal disease change measures on baseline 25(OH)D concentrations were examined, and assumptions for statistical methods were checked and met. In the full analytic sample (n = 655), linear regression was used to regress periodontal disease change measures on baseline 25(OH)D (Table 3). Regression coefficients, standard errors (SEs), and affiliated P values for the baseline 25(OH)D per each 10-nmol/L increment are presented. Regression coefficients represent a 1-mm change in ACH, CAL, or PD or a 1-unit change in the percent of gingival sites that bled for a 10-nmol/L difference in 25(OH)D. The authors examined whether adjustment for common periodontal disease risk factors, as noted in the literature (Table 2), confounded the regression models. A forward selection process was used where confounder selection was based on confounders that changed the β-coefficient ≥10%. Multivariable regression models were adjusted for the following covariates assessed at baseline: age, education, frequency of dental visits, smoking status, self-reported history of diabetes, and current use of osteoporosis-related medications or bone therapies (e.g., current use of hormone therapy drugs, bone drugs [miacalcin, alendronate], or selective estrogen receptor modulator drugs [raloxifene]). Models examining ACH side-by-side change were also adjusted for baseline whole-mouth mean ACH. Models examining CAL or PD whole-mouth mean change were also adjusted for baseline whole-mouth mean CAL or PD, respectively; models examining change in the percentage of all gingival sites in the mouth that bled were also adjusted for the baseline measure of percentage of gingival sites that bled. Adjustment for baseline measures of periodontal disease helps to differentiate between cross-sectional and longitudinal relationships. Tests were

** LIAISON 25-OH Vitamin D TOTAL Assay, DiaSorin, Stillwater, MN.
†† SAS for Windows, v.9.3, SAS Institute, Cary, NC.

Table 2.

Mean and SD of Baseline Plasma 25(OH)D Concentrations by Baseline Characteristics: The Buffalo OsteoPerio Study (n = 655)

Baseline Characteristic	Baseline Plasma 25(OH)D		
	n	Mean (SD)	*P**
Overall sample	655	60.64 (21.9)	
Categories of vitamin D status in 25(OH)D (nmol/L)			<0.0001
Deficient (<30)	47	22.94 (1.3)	
Inadequate (≥30 to <50)	159	41.36 (0.7)	
Adequate (≥50 to <75)	300	61.74 (0.5)	
Adequate (≥75)	149	90.88 (0.7)	
ACH definition of periodontal disease[20]			0.367
None	175	61.93 (22.4)	
Mild/moderate	335	59.45 (20.7)	
Severe	145	61.82 (24.1)	
CDC/AAP definition of periodontal disease[23]			0.297
None/mild	145	62.98 (23.6)	
Moderate	404	59.70 (21.3)	
Severe	106	61.01 (21.9)	
Percentage of gingival sites that bled at baseline (%)			0.004
Tertile 1 (0 to 22.2)	223	61.51 (22.3)	
Tertile 2 (22.6 to 45.0)	214	63.64 (22.0)	
Tertile 3 (45.1 to 100)	218	56.80 (20.9)	
Number of teeth at baseline			0.038
6 to 14	51	53.46 (24.8)	
15 to 24	227	62.13 (22.7)	
25 to 28	377	60.71 (20.9)	
Age (years)			0.018
<60	143	64.30 (22.3)	
60 to <70	322	60.88 (22.3)	
≥70	190	57.47 (20.6)	
Race			0.806
White	646	60.66 (21.8)	
Other	9	58.85 (28.5)	
Education			0.310
High school diploma or equivalent or less	134	58.92 (22.8)	
School after high school	521	61.08 (21.7)	
Cigarette smoking status			0.598
Never	364	60.25 (22.2)	
Former	276	61.38 (21.1)	
Current	15	56.25 (30.0)	
Waist circumference (cm)†			<0.0001
Tertile 1 (61 to 78)	223	68.47 (22.3)	
Tertile 2 (79 to 87)	214	60.33 (20.4)	
Tertile 3 (88 to 135)	209	52.92 (20.1)	

Table 2. (continued)

Mean and SD of Baseline Plasma 25(OH)D Concentrations by Baseline Characteristics: The Buffalo OsteoPerio Study (n = 655)

Baseline Characteristic	Baseline Plasma 25(OH)D		
	n	Mean (SD)	*P**
Waist-to-hip ratio†			<0.0001
Tertile 1 (0.65 to 0.77)	214	66.66 (22.5)	
Tertile 2 (0.77 to 0.82)	216	58.43 (18.9)	
Tertile 3 (0.82 to 1.20)	215	57.29 (22.9)	
BMI (kg/m^2)			<0.0001
Underweight or normal (<25)	289	67.34 (22.3)	
Overweight (25 to <30)	233	58.17 (20.9)	
Obese (≥30)	133	50.39 (17.7)	
Recreational physical activity (MET hours/week)‡			<0.0001
None	97	53.71 (21.9)	
<12.5	275	58.17 (21.1)	
≥12.5	283	65.40 (21.7)	
Hormone therapy use†			0.035
Never	194	58.25 (23.1)	
Past only	116	58.17 (19.9)	
Current	338	62.64 (21.4)	
Osteoporosis-related medication use or bone therapies§			0.004
No	288	57.85 (21.6)	
Yes	367	62.82 (22.0)	
Self-reported history of osteoporosis			0.146
No	573	60.17 (21.8)	
Yes	82	63.93 (22.7)	
Worst-site T score			0.019
Normal	118	61.26 (21.4)	
Low bone density	300	62.85 (21.2)	
Osteoporosis	237	57.52 (22.8)	
Self-reported history of diabetes			0.009
No	632	61.06 (22.0)	
Yes	23	48.90 (15.8)	
Days since last dental cleaning†			0.567
≤90	254	61.27 (21.4)	
>90	386	60.26 (22.4)	
Frequency of dental visits			0.017
Never or only with a problem	50	52.47 (20.0)	
Once a year	86	59.66 (20.5)	
More than once a year	519	61.58 (22.2)	
Frequency of flossing teeth†			0.083
Not every week	116	61.51 (23.5)	
Once a week	62	62.80 (17.6)	
More than once a week	189	57.12 (23.0)	
Every day	287	61.90 (21.0)	

* *P* values from analysis of variance of mean baseline plasma 25(OH)D concentrations across level of baseline characteristics.
† Sample size does not add up to 655 due to missing data.
‡ MET, metabolic equivalent task.
§ Current use of osteoporosis-related medications or bone therapies include women taking current hormone therapy drugs, bone drugs (miacalcin, alendronate), or selective estrogen receptor modulator drugs (raloxifene).

Table 3.

β-Coefficients and SEs for Changes in Periodontal Disease Measures Corresponding to a 10-nmol/L Difference in Baseline 25(OH) D Concentrations (nmol/L) Among all Women With Baseline 25(OH)D Measures and Limited to Women With Stable 25(OH)D Concentrations With Time: The Buffalo OsteoPerio Study

	Baseline 25(OH)D (n = 655)		Baseline 25(OH)D Among Participants Who Changed <20 nmol/L in 5 Years (n = 442)	
Value	β-Coefficient (SE)	*P**	β-Coefficient (SE)	*P**
ACH side-by-side mean change (mm)				
Age-adjusted model	–0.001 (0.004)	0.724	–0.003 (0.005)	0.579
Multivariable model 1†§	–0.003 (0.004)	0.474	–0.005 (0.006)	0.397
Multivariable model 2‡§	–0.002 (0.004)	0.593	–0.004 (0.006)	0.530
CAL whole-mouth mean change (mm)				
Age-adjusted model	–0.001 (0.009)	0.889	0.003 (0.012)	0.788
Multivariable model 1†	0.001 (0.008)	0.905	0.005 (0.012)	0.686
Multivariable model 2‡	0.001 (0.009)	0.882	0.008 (0.012)	0.501
PD whole-mouth mean change (mm)				
Age-adjusted model	0.009 (0.007)	0.214	0.013 (0.009)	0.157
Multivariable model 1†	0.0009 (0.006)	0.883	0.006 (0.008)	0.432
Multivariable model 2‡	0.0005 (0.006)	0.930	0.007 (0.008)	0.429
Change in the percentage of gingival sites that bled on assessment (%)				
Age-adjusted model	0.864 (0.433)	0.046	0.389 (0.572)	0.497
Multivariable model 1†	0.034 (0.281)	0.903	–0.209 (0.366)	0.568
Multivariable model 2‡	0.112 (0.295)	0.704	–0.155 (0.382)	0.685

* *P* value for associated β-coefficient.
† Model 1 is adjusted for age, education, frequency of dental visits, smoking status, diabetes status, current use of osteoporosis-related medications or bone therapies, and baseline periodontal disease measure.
‡ Model 2 is equivalent to model 1, but further adjusted for BMI and recreational physical activity.
§ Analyses for baseline 25(OH)D include only 651 women and analyses for stable 25(OH)D include only 439 women because some women's cemento-enamel junctions were not visible on their baseline radiograph to determine baseline whole-mouth mean ACH.

considered statistically significant with *P* values of <0.05 (two-sided).

The multivariable model is also shown with and without further adjustment for body mass index (BMI) and self-reported recreational physical activity, both of which are strong predictors of vitamin D status.[26,27] Inclusion of these variables in the model will explain variation in 25(OH)D and could result in overadjustment; therefore, the results are presented with and without their inclusion in the model. The authors also examined these analyses stratified by baseline periodontal disease status for ACH, CAL, and PD change measures and stratified by tertiles of baseline measures of percentage of gingival sites that bled on assessment (Table 4). *P* values for interaction between baseline 25(OH)D and baseline periodontal disease status were estimated by addition of an interaction term to the logistic regression model. *P* values <0.20 (two-sided) for interactions were considered statistically significant.

Further, sensitivity analyses were conducted to explore the effect of teeth lost between baseline and follow-up. Lost teeth resulted in missing measurements that could lead to biased estimates of periodontal disease change. To address this concern, the authors conducted a sensitivity analysis by imputing a wide range of plausible values (from 2 to 10 mm of change) to estimate the extent that tooth loss may have influenced results for whole-mouth mean change values.

Further exploratory analyses using linear regression were also conducted to examine the association between periodontal disease–change measures and baseline 25(OH)D among participants with stable vitamin D status with time [25(OH)D change <20 nmol/L (n = 422)]. An increase in 25(OH)D concentrations of ≈20 nmol/L, in the absence of sunlight, requires a substantial increase in vitamin D intake per day (≈1,000 IU/day in supplementation).[28,29] Because two-thirds of the sample did not increase or decrease their 25(OH)D concentrations beyond 20 nmol/L, the authors considered women outside this range (±20 nmol/L) to have greatly changed their vitamin D status with time.

Table 4.

Adjusted β-Coefficients and SEs for Changes in Periodontal Disease Measures Corresponding to a 10-nmol/L Difference in Baseline 25(OH)D Concentrations (nmol/L) Stratified by Baseline Periodontal Disease Status (n = 655): The Buffalo OsteoPerio Study

Value	n	β-Coefficient (SE)	*P**
ACH side-by-side mean change (mm)†			
None	175	0.003 (0.004)	0.473
Moderate	332	–0.0009 (0.005)	0.857
Severe	144	–0.012 (0.014)	0.385
P for interaction			0.319
CAL whole-mouth mean change (mm)			
None/Mild	145	–0.014 (0.013)	0.286
Moderate	404	0.010 (0.011)	0.354
Severe	106	–0.037 (0.030)	0.219
P for interaction			0.317
PD whole-mouth mean change (mm)			
None/Mild	145	–0.007 (0.010)	0.531
Moderate	404	0.005 (0.007)	0.517
Severe	106	–0.026 (0.024)	0.291
P for interaction			0.586
Change in the percentage of gingival sites that bled on assessment (%)			
Tertile 1 (0 to 22.2)	223	–0.468 (0.431)	0.279
Tertile 2 (22.6 to 45.0)	214	0.516 (0.509)	0.312
Tertile 3 (45.1 to 100)	218	0.417 (0.617)	0.500
P for interaction			0.542

Models are adjusted for age, education, frequency of dental visits, smoking status, diabetes status, current use of osteoporosis-related medications or bone therapies, baseline periodontal disease measures, BMI, and recreational physical activity. Periodontal disease status is defined using the whole-mouth mean ACH and self-reported tooth loss due to periodontitis[20] when analyzing the ACH side-by-side mean change measures. Periodontal disease status is defined using CDC/AAP defined categories[23] when analyzing the CAL and PD whole-mouth mean change measures. Change in the percentage of gingival sites that bled on assessment is stratified by tertiles of percent of gingival sites that bled at baseline.

* *P* value for associated β-coefficient.

† Analyses include only 651 women because four women's cemento-enamel junctions were not visible on their baseline radiograph to determine baseline whole-mouth mean ACH.

RESULTS

The authors examined characteristics of women included (n = 655) and excluded (n = 265) from these analyses. Women excluded had slightly lower mean 25(OH)D concentrations (mean [SD] = 58.22 [24.4] nmol/L) than women included (60.64 [21.9] nmol/L), although this difference was not statistically significant ($P = 0.14$). A greater percentage of excluded women had severe periodontal disease based on measures of ACH and tooth loss due to periodontal disease (30.2% versus 22.1%, $P = 0.03$) and the CDC/AAP definition of periodontal disease (20.0% versus 16.2%, $P = 0.25$), although these differences were only statistically significant for disease based on ACH and tooth loss. Women excluded versus included had fewer teeth at baseline (mean [SD] 22.48 [5.5] versus 23.72 [5.0], $P = 0.001$), were older (68.91 [7.5] versus 65.60 [6.6], $P < 0.0001$), were more likely to be a race other than white (4.9% versus 1.4%, $P = 0.002$), were more likely to be current smokers at baseline (5.3% versus 2.3%, $P = 0.01$), and were more likely to have osteoporosis at baseline (42.6% versus 36.2%, $P = 0.03$) and self-reported diabetes at baseline (6.8% versus 3.5%, $P = 0.03$). Statistically significant differences with respect to baseline measures of percentage of gingival sites that bled, education, BMI, waist circumference, recreational physical activity, hormone therapy use, current use of osteoporosis-related medications or bone therapies, and measures of dental hygiene were not observed (data not shown).

Summaries of the changes in periodontal disease measures during 5 years are shown in Table 1. The mean ACH side-by-side change indicates loss, on average, in alveolar bone with time. For the other periodontal disease–change measures based on CAL, PD, and gingival bleeding, mean changes were negative, suggesting that on average, there was a slight improvement in these measures with time.

At baseline, 25(OH)D concentrations ranged from 5.91 to 146.01 nmol/L with 7% (n = 47) of the sample having deficient [25(OH)D <30 nmol/L] and 24% (n = 159) having inadequate [25(OH)D <50 nmol/L] vitamin D status[30] (Table 2). Mean baseline 25(OH)D concentrations did not vary significantly by baseline periodontal disease status as defined using the ACH or CDC/AAP categorical definitions. Twenty-two percent of women were defined as having none/mild and 16% severe periodontal disease at baseline based on the CDC/AAP definition. The majority of the sample (62%) had moderate disease. Mean baseline 25(OH)D concentrations were lower among women with a greater percentage of gingival sites that bled at baseline (tertile 3) compared with women with fewer sites that bled (tertile 1) and among women with fewer compared to more teeth at baseline. Mean 25(OH)D concentrations were lower for older women (≥70 years) and women with greater waist circumferences, waist-to-hip ratios, and BMIs and women who self-reported not engaging in recreational physical activity. Mean concentrations were also lower for women who reported never using hormone therapy or using it in the past, and concentrations were lower in women who did not take osteoporosis-related medications or bone therapies. Women who had osteoporosis by dual x-ray absorptiometry or self-reported a history of diabetes also had lower 25(OH)D concentrations, as did women who never frequented the dentist or went only with a problem.

Table 3 shows regression coefficients and standard errors (SEs) for change in periodontal disease measures regressed on baseline 25(OH)D. In the age-adjusted model, only change in the percentage of gingival sites that bled on assessment was associated with 25(OH)D status. A 10-nmol/L greater baseline 25(OH)D concentration was associated with ≈0.9 percentage points more gingival sites that bled at follow-up than at baseline ($P = 0.046$). Adjustment for additional covariates attenuated this association (Model 1 β-coefficient [SE] = 0.034 [0.281], $P = 0.903$). There were no other statistically significant associations observed between baseline 25(OH)D and change in any other periodontal disease measure.

Among the 655 women in these analyses, 6.7% (n = 24) self-reported loss of ≥1 tooth due to periodontal disease. Imputing 2, 5, or 10 mm for ACH, CAL, and PD change measures for teeth lost because of periodontal disease during follow-up did not greatly influence the results. The 25(OH)D β-coefficients (SEs) and *P* values for Model 2 with a 10-mm imputation were: –0.007 (0.008), $P = 0.381$ for ACH; 0.004 (0.011), $P = 0.722$ for CAL; and 0.002 (0.009), $P = 0.803$ for PD. Table 3 also shows exploratory analyses where the analyses of baseline 25(OH)D were restricted to include only participants whose vitamin D status was stable with time (change <20 nmol/L from baseline to follow-up). This slightly strengthened the β-coefficients when examining change measures for ACH, CAL, and PD. The β-coefficient in the model examining change in the percentage of gingival sites that bled became negative. Even so, vitamin D status remained unrelated to periodontal disease progression.

Table 4 shows associations between baseline 25(OH)D and periodontal disease change measures stratified by baseline periodontal disease status. Among women with severe periodontal disease at baseline, greater concentrations of baseline 25(OH)D were consistently associated with less periodontal disease progression defined using measures of ACH, CAL, and PD, but these associations were not statistically significant.

DISCUSSION

This is the largest prospective study to date on vitamin D status and progression of periodontal disease in postmenopausal women. The authors observed no associations between vitamin D status, assessed with baseline 25(OH)D concentrations and subsequent 5-year change in periodontal disease measures inclusive of changes in ACH, CAL, PD, and gingival bleeding. These results did not vary greatly by baseline periodontal disease status. There was some suggestion that among women with severe periodontal disease at baseline, higher baseline 25(OH)D concentrations may protect against periodontal disease progression, defined using ACH, CAL, and PD, but these results were not statistically significant. The authors cannot say for certain that the results differed by baseline disease severity. Additionally, these results do not support the previous cross-sectional findings in the same cohort of women[7] where adequate compared to inadequate or deficient vitamin D status was associated with decreased odds of periodontal disease.

Among the 655 participants, 16.2% and 61.7% of the sample had severe and moderate periodontal disease, respectively, at baseline (1997 to 2000). This is comparable to recent estimates of 11.7% (aged 50 to 64 years) and 11.2% (aged ≥65 years) of United States adults with severe disease and 37.7% (aged 50 to 64 years) and 53.0% (≥65 years) of United States adults with moderate disease as reported using nationally representative data.[1] Although the baseline prevalence estimates of periodontal disease are comparable to national prevalence estimates, previous data from the cohort showed small changes in periodontal disease measures during 5 years.[21] The authors speculated that this was attributable to a low prevalence of periodontal disease risk

factors (e.g., minimal current smokers, low prevalence of diabetes) in this cohort of postmenopausal women.[21] It is possible that the null results are explained by the minimal progression of disease during 5 years (e.g., 0.18-mm loss in alveolar bone on average). Perhaps a longer follow-up period is needed to observe meaningful changes in disease status. A previous report of disease progression during 10 years cites 0.7 to 1.4 mm of alveolar bone loss among individuals 25 to 65 years old at baseline and 2.8 mm among those 60 to 70 years old.[31]

The null results are not likely explained by a lack of variation in vitamin D status. In the sample, 7% of women demonstrated deficient baseline vitamin D status [i.e., 25(OH)D <30 nmol/L]. This is similar to the 3% to 5% of older women reported to have deficient vitamin D status [defined as 25(OH)D <25 nmol/L] in a nationally representative dataset[32] in which associations between vitamin D status and clinical measures of periodontal disease have been observed.[10,11]

Two previously conducted studies[14,17] examined associations between vitamin D intake and repeat measures of periodontal disease with time. Garcia et al.[14] conducted a 12-month study among 51 patients in a periodontal disease maintenance program consisting of postmenopausal women and men 50 to 80 years old. They showed that supplement users, defined as users of both calcium (≥ 1,000 mg/day) and vitamin D (≥400 IU/day) supplements for >18 months at baseline, had borderline statistically significant (P = 0.058) better periodontal disease measures (assessed collectively as attachment loss, bleeding on probing, gingival index, PD, and furcation involvement) at 12 months than non-supplement users (who did not use vitamin D or calcium supplements and had low dietary intake of these nutrients). Supplement users, although not different with respect to ACH from non-users, had denser oral bone at 6 and 12 months. Follow-up in this study design was short, and supplement users of calcium were not examined differently from users of vitamin D. In the Department of Veterans Affairs Dental Longitudinal Study of men (mean age 62.9 years), vitamin D intake of ≥800 compared to <400 IU/day was associated with a respective 33% and 46% decreased odds of severe periodontal disease, defined with measures of CAL and PD, and moderate-to-severe disease, defined using measures of alveolar bone loss.[17] Data collected on diet and periodontal disease one to four times from 1986 to 1998 was used in a repeated-measures cross-sectional design. Neither of these studies examined progression of disease over time in relation to vitamin D intake.

In a different study conducted by Krall,[15] in men aged ≥65 years, high compared to low dietary plus supplemental calcium, but not vitamin D intake, was shown to protect against the 7-year progression of periodontal disease as defined by alveolar bone loss. It is possible that the null results with vitamin D intake were explained in part by misclassification of vitamin D status when using the measure of oral vitamin D intake,[26] or their results support what the present authors observed, i.e., that vitamin D status is not associated with changes in alveolar bone loss. It is also possible that vitamin D intake is influential, but only among persons with low sun exposure, which is not explored in this study. Differently, Jimenez et al.[18] observed a lower incidence of self-reported tooth loss and periodontitis in a 20-year prospective cohort of 42,730 male health professionals with high compared to low predicted 25(OH)D concentrations. The multivariable model was adjusted for age, smoking, pipe use, chewing tobacco use, multivitamin use, vitamin E, vitamin C, dental profession, alcohol consumption, routine physical examination, and diabetes status. After further adjustment for BMI, race, and physical activity [factors used to develop the 25(OH)D predictor score], the periodontitis, but not the tooth loss, association was no longer statistically significant. This model may have been overadjusted, or as the authors suggested, self-reported periodontitis compared to self-reported tooth loss may be more susceptible to misclassification and led to the attenuation of the observed association. The predictor score may also reflect a healthy lifestyle score rather than a true indicator of vitamin D status. In a third study, a 3-year randomized clinical trial of combined vitamin D and calcium supplementation, a protective effect of supplementation on tooth loss was observed.[16] Unfortunately, that study cannot differentiate the effect of one nutrient from another. It is also possible that the difference between Krall's and Jimenez et al.'s study findings compared to others could be explained by the outcome of tooth loss, which is representative of end-stage periodontal disease compared to incident periodontitis or changes in PD, CAL, ACH, and gingival bleeding.

Whether the present results are generalizable to other populations remains unknown. The study consists of well-educated, primarily white women. It is possible that loss of participants due to follow-up may have contributed to the null results because women who were excluded from the current analyses had slightly lower 25(OH)D concentrations and more severe periodontal disease. It is also questionable whether the follow-up period was long enough; however, this study is still the largest and longest prospective analysis of vitamin D status and periodontal disease progression in postmenopausal women. The participants all had standardized

full-mouth oral exams and oral radiographs at baseline and follow-up. The authors were able to assess change in a varied set of periodontal disease measures using matched sites at both time points.

CONCLUSIONS

The results of the present study suggest that baseline plasma vitamin D status does not influence the subsequent 5-year change in chronic periodontal disease measures in postmenopausal women. Thus, supplementation of vitamin D for prevention of periodontal disease progression is not warranted at this time. Further replication of these findings is needed. The authors recommend that these associations be examined in studies with a longer follow-up period of periodontal disease progression.

ACKNOWLEDGMENTS

This research is supported by National Institutes of Health (NIH) grants 1R21DE020918 (awarded to AEM) and 1R01DE13505 (awarded to JW-W) from the National Institute of Dental and Craniofacial Research (NIDCR) and a grant awarded to JW-W from the Department of Defense (DAMD179616319).

The Women's Health Initiative (WHI) program is funded by the National Heart, Lung, and Blood Institute, NIH, US Department of Health and Human Services, through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C.

Program Office: (National Heart, Lung, and Blood Institute, Bethesda, Maryland) Jacques Rossouw, Shari Ludlam, Dale Burwen, Joan McGowan, Leslie Ford, and Nancy Geller.

Clinical Coordinating Center: (Fred Hutchinson Cancer Research Center, Seattle, Washington) Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg.

Investigators and Academic Centers: (Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts) JoAnn E. Manson, (MedStar Health Research Institute/Howard University, Washington, DC) Barbara V. Howard, (Stanford Prevention Research Center, Stanford, California) Marcia L. Stefanick, (The Ohio State University, Columbus, Ohio) Rebecca Jackson, (University of Arizona, Tucson/Phoenix, Arizona) Cynthia A. Thomson, (University at Buffalo, Buffalo, New York) Jean Wactawski-Wende, (University of Florida, Gainesville/Jacksonville, Florida) Marian Limacher, (University of Iowa, Iowa City/Davenport, Iowa) Robert Wallace, (University of Pittsburgh, Pittsburgh, Pennsylvania) Lewis Kuller, (Wake Forest University School of Medicine, Winston-Salem, North Carolina) Sally Shumaker.

WHI Memory Study: (Wake Forest University School of Medicine, Winston-Salem, North Carolina) Sally Shumaker.

AEM is currently a co-investigator on a vitamin D grant (#10008) funded by the Mushroom Council, 2880 Zanker Road, Suite 203, San Jose, California, 95134. The other authors report no conflicts of interest related to this study.

REFERENCES

1. Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ; CDC Periodontal Disease Surveillance workgroup. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J Dent Res* 2012;91:914-920.
2. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol* 2012;83:1449-1454.
3. Hildebolt CF. Effect of vitamin D and calcium on periodontitis. *J Periodontol* 2005;76:1576-1587.
4. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: Vitamins A and D take centre stage. *Nat Rev Immunol* 2008;8:685-698.
5. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004;173:2909-2912.
6. Institute of Medicine. Dietary Reference Intakes for Adequacy. In: *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy Press; 2011:366-370.
7. Millen AE, Hovey KM, LaMonte MJ, et al. Plasma 25-hydroxyvitamin D concentrations and periodontal disease in postmenopausal women. *J Periodontol* 2013;84:1243-1256.
8. Jabbar S, Drury J, Fordham J, Datta HK, Francis RM, Tuck SP. Plasma vitamin D and cytokines in periodontal disease and postmenopausal osteoporosis. *J Periodontal Res* 2011;46:97-104.
9. Boggess KA, Espinola JA, Moss K, Beck J, Offenbacher S, Camargo CA Jr. Vitamin D status and periodontal disease among pregnant women. *J Periodontol* 2011;82:195-200.
10. Dietrich T, Joshipura KJ, Dawson-Hughes B, Bischoff-Ferrari HA. Association between serum concentrations of 25-hydroxyvitamin D3 and periodontal disease in the US population. *Am J Clin Nutr* 2004;80:108-113.
11. Dietrich T, Nunn M, Dawson-Hughes B, Bischoff-Ferrari HA. Association between serum concentrations of 25-hydroxyvitamin D and gingival inflammation. *Am J Clin Nutr* 2005;82:575-580.
12. Miley DD, Garcia MN, Hildebolt CF, et al. Cross-sectional study of vitamin D and calcium supplementation effects on chronic periodontitis. *J Periodontol* 2009;80:1433-1439.
13. Liu K, Meng H, Tang X, et al. Elevated plasma calcifediol is associated with aggressive periodontitis. *J Periodontol* 2009;80:1114-1120.
14. Garcia MN, Hildebolt CF, Miley DD, et al. One-year effects of vitamin D and calcium supplementation on chronic periodontitis. *J Periodontol* 2011;82:25-32.
15. Krall EA. The periodontal-systemic connection: Implications for treatment of patients with osteoporosis and periodontal disease. *Ann Periodontol* 2001;6:209-213.

16. Krall EA, Wehler C, Garcia RI, Harris SS, Dawson-Hughes B. Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am J Med* 2001;111:452-456.
17. Alshouibi EN, Kaye EK, Cabral HJ, Leone CW, Garcia RI. Vitamin D and periodontal health in older men. *J Dent Res* 2013;92:689-693.
18. Jimenez M, Giovannucci E, Krall Kaye E, Joshipura KJ, Dietrich T. Predicted vitamin D status and incidence of tooth loss and periodontitis. *Public Health Nutr* 2014; 17:844-852.
19. Institute of Medicine. Overview of Vitamin D. In: *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy Press; 2011: 81-83.
20. Wactawski-Wende J, Hausmann E, Hovey K, Trevisan M, Grossi S, Genco RJ. The association between osteoporosis and alveolar crestal height in postmenopausal women. *J Periodontol* 2005;76(Suppl. 11):2116-2124.
21. LaMonte MJ, Hovey KM, Genco RJ, Millen AE, Trevisan M, Wactawski-Wende J. Five-year changes in periodontal disease measures among postmenopausal females: The Buffalo OsteoPerio study. *J Periodontol* 2013;84: 572-584.
22. Brennan RM, Genco RJ, Hovey KM, Trevisan M, Wactawski-Wende J. Clinical attachment loss, systemic bone density, and subgingival calculus in postmenopausal women. *J Periodontol* 2007;78:2104-2111.
23. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. *J Periodontol* 2007;78(Suppl. 7):1387-1399.
24. Hausmann E, Allen K, Carpio L, Christersson LA, Clerehugh V. Computerized methodology for detection of alveolar crestal bone loss from serial intraoral radiographs. *J Periodontol* 1992;63:657-662.
25. Meng JE, Hovey KM, Wactawski-Wende J, et al. Intra-individual variation in plasma 25-hydroxyvitamin D measures 5 years apart among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 2012;21: 916-924.
26. Millen AE, Wactawski-Wende J, Pettinger M, et al. Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: The Women's Health Initiative Calcium plus Vitamin D clinical trial. *Am J Clin Nutr* 2010;91:1324-1335.
27. Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst* 2006;98:451-459.
28. Institute of Medicine. Overview of Vitamin D. In: *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy Press; 2011:97-100.
29. Smith SM, Gardner KK, Locke J, Zwart SR. Vitamin D supplementation during Antarctic winter. *Am J Clin Nutr* 2009;89:1092-1098.
30. Institute of Medicine. Summary. In: *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academy Press; 2011:1-14.
31. Papapanou PN, Wennström JL, Gröndahl K. A 10-year retrospective study of periodontal disease progression. *J Clin Periodontol* 1989;16:403-411.
32. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* 2002;30:771-777.

Correspondence: Dr. Amy E. Millen, Department of Epidemiology and Environmental Health, School of Public Health and Health Professions, University at Buffalo, The State University of New York, 270 Farber Hall, Buffalo, NY 14214-8001. Fax: 716/829-2979; e-mail: aemillen@buffalo.edu.

Submitted November 18, 2013; accepted for publication March 5, 2014.