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Plasma 25-hydroxyvitamin D concentrations and periodontal disease in postmenopausal women

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Abstract

Background—Vitamin D has anti-inflammatory and anti-microbial properties that, together with its influence on bone health, may confer periodontal benefit.

Methods—We investigated cross-sectional associations (1997–2000) between plasma 25-hydroxyvitamin D concentrations [25(OH)D] and periodontal measure among 920 postmenopausal women. Chronic measures of disease were defined based on: 1) alveolar crestal height (ACH) measures from intraoral radiographs and tooth loss, and the 2) Center for Disease Control and Prevention (CDC)/American Academy of Periodontology (AAP) criteria using measures of clinical attachment level (CAL) and probing pocket depth (PD). Acute oral inflammation was assessed by the % of gingival sites that bled upon assessment with a probe. Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (CIs) for periodontal disease among participants with adequate ([25(OH)D] ≥ 50 nmol/L) compared to deficient/inadequate ([25(OH)D] < 50 nmol/L) vitamin D status adjusted for age, dental visit frequency, and body mass index.

Results—No association was observed between vitamin D status and periodontal disease defined by ACH and tooth loss (adjusted OR=0.96, 95% CI: 0.68–1.35). In contrast, women with adequate compared to deficient/inadequate vitamin D status had a 33% lower odds (95% CI: 5%–53%) of periodontal disease defined using the CDC/AAP definition and a 42% lower odds (95% CI: 21%–58%) of having ≥ 50% of gingival sites that bled.

Conclusion—Vitamin D status was inversely associated with gingival bleeding, an acute measure of oral health and inflammation and inversely associated with clinical categories of chronic periodontal disease that incorporated PD, an indicator of oral inflammation. However, vitamin D was not associated with chronic periodontal disease based on measures of ACH in combination with tooth loss.

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Keywords

vitamin D; 25-hydroxyvitamin D; periodontal diseases; postmenopausal period; epidemiology; women

INTRODUCTION

Periodontal disease, the leading cause of tooth loss in adults ¹, is estimated to be prevalent in approximately 35% of the US population (aged 30 to 90), with 12.6% having a moderate to severe form ². Prevalence increases with age and is estimated to occur in 12.97% of men and 8.56% of women over 65 years ³. Prevention of periodontal disease is important because poor oral health and tooth loss may impede consumption of a nutritious diet ⁴⁻⁶, reduces quality of life and is associated with systemic diseases such as type 2 diabetes ⁷ and cardiovascular disease ⁸. Periodontal disease is a chronic disease of inflammation thought to result from oral bacterial infection ⁹. The body's immune response to infection can lead to tissue (gingival and bone) destruction ⁹.

It is hypothesized that vitamin D status could modify risk for periodontal disease ¹⁰⁻¹² by preventing alveolar bone loss ¹³ or by mediation of the host's immune response to infection ¹⁴ and resultant inflammatory response ¹⁵. It is well known that the active vitamin D hormone, 1,25-dihydroxyvitamin D (1,25(OH)₂D), is needed for calcium homeostasis in the blood ¹⁶. If plasma calcium concentrations decrease, calcium reabsorption from bone ensues leading to decreased bone mineralization. More recently, 1,25(OH)₂D has been shown to increase transcription of anti-microbial peptides ^{14, 17} which fight foreign invaders (e.g., pathogenic oral bacteria). Vitamin D may also protect against periodontitis because of anti-inflammatory properties of 1,25(OH)₂D (reviewed in ¹⁸⁻²⁰). For example, the vitamin D receptor is expressed on a number of human immune cells ¹⁸⁻²⁰ and 1,25(OH)₂D has been shown to decrease proliferation T and B lymphocytes ^{21, 22} and inhibit the T-helper (Th)1 and Th17 (pro-inflammatory) cell response ²³ while promoting a Th2 (anti-inflammatory) cell response ^{24, 25}. Vitamin D may help suppress local inflammation at the site of oral infection.

Few studies have investigated the association between vitamin D status, assessed with a blood biomarker, and periodontal disease. Of those conducted, the majority were small case-control studies ²⁶⁻²⁸. Differently, two large, cross-sectional studies conducted using the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) observed an inverse association between serum 25-hydroxyvitamin D concentrations [25(OH)D] and gingival inflammation ¹¹ and periodontal clinical attachment level ¹². Additional studies are needed to confirm the observed associations seen in NHANES III with respect to both less severe periodontal disease defined by measures of gingival inflammation and more severe periodontal disease defined by clinical attachment loss. NHANES did not include assessment of alveolar crestal height. A better understanding of vitamin D status in relation to both acute oral inflammation (e.g., gingival bleeding and probing pocket depth) and measures of past destructive periodontal disease (e.g., alveolar crestal height and clinical attachment level) is needed. This will help determine whether vitamin D status may influence a specific part of the disease process leading to periodontitis.

Using baseline (1997-2000) data from the Buffalo Osteoporosis and Periodontal Disease (OsteoPerio) ancillary study of the Women's Health Initiative Observational Study (WHIOS) we conducted a cross-sectional analysis on the association between plasma [25(OH)D] and prevalent periodontal disease in postmenopausal women. We hypothesized

that lower prevalence of periodontal disease and better periodontal measures would be associated with higher plasma [25(OH)D].

MATERIALS AND METHODS

Study Sample

The WHIOS is a multi-center study focused on assessing the major causes of morbidity and mortality of postmenopausal women^{29, 30}. Between October 1993 and December 1998, 93,676 women (ages 50–79 years) were enrolled in the WHIOS from 40 clinic centers across the United States³¹. Of these, 2,249 WHIOS participants enrolled in the Buffalo, NY clinic center. The OsteoPerio study is a WHIOS ancillary study conducted at the Buffalo, NY clinic center and designed to investigate associations between skeletal bone density and oral bone health, the details of which were described previously³². The OsteoPerio baseline examination was conducted at the University at Buffalo from 1997–2000 and scheduled to coincide with WHIOS year 3 clinic visit. Of the 2,249 baseline participants, 549 women were unable to be reached, not interested, deceased, canceled after accepting, or were temporarily ineligible and 338 women did not meet the specified inclusion criteria (had fewer than 6 teeth [n=162]; history of bone disease [n=2]; both hips replaced [n=16]; cancer diagnosed in the last 10 years [n=106]; and had other serious illnesses [n=52]). Among the remaining 1,362 individuals, 5 did not complete or return study questionnaires. An additional 39 were missing data on outcome measures (oral radiographs [n=16], clinical attachment level [n=18], probing pocket depth [n=17] and gingival bleeding measures [n=11]). Of the remaining 1,318 participants 921 had plasma samples available for assessment of [25(OH)D]. One woman was excluded because her [25(OH)D] value was determined to be an extreme out of range value (530 nmol/L), leaving a sample of 920 for these analyses.

All OsteoPerio study participants signed informed consent and 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.

Data Collection

Oral Health Exam and Outcome Measures—All participants underwent oral clinical examinations from trained and calibrated dental examiners using standardized protocols modeled after the National Institute of Dental Research Diagnostic criteria for dental examinations³³ (as previously described^{32, 34, 35}). Reliability of PD measurements for our dental team was previously presented³⁶. The inter- and intraexaminer variability for the tooth-mean standard error of measurement were 0.24 mm ($\pm$ 0.05 standard error) and 0.38 mm ($\pm$ 0.07), respectively.

Alveolar crestal height (ACH): Measures of ACH were determined from standardized intraoral radiographs* as previously described³². Briefly, a maximum of 11 radiographs (including four vertical) bitewings were taken. ACH was measured as the absolute distance (mm) along the long axis of the tooth from the cemento-enamel junction to the most coronal point of the alveolar crest immediately adjacent to the root surface. ACH was measured mesially and distally for each tooth present, with the exception of third molars and canines (maximum 24 teeth and 48 sites measured). If a vertical defect existed, the distance from the cement-enamel junction to the point immediately adjacent to the root surface at the base of

*Bennet HFQ 300, Bennet X-Ray Corp., Copaque, NY.

the defect was used. Larger ACH values correspond to greater bone loss surrounding the tooth.

Continuous measures of worst ACH site (mm) and whole-mouth mean ACH (mm), the average loss of bone height in all sites measured, were determined. Using whole-mouth mean ACH levels and reported tooth loss to periodontitis participants were grouped into none, mild/moderate or severe categories of periodontal disease, as previously described³² (Table 1).

Probing pocket depth (PD) and clinical attachment level (CAL): PD, the distance from the gingival margin to the base of the pocket, was assessed using a constant-force electronic periodontal probing system[†]. The distance from the cemento-enamel junction to the gingival margin was measured with a Michigan O periodontal probe. These measurements were taken on six surfaces of each tooth (disto-buccal, mid-buccal, mesio-buccal, disto-lingual, mid-lingual, and mesio-lingual). CAL was calculated for each of the six surfaces per tooth, as previously described³⁴.

Continuous measures of whole-mouth mean (mm) and worst site (mm) PD and CAL were obtained³⁴. The whole-mouth mean PD and CAL measures were used to classify participants into categories of none/mild, moderate, and severe prevalent periodontal disease (Table 1) using the definition developed by the Centers for Disease Control and Prevention (CDC) and the American Academy of Periodontology (AAP) Working Group³⁷ (Table 1).

Gingival Assessment: Gingival assessment was conducted using the Michigan O periodontal probe with insertion of the probe approximately 2 mm into the gingival sulcus/pocket while being held parallel to the long axis of the tooth and moved in a horizontal direction along the arch at the gum line. Three gingival units per tooth (buccal, mesio-buccal, and lingual) were assessed for each tooth present, except for the third molars. Each gingival unit was scored a 0 or 1 according to the absence (0) or presence (1) of gingival bleeding regardless of the quantity of bleeding in that unit. The mean of all gingival assessment scores for all sites measured was computed and ranged from 0 to 1. This represents the proportion (%) of all sites in the mouth that bled upon assessment. This value was also dichotomized to represent women who had < 50% or ≥ 50% of all sites in their mouth that bled upon assessment.

Assessment of Oral Bacterial Species: At each oral exam, subgingival plaque samples were taken using a paper point technique with fine paper points[‡] or fine absorbent point #504[§], as previously described³⁸. Samples were taken for a total of 12 teeth, and fixed in formalin. Bacteria species were assessed by indirect immunofluorescence microscopy using species-species polyclonal and monoclonal serodiagnostic reagents as described by Bonta et al.³⁹ and Zambon et al.⁴⁰. In the OsteoPerio Study, five periodontal pathogens were assessed (*P. intermedia*, *T. forsythensis*, *C. rectus*, *P. gingivalis*, and *F. nucleatum*) and used to form a dichotomous variable of any versus no prevalent pathogenic oral bacteria. Data on oral bacterial species was only available in 845 of the 920 participants.

Other Health Outcome Data—Participants completed questionnaires which included data on personal information, family history, medical history update, personal habits, daily life, current medication and supplement use, physical measurements, physical activity, osteoporosis risk factors and oral health history. Additionally, women underwent dual-

[†] The Florida Probe System[®], Florida Probe, Gainesville, FL, USA.

[‡] Johnson & Johnson, East Windsor, NJ

[§] Henry Schein, Melville, NY

energy x-ray absorptiometry^{||} to determine systemic bone mineral density and T-scores were calculated using this data.

Assessment of Plasma 25-hydroxyvitamin D—Plasma samples were taken at the time of the oral examination and stored in 0.5 ml straws in liquid nitrogen at -196°C until the summer of 2010. At this time they were pulled from liquid nitrogen and put in a -80°C freezer, then thawed and aliquoted under standard protocol into cryovials, refrozen and shipped on dry ice to the laboratory[¶] for assessment of plasma [25(OH)D] (as previously described⁴¹). This all occurred over a consistent 4 month period. Samples were assessed for [25(OH)D] by competitive chemiluminescence immunoassay[#]. The within pair CV was 4.9% using the investigators' blinded duplicate quality control samples nested in each batch.

Statistical Analysis

Concentrations of plasma 25(OH)D were adjusted for season of blood draw. Residuals from regression of [25(OH)D] on the day of the year of blood draw using PROC LOESS in SAS[®] were added to the sample mean for [25(OH)D] (59.7 nmol/L)⁴² and these season-adjusted [25(OH)D] were used in all subsequent analyses. These data were also categorized dichotomously as adequate ([25(OH)D] ≥ 50 nmol/L) or deficient/inadequate ([25(OH)D] < 50 nmol/L) vitamin D status, as the Institute of Medicine's summary conclusions of vitamin D deficiency in relation to bone health reviewed that "[p]ractically all persons are sufficient at serum 25OHD levels of at least 50 nmol/L (20 ng/mL)".⁴³ However, the relevance of these specific cutpoints for blood [25(OH)D] is less clear for some of the other known biological actions of vitamin D (e.g., ant-inflammatory properties). Participants' [25(OH)D] were also ranked into quintiles.

Participant's characteristics and periodontal disease risk factors were compared across quintiles of [25(OH)D] using analysis of variance for continuous variables and chi-square tests for categorical variables. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) for periodontal disease among women with adequate (≥ 50 nmol/L) compared to deficient/inadequate (< 50 nmol/L: reference group) [25(OH)D] and among women in [25(OH)D] quintile 5, 4, 3, or 2 compared to quintile 1 (reference group). Analyses were conducted for prevalent periodontal disease outcomes defined as 1) any, mild/moderate or severe using the ACH definition, 2) any, moderate or severe using the CDC/AAP definition, and 3) $\geq 50\%$ of gingival sites that bled upon assessment. P for trend was computed using continuous [25(OH)D] as the independent variable in logistic regression models.

Next, linear regression was used to regress continuous measures of periodontal disease (both whole-mouth mean and worst site for ACH, CAL, and PD and the percent of gingival sites that bled upon assessment) on [25(OH)D]. Variance stabilization of the response variable of gingival sites that bled upon assessment was not pursued because the number of sites measured for nearly all subjects was large (at least 60) and the percentage of sites that bled for nearly all subjects was not close to 0% or 100%. Beta-coefficients (β) and their 95% CIs and p-values were estimated for each periodontal disease measure. Reported regression coefficients represent the difference in periodontal disease measures for a 10 nmol/L difference in [25(OH)D]. All logistic and linear regression models were minimally adjusted for age.

^{||}QDR-4500A, Hologic, Bedford, MA.

[¶]Heartland Assays, LLC (Ames, IA)

[#]DiaSorin LIAISON 25-OH Vitamin D TOTAL Assay (Stillwater, MN)

The following factors, measured at OsteoPerio baseline, were assessed as potential confounders of the association between periodontal disease and [25(OH)D]: age, education (high school diploma or equivalent or school after high school), race (Caucasian, non-Caucasian), smoking status (ever, never), frequency of dental visits (never or only with a problem, once a year, greater than once a year), hormone therapy use of estrogen and/or progestin (never, past, current), self-reported history of diabetes (yes, no), body mass index (BMI) in kg/m² (<25, 25 to <30, 30), and total recreational physical activity energy expenditure in metabolic equivalent task hours per week (MET-hrs/wk in quintiles). Additionally, a composite variable for current use of osteoporosis related medications or bone therapies were investigated as a potential confounder. Individuals were considered users of these medications or therapies if they reported yes to current use of hormone therapy, current use of the bone drugs micacalcin or alendronate, or reported current use of the selective estrogen receptor modulator (SERM) drug raloxifene. For all regression models, each potential confounder was added to the model to assess its influence on the OR (for logistic regression) or the β (for linear regression). If the potential confounder changed the OR or β 10% or more then this variable was retained in the model. Next, all remaining potential confounders were added to this minimally adjusted model to examine their influence on the OR or β . This procedure was repeated until addition of other potential confounders did not influence the OR or β by 10% or more. Using this approach, a final multivariable adjusted model was chosen and included, age, frequency of dental visits and BMI. Smoking status was not identified as a potential confounder in this study group.

In exploratory analyses we further investigated presence of pathogenic oral bacteria as a pathway variable explaining the association between vitamin D status and prevalent periodontal disease in a subset of participants with available data on pathogenic oral bacteria (n=845). In sensitivity analyses, we also explored whether removal of participants with prevalent self-reported co-morbidities (osteoporosis, diabetes, and cardiovascular disease) thought to share common etiologic pathways with periodontal disease^{7, 8, 44} influenced our results. These individuals may have previously changed their behaviors after diagnosis with chronic disease in ways that could change vitamin D status (e.g., increased or decreased physical activity; increased multivitamin use) and biased our results. Data was analyzed with statistical software^{**}.

RESULTS

In quintile 1, [25(OH)D] ranged from 5.98 to 40.73 nmol/L and in quintile 5 from 77.55 to 193.56 nmol/L. Overall 33% of women were considered to have deficient/inadequate (<50 nmol/L) [25(OH)D]. Women with high [25(OH)D] (quintile 5) were more likely to be younger, have smaller waist circumferences, have lower BMIs, and reported participating in greater amounts of physical activity compared to women with low [25(OH)D] (quintile 1) (Table 2).

Women with high [25(OH)D] were also more likely to report ever using hormone therapy and to report current use of osteoporosis related medications or bone therapies and were less likely to self-report a history of diagnosed diabetes than women with low [25(OH)D] (Table 2). Women with higher [25(OH)D] also reported a greater frequency of dental visits and were less likely to have osteoporosis based on worst site T-score (from dual energy x-ray absorptiometry scans) compared to women with low [25(OH)D]. Very few women in our sample were current smokers (3.2%, n=29) whereas 43.7% (n=402) were former smokers and 53.1% (n=489) reported never smoking. No statistically significant differences in periodontal disease measures by quintile of [25(OH)D] were observed except for percent of

^{**}SAS[®] for windows version 9.2 and SPSS for windows version 18.

gingival bleeding sites upon assessment. Women with higher [25(OH)D] had lower mean % of gingival bleeding sites than women with lower [25(OH)D].

The odds of prevalent periodontal disease among women with adequate compared to deficient/inadequate vitamin D status were assessed after adjustment for age, frequency of dental visits and BMI (Table 3). Vitamin D status was not significantly associated with periodontal disease based on the ACH definition. Differently, when based on the CDC/AAP definition, the odds of any periodontal disease were 33% lower in women with adequate compared to deficient/inadequate vitamin D status. A significant inverse association also was seen between vitamin D status and presence of moderate periodontal disease. Vitamin D status was inversely associated with severe periodontal disease, though not statistically significant. The odds of having 50% of gingival sites that bled were also lower, by 42% (Table 3). Further adjustment for smoking status did not influence Table 3 results and in a subset of participants with available data, further adjustment for presence of pathogenic oral bacteria minimally attenuated the results (data not shown). In sensitivity analyses, removal of participants with prevalent self-reported co-morbidities also did not change the results (data not shown).

Analyses were also conducted to examine the odds of periodontal disease by quintile of [25(OH)D] to better understand the pattern of the association between vitamin D status and periodontal disease (Table 4). In multivariable models, there was no statistically significant association between [25(OH)D] and periodontal disease defined using ACH, although results were more inverse for mild/moderate disease than any or severe disease. Overall the patterns of association between [25(OH)D] quintiles and the CDC/AAP definition of periodontal disease (any, moderate, or severe) trend inversely. Results were borderline statistically significant for any periodontal disease, statistically significant for moderate disease, and inverse but not statistically significant for severe disease. There was also an inverse association between [25(OH)D] and % of gingival bleeding sites. The OR (95% CI) was statistically significant for women in quintiles 3, 4 and 5 compared to 1, with a 45% lower odds of 50% of gingival bleeding sites in quintile 5 compared to 1 (p for linear trend=0.005). To examine if this association remained in individuals with minor gingivitis, as previously conducted in another study¹¹, the association between vitamin D status and % of gingival bleeding sites was repeated including only individuals with whole-mouth mean CAL ≥ 2 mm ($n=207$). The statistically significant inverse association remained (OR (95% CI) for quintile 5 vs. 1=0.16 (0.03, 0.81), p for linear trend=0.017).

Table 5 shows continuous measures of periodontal disease (whole-mouth means and worst sites) regressed on continuous measures of [25(OH)D]. The association between whole-mouth mean ACH and [25(OH)D] was positive and borderline statistically significant. There was no statistically significant association with worst site ACH. There was also no observed association between whole-mouth mean CAL, worst site CAL, or whole-mouth mean PD, but the association between worst site PD and [25(OH)D] concentrations was inverse and borderline statistically significant. Supporting previous observations, there was a statistically significant inverse association between % of gingival sites that bled and [25(OH)D] with a -0.83 percentage points per 10 nmol/L higher [25(OH)D].

DISCUSSION

This is one of the first large epidemiologic studies to report associations between [25(OH)D], a blood biomarker of vitamin D status, and multiple measures of periodontal disease in a well-characterized cohort of postmenopausal women. To our knowledge, no previous studies have examined measures of [25(OH)D] with respect to radiographic measures of ACH. In this cross-sectional analysis of generally healthy postmenopausal

women we observed no association between [25(OH)D] and prevalent periodontal disease defined using ACH and tooth loss. Differently, we found a 33% lower odds of prevalent periodontal disease, defined using the CDC/AAP definition, among women with adequate (≥50 nmol/L) compared to deficient/inadequate (<50 nmol/L) vitamin D status. Further investigations of associations between [25(OH)D] and continuous measures of CAL and PD showed a borderline statistically significant inverse association between worst site PD and [25(OH)D], but not CAL. We also observed a negative association between vitamin D status and % of gingival sites that bled upon assessment. There was a 42% lower odds of 50% of sites that bled among women with adequate compared to deficient/inadequate vitamin D status which was supported by linear regression analyses showing inverse association between [25(OH)D] and % of gingival sites that bleed upon assessment. Together these data suggest that vitamin D status may influence periodontal disease health, but the association appears to be related to reducing acute measures of periodontal inflammation (PD and gingival bleeding upon assessment) rather than measures that reflect past destructive periodontal disease (ACH and tooth loss or CAL) ³⁷.

Other previous studies have investigated associations between [25(OH)D] and periodontal disease ^{11, 12, 26–28}. In two previous case-control studies, one conducted in postmenopausal women with osteoporosis ²⁶ and another in pregnant women ²⁷, lower serum [25(OH)D] were observed among women with periodontal disease. Differently, in a third clinic-based case-control study ²⁸, cases of aggressive but not chronic periodontal disease, defined with measures of CAL and PD, had higher [25(OH)D] than controls. Because aggressive disease was defined as onset at <35 years of age, these findings are likely not applicable to our results of chronic disease in postmenopausal women. All studies were limited by small samples sizes (<200 cases) and none were population-based. Only one study has examined this association in postmenopausal women, but they used self-reported outcome data ²⁶. A strength of our study is the use of objective measures of periodontal disease assessment.

Two previous studies in NHANES III examined the association between serum [25(OH)D] and measures of bleeding upon probing ¹¹ and CAL ¹². The odds of bleeding upon probing was 20% less among participants in the highest compared to the lowest quintile of [25(OH)D] ¹¹. These analyses were limited to non-smokers and individuals with CAL ≥2 mm. Similarly, our results showed lower odds of gingival bleeding among women with high compared to low [25(OH)D]. Our study was comprised primarily of non-smokers and this inverse association remained after exclusion of more severe cases of periodontal disease (those with a whole-mouth mean CAL >2 mm). Dietrich et al. ¹² also observed a 0.26 mm lower mean CAL among women ≥50 years old in the quintile 5 vs. 1 of [25(OH)D]. These data also support our observations in postmenopausal women of a protective association between [25(OH)D] and the CDC/AAP definition of periodontal disease using measures of CAL and PD. However, we did not observe associations between [25(OH)D] and continuous measures of worst site or whole-mouth mean CAL.

Other studies have examined associations between periodontal disease and intake of vitamin D from foods and/or supplements which support our current observations. A different study using NHANES III ⁴⁵ found a 41% lower prevalence of periodontitis, but no association with gingival bleeding upon probing, among those with high compared to low consumption of dairy products. Milk, a dairy product, is a major contributor of vitamin D in the US diet ^{46, 47}. However, dietary vitamin D intake alone may not adequately reflect a person's 25(OH)D concentrations ⁴⁸ as other sources contribute to vitamin D status (e.g., supplements and sunlight).

Results of a three year double-blind, randomized, placebo-controlled trial of calcium (500 mg/day) and vitamin D (700 IU/day) supplementation on hip bone loss ⁴⁹ (n=145) showed a

60% lower odds of tooth loss in the supplemented compared to the placebo group. Further analysis of 2-year follow-up data showed significant associations between reduced odds of tooth loss with high compared to low calcium but not vitamin D intake. Although the clinical trial could not differentiate whether the influence of supplementation from calcium or vitamin D was most influential, the follow-up analyses suggest that calcium intake may be more important than vitamin D in preventing tooth loss.

Only one other study to our knowledge has examined associations between vitamin D and alveolar bone loss. This study⁵⁰ was conducted examining food and supplement sources of vitamin D in relation to periodontal disease progression in aging men. A 7-year longitudinal study observed less teeth with alveolar bone loss in men with self-reported high (>1,000 mg/day) compared to low (<1,000 mg/day) total calcium intake, but not total vitamin D intake (>450 IU/day compared to <450 IU/day). Similarly, our study did not observe a statistically significant association between measures of alveolar bone loss and vitamin D status.

Vitamin D is thought to potentially influence risk of periodontal disease by three mechanisms: maintenance of oral bone health, reducing oral inflammation, and promoting production of antimicrobial peptides. Vitamin D is needed for uptake of calcium from the gut and reabsorption of calcium from the kidneys and bone in order to maintain adequate plasma calcium concentrations for bone mineralization¹⁶. The recent Institute of Medicine's report on the Dietary Reference Intakes for vitamin D and calcium⁵¹ found evidence to support a role of vitamin D in skeletal health. Presumably this incorporates oral bone health, although the data to support vitamin D in oral bone health is minimal. Results from our study do not suggest that measures of 25(OH)D are associated with measures of ACH at least in a relatively healthy, Caucasian cohort of postmenopausal women.

Our data suggest that vitamin D may influence measures of more acute periodontal disease. It is hypothesized that vitamin D acts through anti-inflammatory or antimicrobial mechanisms or both. Vitamin D has been shown to suppress the body's adaptive immune response (reviewed in^{18–20}), perhaps reducing local inflammation in oral tissues. Vitamin D may also influence periodontal health at an earlier step in the etiologic pathway of periodontal disease, by reducing overall pathogenic oral infection. Oral infection of pathogenic bacteria species leading to gingivitis can then develop into more severe forms of periodontal disease. The active hormone of 1,25(OH)₂D has been shown to increase synthesis of anti-microbial peptides (e.g., cathelicidin)^{14, 17}. The data in this manuscript will aid in establishing whether vitamin D is associated with non-skeletal outcomes (e.g., gingival inflammation), an area highlighted as a research need by the IOM Committee.⁵²

Our study is limited by its cross-sectional design. We cannot determine temporality of the observed vitamin D and periodontal disease associations. Our study is also not generalizable outside of relatively healthy, Caucasian postmenopausal women. Additionally, if long-term vitamin D status (e.g., over decades) is most relevant to alveolar bone loss, it is possible that variability in measures of 25(OH)D over decades attenuated our observed associations. Although previous analysis of this data showed that there is moderate variability of [25(OH)D] over a 5 year period⁴¹, [25(OH)D] may not reflect status over a longer time period than 5 years. Long-term exposure may be more relevant to oral bone health. Although our results were adjusted for other lifestyle factors that were related to [25(OH)D] and periodontal disease measures, it is still possible that residual confounding exists.

Our study is one of the largest study to date to investigate associations between blood [25(OH)D] and periodontal disease. Further, our study had multiple outcome measures of periodontal disease defined by clinical measures of CAL, PD and % of gingival sites that bled, as well as radiographic assessment of ACH. Our study adds to the current minimal

body of literature on associations between vitamin D and alveolar bone loss. The OsteoPerio Study was able to control for a number of potential confounding variables, inclusive of oral hygiene measures. We were also able to explore the presence of pathogenic oral bacteria as a pathway variable in the observed analyses. We also had too few current smokers to investigate effect modification by smoking status.

In conclusion, vitamin D status appears to be associated with more acute measures of periodontal health (e.g., % of gingival sites that bleed and PD) rather than measures of CAL or oral bone loss among generally healthy postmenopausal women. Prospective studies of vitamin D status in relation to changes in alveolar bone health over time will help clarify vitamin D's role in oral bone health or chronic measures of periodontal disease outcomes. Future studies are needed to focus on populations at greater risk for vitamin D (e.g., African Americans) who are also at increased risk for periodontal disease. Additionally, future studies should attempt to determine potential mechanisms (e.g., reduction of inflammation or anti-microbial effects) through which vitamin D status influences acute measures of oral health.

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Study summary

In our cross-sectional study in postmenopausal women, vitamin D status was inversely associated with acute measures of periodontal disease (e.g., probing pocket depth and gingival bleeding) but not associated with measures of past destructive periodontal disease (e.g., alveolar crestal height and tooth loss or clinical attachment level).

Periodontal Disease Categories Based on Measures of ACH ³² or the CDC/AAP Classification based on PD and CAL ³⁷

Table 1

ACH Definition	Operational Definition
None	Whole-mouth mean ACH < 2 mm; no site 4mm; no reported tooth loss due to periodontal disease.
Mild/Moderate	Whole-mouth mean ACH 2 to <3 mm, or 1 tooth with an ACH 4mm, but no reported tooth loss due to periodontal disease.
Severe	Whole-mouth mean ACH 3 mm or 2 teeth with ACH 5 mm, or tooth loss (1) due to periodontal disease.
CDC/AAP Definition	Operational Definition
None/Mild	Neither "moderate" nor "severe" periodontitis
Moderate	2 Interproximal sites with CAL 4 mm (not on same tooth) or 2 interproximal sites with PD 5 mm (not on same tooth)
Severe	2 Interproximal sites with CAL 6 mm (not on same tooth) and 1 interproximal site with PD 5 mm

Table 2

Baseline (1997–2000) characteristics by quintile of plasma [25(OH)D] in nmol/L: The Buffalo OsteoPerio Study (n=920)

Demographic, Lifestyle, or Health Outcome Characteristic	Overall Sample	Quintiles (Q) of Plasma 25(OH)D (nmol/L)				
		Q1	Q2	Q3	Q4	Q5
		(5.98–40.73)	(40.79–53.72)	(53.73–63.53)	(63.54–77.41)	(77.55–193.56)
	N=920	n=184	n=184	n=184	n=184	n=184
Age (years), mean ± SD	66.6 ± 7.0	67.1 ± 7.2	66.8 ± 6.8	67.9 ± 7.0	65.9 ± 6.8	65.1 ± 7.2
Race/Ethnicity, n (%) Caucasian	898 (97.6)	175 (95.1)	179 (97.3)	182 (98.9)	181 (98.4)	181 (98.4)
Education, n (%) school after high school	721 (79.2)	135 (74.2)	148 (80.4)	147 (80.3)	143 (78.6)	148 (82.7)
Smoking status, n (%) ever smoke	431 (46.8)	84 (45.7)	84 (45.7)	83 (45.1)	92 (50.0)	88 (47.8)
Waist circumference (cm), mean ± SD	84.3 ± 12.2	89.9 ± 13.2	86.5 ± 11.8	84.1 ± 11.4	81.8 ± 10.7	79.5 ± 11.1
Body mass index (kg/m ²), mean ± SD	26.6 ± 5.0	28.5 ± 5.5	27.9 ± 5.1	26.3 ± 4.4	25.6 ± 4.3	25.0 ± 4.8
Total recreational physical activity (MET hrs/week), mean ± SD	13.9 ± 14.0	10.5 ± 12.9	12.0 ± 13.1	13.9 ± 14.5	16.0 ± 13.7	16.8 ± 14.8
Hormone therapy use, n (%) never	284 (31.1)	69 (37.7)	62 (33.9)	61 (33.3)	44 (24.3)	48 (26.2)
Osteoporosis related medication use or bone therapies [†] , n (%) current use	501 (54.5)	76 (41.3)	99 (53.8)	100 (54.4)	114 (62.0)	112 (60.9)
Self-reported history of diabetes, n (%) yes	41 (4.5)	12 (6.5)	15 (8.2)	7 (3.8)	5 (2.7)	2 (1.1)
Self-reported history of osteoporosis, n (%) yes	132 (14.3)	24 (13.0)	22 (12.0)	32 (17.4)	24 (13.0)	30 (16.3)
Worst site T score, n (%)						
Normal	149 (16.2)	25 (13.6)	34 (18.5)	26 (14.1)	28 (15.2)	36 (19.6)
Osteopenia	421 (45.8)	71 (38.6)	90 (48.9)	80 (43.5)	94 (51.1)	86 (46.7)
Osteoporosis	350 (38.0)	88 (47.8)	60 (32.6)	78 (42.4)	62 (33.7)	62 (33.7)
Days since last dental cleaning, n (%) > 90 days	556 (62.1)	115 (65.0)	114 (63.7)	111 (62.4)	114 (62.3)	102 (57.3)
Frequency of dental visits, n (%)						
Never or only with a problem	81 (8.8)	30 (16.3)	12 (6.5)	16 (8.7)	12 (6.5)	11 (6.0)
Once a year	126 (13.7)	27 (14.7)	29 (15.8)	17 (9.2)	28 (15.2)	25 (13.6)
More than once a year	713 (77.5)	127 (69.0)	143 (77.7)	151 (82.1)	144 (78.3)	148 (80.4)
Antibiotic use in the past month, n (%) yes	225 (24.5)	48 (26.1)	47 (25.5)	47 (25.5)	35 (19.0)	48 (26.1)
Pathogenic Oral Bacteria, n (%) yes	488 (57.8)	113 (65.3)	96 (59.3)	92 (54.8)	92 (54.8)	95 (54.6)
ACH Definition of Periodontal Disease, n (%) with outcome						

Demographic, Lifestyle, or Health Outcome Characteristic	Overall Sample	Quintiles (Q) of Plasma 25(OH)D (nmol/L)					P-value *
		Q1	Q2	Q3	Q4	Q5	
	N=920	(5.98–40.73) n=184	(40.79–53.72) n=184	(53.73–63.53) n=184	(63.54–77.41) n=184	(77.55–193.56) n=184	
None	233 (25.3)	45 (24.5)	46 (25.0)	45 (24.5)	43 (23.4)	54 (29.3)	0.848
Mild/moderate	462 (50.2)	92 (50.0)	99 (53.8)	93 (50.5)	95 (51.6)	83 (45.1)	
Severe	225 (24.5)	47 (25.5)	39 (21.2)	46 (25.0)	46 (25.0)	47 (25.5)	
CDC/AAP Definition of Periodontal Disease, n (%) with outcome							
None/mild	208 (22.6)	34 (18.5)	41 (22.3)	43 (23.4)	42 (22.8)	48 (26.1)	0.659
Moderate	553 (60.1)	116 (63.0)	118 (64.1)	107 (58.2)	110 (59.8)	102 (55.4)	
Severe	159 (17.3)	34 (18.5)	25 (13.6)	34 (18.5)	32 (17.4)	34 (18.5)	
% of Gingival Sites that Bled Upon Assessment, mean $\pm$ SD							
Whole-mouth mean ACH (mm), mean $\pm$ SD	36.3 $\pm$ 23.7	43.0 $\pm$ 25.9	37.1 $\pm$ 25.5	35.9 $\pm$ 22.4	31.0 $\pm$ 19.8	34.4 $\pm$ 22.9	<0.001
Worst site ACH (mm), mean $\pm$ SD	2.4 $\pm$ 0.8	2.4 $\pm$ 0.7	2.3 $\pm$ 0.7	2.4 $\pm$ 0.8	2.4 $\pm$ 0.7	2.4 $\pm$ 0.8	0.709
Whole-mouth mean CAL † (mm), mean $\pm$ SD	4.6 $\pm$ 1.5	4.6 $\pm$ 1.5	4.4 $\pm$ 1.4	4.6 $\pm$ 1.6	4.6 $\pm$ 1.7	4.6 $\pm$ 1.6	0.712
Worst site CAL (mm), mean $\pm$ SD	2.5 $\pm$ 0.7	2.6 $\pm$ 0.6	2.4 $\pm$ 0.6	2.4 $\pm$ 0.7	2.4 $\pm$ 0.6	2.5 $\pm$ 0.8	0.319
Whole-mouth mean PD ‡ (mm), mean $\pm$ SD	5.8 $\pm$ 1.8	6.0 $\pm$ 1.9	5.7 $\pm$ 1.7	5.8 $\pm$ 1.7	5.6 $\pm$ 1.8	5.9 $\pm$ 2.0	0.531
Worst site PD (mm), mean $\pm$ SD	2.2 $\pm$ 0.4	2.2 $\pm$ 0.4	2.3 $\pm$ 0.4	2.2 $\pm$ 0.4	2.2 $\pm$ 0.4	2.2 $\pm$ 0.4	0.456
Worst site PD (mm), mean $\pm$ SD	4.9 $\pm$ 1.4	5.0 $\pm$ 1.5	5.0 $\pm$ 1.3	4.9 $\pm$ 1.3	4.7 $\pm$ 1.3	4.8 $\pm$ 1.4	0.209

* P-values from analyses of variance for continuous data and from chi square tests (or Fisher's tests) for categorical data. Tests compared means and percentages across all five quintiles.

† 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 (SERM) drugs (raloxifene).

Table 3

Adjusted ORs and 95% CIs for prevalent periodontal disease among women with adequate ([25(OH)D] ≥ 50 nmol/L) compared to deficient/inadequate ([25(OH)D] < 50 nmol/L) vitamin D status: The Buffalo OsteoPerio Study, N=920

Periodontal Disease	Inadequate Vitamin D Status [25(OH)D] <50 nmol/L (referent)	Adequate Vitamin D Status [25(OH)D] ≥ 50 nmol/L
ACH Definition		
<i>Any vs. None</i>		
# with outcome/total #	230/304	457/616
Age-adjusted Model	1.00	0.98 (0.70, 1.35)
Multivariable Model *	1.00	0.96 (0.68, 1.35)
<i>Mild/moderate vs. None</i> †		
# with outcome/total #	158/232	304/463
Age-adjusted Model	1.00	0.96 (0.68, 1.35)
Multivariable Model	1.00	0.90 (0.62, 1.29)
<i>Severe vs. None</i> †		
# with outcome/total #	72/146	153/312
Age-adjusted Model	1.00	1.00 (0.66, 1.52)
Multivariable Model	1.00	1.06 (0.70, 1.63)
CDC/AAP Definition		
<i>Any vs. None/Mild</i>		
# with outcome/total #	247/304	465/616
Age-adjusted Model	1.00	0.72 (0.51, 1.02)
Multivariable Model	1.00	0.67 (0.47, 0.95)
<i>Moderate vs. None/Mild</i> †		
# with outcome/total #	197/254	356/507
Age-adjusted Model	1.00	0.70 (0.49, 1.00)
Multivariable Model	1.00	0.63 (0.43, 0.91)
<i>Severe vs. None/Mild</i> †		
# with outcome/total #	50/107	109/260
Age-adjusted Model	1.00	0.84 (0.54, 1.33)
Multivariable Model	1.00	0.85 (0.52, 1.36)
% of Gingival Sites that Bled Upon Assessment (≥ 50% vs. <50%)		
# with outcome/total #	110/304	137/616
Age-adjusted Model	1.00	0.51 (0.38, 0.69)
Multivariable Model	1.00	0.58 (0.42, 0.79)

* Adjusted for age, frequency of dental visits and body mass index.

† Analyses for the outcome mild/moderate periodontal disease (ACH definition) excluded individuals with severe disease and analyses for the outcome severe periodontal disease (ACH definition) excluded individuals with mild/moderate disease. Analyses for moderate periodontal disease

(CAL/PD definition) excluded individuals with severe disease and analyses for the outcome severe periodontal disease (CAL/PD definition) excluded individuals with moderate disease.

Table 4

ORs and 95% CIs for any prevalent periodontal disease by quintile of plasma [25(OH)D] in nmol/L: The Buffalo OsteoPerio Study, N=920

Periodontal Disease	Quintiles of Plasma 25(OH)D (nmol/L)					P for linear trend *
	Q1 (5.98–40.73) referent	Q2 (40.79–53.72) OR (95% CI)	Q3 (53.73–63.53) OR (95% CI)	Q4 (63.54–77.41) OR (95% CI)	Q5 (77.55–193.56) OR (95% CI)	
ACH Classification						
<i>Any vs. None</i>						
# with outcome/total #	139/184	138/184	139/184	141/184	130/184	
Age-adjusted Model	1.00	0.98 (0.61, 1.60)	0.94 (0.58, 1.54)	1.17 (0.71, 1.91)	0.91 (0.56, 1.46)	0.609
Multivariable Model [†]	1.00	0.99 (0.60, 1.61)	0.93 (0.56, 1.53)	1.16 (0.70, 1.92)	0.90 (0.54, 1.48)	0.568
<i>Mild/moderate vs. None [‡]</i>						
# with outcome/total #	92/137	99/145	93/138	95/138	83/137	
Age-adjusted Model	1.00	1.07 (0.64, 1.79)	0.97 (0.58, 1.63)	1.24 (0.74, 2.09)	0.86 (0.52, 1.44)	0.398
Multivariable Model	1.00	1.03 (0.61, 1.73)	0.91 (0.54, 1.54)	1.15 (0.67, 1.96)	0.78 (0.46, 1.34)	0.227
<i>Severe vs. None [‡]</i>						
# with outcome/total #	47/92	39/85	46/91	46/89	47/101	
Age-adjusted Model	1.00	0.79 (0.42, 1.47)	0.92 (0.50, 1.71)	1.02 (0.55, 1.89)	0.92 (0.51, 1.68)	0.942
Multivariable Model	1.00	0.82 (0.44, 1.54)	0.97 (0.52, 1.81)	1.15 (0.61, 2.17)	0.98 (0.53, 1.82)	0.834
CDC/AAP Definition						
<i>Any vs. None/Mild</i>						
# with outcome/total #	150/184	143/184	141/184	142/184	136/184	
Age-adjusted Model	1.00	0.80 (0.48, 1.33)	0.73 (0.44, 1.21)	0.79 (0.48, 1.32)	0.68 (0.41, 1.12)	0.099
Multivariable Model	1.00	0.79 (0.47, 1.32)	0.68 (0.40, 1.13)	0.74 (0.44, 1.24)	0.61 (0.36, 1.03)	0.045
<i>Moderate vs. None/Mild [‡]</i>						
# with outcome/total #	116/150	118/159	107/150	110/152	102/150	
Age-adjusted Model	1.00	0.86 (0.51, 1.45)	0.71 (0.42, 1.20)	0.81 (0.48, 1.37)	0.66 (0.39, 1.11)	0.070
Multivariable Model	1.00	0.85 (0.50, 1.44)	0.65 (0.38, 1.11)	0.73 (0.42, 1.26)	0.57 (0.33, 0.99)	0.023
<i>Severe vs. None/Mild [‡]</i>						
# with outcome/total #	34/68	25/66	34/77	32/74	34/82	

Periodontal Disease	Quintiles of Plasma 25(OH)D (nmol/L)					P for linear trend *
	Q1 (5.98–40.73) referent	Q2 (40.79–53.72) OR (95% CI)	Q3 (53.73–63.53) OR (95% CI)	Q4 (63.54–77.41) OR (95% CI)	Q5 (77.55–193.56) OR (95% CI)	
Age-adjusted Model	1.00	0.59 (0.30, 1.19)	0.79 (0.41, 1.52)	0.78 (0.40, 1.52)	0.76 (0.39, 1.46)	0.583
Multivariable Model	1.00	0.59 (0.29, 1.18)	0.79 (0.40, 1.53)	0.79 (0.40, 1.56)	0.79 (0.39, 1.57)	0.650
% of Gingival Sites that Bled Upon Assessment (50% vs. <50%)						
# with outcome/total #	70/184	58/184	45/184	35/184	39/184	
Age-adjusted Model	1.00	0.75 (0.49, 1.16)	0.52 (0.33, 0.82)	0.39 (0.24, 0.62)	0.45 (0.28, 0.72)	<0.001
Multivariable Model	1.00	0.80 (0.51, 1.24)	0.59 (0.37, 0.93)	0.45 (0.28, 0.73)	0.55 (0.34, 0.89)	0.005

* Logistic regression was used to calculate p for linear trend with continuous 25(OH)D concentrations as the independent variable in each model.

† Adjusted for age, frequency of dental visits and body mass index.

‡ Analyses for the outcome mild/moderate periodontal disease (ACH definition) excluded individuals with severe disease and analyses for the outcome severe periodontal disease (ACH definition) excluded individuals with mild/moderate disease. Analyses for moderate periodontal disease (CAL/PD definition) excluded individuals with severe disease and analyses for the outcome severe periodontal disease (CAL/PD definition) excluded individuals with moderate disease.

Table 5

β -coefficient for differences in ACH, CAL, and PD in mm corresponding to a 10 nmol/L difference in plasma [25(OH)D] in nmol/L; The Buffalo OsteoPerio (n=920)

Periodontal disease measure	Mean $\pm$ standard deviation	β -coefficient (Standard error) for 10 nmol/L difference in [25(OH)D]	P-value for β -coefficient
Whole-mouth mean ACH (mm)	2.4 $\pm$ 0.8		
Age-adjusted Model		0.02 (0.01)	0.135
Multivariable Model *		0.02 (0.01)	0.057
Worst site ACH (mm)	4.6 $\pm$ 1.5		
Age-adjusted Model		0.03 (0.02)	0.255
Multivariable Model		0.03 (0.02)	0.135
Whole-mouth mean CAL (mm)	2.5 $\pm$ 0.7		
Age-adjusted Model		-0.002 (0.01)	0.808
Multivariable Model		0.003 (0.01)	0.770
Worst site CAL (mm)	5.8 $\pm$ 1.8		
Age-adjusted Model		0.004 (0.03)	0.868
Multivariable Model		0.01 (0.03)	0.654
Whole-mouth mean PD (mm)	2.2 $\pm$ 0.4		
Age-adjusted Model		-0.01 (0.006)	0.082
Multivariable Model		-0.008 (0.006)	0.166
Worst site PD (mm)	4.9 $\pm$ 1.4		
Age-adjusted Model		-0.04 (0.02)	0.057
Multivariable Model		-0.04 (0.02)	0.063
% of Gingival Sites that Bled Upon Assessment	36.3 $\pm$ 23.7		
Age-adjusted Model		-1.3 (0.34)	<0.001
Multivariable Model		-0.83 (0.35)	0.018

* Model adjusted for age, frequency of dental visits and body mass index.