Association between serum concentrations of 25-hydroxyvitamin D and gingival inflammation\textsuperscript{1–3}

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ABSTRACT
Background: Vitamin D has been shown to have immunomodulatory effects in vitro and in animal studies. However, data from clinical studies of inflammatory diseases are scarce.
Objective: The purpose of this study was to evaluate the association between serum concentrations of 25-hydroxyvitamin D [25(OH)D] and gingival inflammation.
Design: We analyzed data from 77,503 gingival units (teeth) in 6,700 never smokers aged 13 to >90 y from the third National Health and Nutrition Examination Survey. Multiple logistic regression models adjusted for subject- and site-specific covariates included age, sex, race-ethnicity, income, body mass index, diabetes, use of oral contraceptives and hormone replacement therapy among women, intake of vitamin C, missing teeth, full crown coverage, presence of calculus, frequency of dental visits, and dental examiner and survey phase. Generalized estimating equations were used to account for correlated observations within subjects.
Results: Compared with sites in subjects in the lowest 25(OH)D quintile, sites in subjects in the highest 25(OH)D quintile were 20% (95% CI: 8%, 31%) less likely to bleed on gingival probing (\(P\) for trend < 0.001). The association appeared to be linear over the entire 25(OH)D range, was consistent across racial or ethnic groups, and was similar among men and women as well as among users and nonusers of vitamin and mineral supplements.
Conclusions: Vitamin D may reduce susceptibility to gingival inflammation through its antiinflammatory effects. Gingivitis may be a useful clinical model to evaluate the antiinflammatory effects of vitamin D. Am J Clin Nutr 2005;82:575–80.

KEY WORDS 25-Hydroxyvitamin D, inflammatory disease, gingivitis, periodontal disease

INTRODUCTION
Vitamin D plays an important role in calcium homeostasis and is essential for bone growth and preservation. More recently, antiinflammatory effects of vitamin D have been described. 1,25-Dihydroxyvitamin D\textsubscript{3} [1,25(OH)\textsubscript{2}D\textsubscript{3}] was shown to inhibit antigen-induced T cell proliferation and cytokine production (1, 2). In animal studies, beneficial effects of vitamin D and its analogues were found for various autoimmune diseases (3). In epidemiologic studies inverse associations between intake of vitamin D and incidence of multiple sclerosis (4) and type I diabetes (5) have been documented. However, evidence for the antiinflammatory effect of vitamin D from clinical studies in humans is scarce (6, 7). Consequently, it is unknown whether vitamin D exerts antiinflammatory effects relevant to human disease. Furthermore, little is known about the range of serum concentrations of 25-hydroxyvitamin D [25(OH)D] that may have antiinflammatory effects in humans.

Some studies have suggested that vitamin D may have beneficial effects on periodontal disease and tooth loss, possibly because of its antiinflammatory effects (8, 9). Another common dental health problem, prevalent across all ages, is chronic marginal gingivitis, a chronic inflammation of the gingival tissues that is induced by bacterial dental plaque. In susceptible patients, this gingival inflammation may eventually lead to the destruction of periodontal ligament and alveolar bone and may thus evolve into periodontal disease. However, the development of marginal gingivitis itself is unrelated to the underlying bone, and any association between vitamin D status and marginal gingivitis must, therefore, be unrelated to the effects of vitamin D on bone.

The susceptibility to gingivitis shows considerable interindividual variation and can be readily assessed with the use of an experimental gingivitis model (10, 11). Thus, marginal gingivitis could be a useful model to evaluate specifically the antiinflammatory effects of vitamin D in humans. However, whether 25(OH)D serum concentrations are associated with gingivitis susceptibility has not been investigated.

The purpose of the present study was to evaluate whether an association exists between serum concentrations of 25(OH)D and gingival inflammation in a large sample of the civilian, noninstitutionalized US population [third National Health and Nutrition Examination Survey (NHANES III)] aged 13 to >90 y. If so, we evaluated whether an identifiable threshold concentration exists above which no association can be observed.

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SUBJECTS AND METHODS

Data source

Data were derived from NHANES III, which was conducted between 1988 and 1994 to assess the health and nutrition of a large representative sample of the civilian, noninstitutionalized US population. The survey was designed as a complex, multi-stage, stratified, clustered sample survey. A detailed description of the survey and the oral health component can be found elsewhere (12). Furthermore, detailed analyses on the periodontal health of the NHANES III population have been previously published (13, 14).

Assessment of 25-hydroxyvitamin D status

Venous blood samples were taken in a standardized fashion. Serum concentrations of 25(OH)D were assayed with a radio-immunoassay kit (Dia-Sorin, Stillwater, MN) (15). The reference range for the assay is 22.5–94 nmol/L and was established in healthy, predominantly white volunteers in the midwestern United States in October, when 25(OH)D concentrations are expected to be lower than those reported in the present study.

Dental assessments

Periodontal indexes were assessed in 2 randomly selected upper and lower quadrants on fully erupted teeth other than wisdom teeth by trained examiners in subjects aged ≥13 y. For assessment of gingival inflammation, a periodontal probe was inserted ≤2 mm into the gingival sulcus and then gently moved into the mesial interproximal area. Gingival bleeding on this stimulus (ie, bleeding on probing) was scored as a dichotomous variable at each site (bleeding present or absent). Bleeding from the gingival sulcus on gentle probing with a periodontal probe (bleeding on probing) is a specific symptom of chronic gingivitis and widely used in clinical dental practice and dental research (16). In addition, the examiner recorded calculus (yes or no), probing pocket depth (in mm), clinical attachment level (in mm), full artificial crown coverage (yes or no), and number of teeth. Details of the extensive quality-control and quality-assurance protocol in the oral component of NHANES III, including reliability statistics for selected periodontal indexes, have been published previously (12, 17).

Data on covariates

Participants were administered several structured interviews, both at home and in the mobile examination center. Respondents were classified as never smokers if they had smoked <100 cigarettes in their lifetime. Furthermore, serum cotinine concentrations were determined with the use of an enzyme immunoassay and a liquid chromatography mass spectrometry method (15).

The poverty-to-income ratio was computed as the ratio of family income compared with the poverty threshold as published annually by the Census Bureau. Missing values for the poverty-to-income ratio were coded as missing.

Furthermore, subjects were classified according to any use of multivitamin and mineral supplements in the preceding month (aged ≥17 y only), diabetes, and frequency of visits to the dentist or dental hygienist. In addition, female respondents were classified according to use of oral contraceptives, hormone replacement therapy, or both. Vitamin C intake was calculated from a 24-h dietary recall. Finally, body mass index (BMI) was calculated from measured height and weight.

Statistical analysis

We conducted a tooth-specific analysis with mesiobuccal sites as the unit of analysis. This approach allowed us to adjust for important site-specific covariates that are strong predictors of gingival bleeding (calculus and full crown coverage). To restrict the analysis to sites with marginal gingivitis, we excluded sites with clinical attachment loss > 2 mm (ie, bone loss resulting from periodontal disease). Furthermore, because smoking suppresses the bleeding response of the gingival tissues (18, 19), the analysis was restricted to subjects who reported to be never smokers and who had serum cotinine concentrations ≤ 15 ng/mL.

Descriptive statistics for covariates by quintiles of 25(OH)D concentrations were calculated with the subject as the unit of analysis. As suggested by a reviewer, P values for crude associations between covariates and 25(OH)D quintiles were calculated with the use of analysis of variance and chi-square statistics.

A multiple logistic regression model was fit to examine the association between gingival bleeding and serum concentrations of 25(OH)D. To account for the dependence of observations (sites) within subjects, generalized estimating equation marginal models with an exchangeable working correlation were used. Serum concentrations of 25(OH)D were categorized according to quintiles. In addition, 25(OH)D concentration was entered as a continuous variable to perform trend tests. The final model adjusted for age (continuous), sex, race-ethnicity (non-Hispanic whites, non-Hispanic blacks, Mexican Americans, other), poverty-to-income ratio (continuous), BMI (continuous), diabetes (yes or no), use of oral contraceptives or hormone replacement therapy among women (never, former, current, missing), intake of vitamin C (continuous), number of missing teeth (continuous), calculus (yes or no), full crown coverage (yes or no), and frequency of dental visits (less than once per year, at least once per year, missing). These variables were adjusted to account for known predictors of gingivitis (eg, calculus) and known modulators of gingivitis expression [eg, estrogens (20), diabetes (21, 22), intake of vitamin C (23)]. We further adjusted for dental examiner and survey phase (24).

Race or ethnicity, sex, and age were a priori considered as potentially important effect measure modifiers. We conducted formal tests of interaction by entering interaction terms in the model and also compared effect estimates from stratified analyses. We also compared effect estimates in supplement users and nonusers. Finally, decimals of 25(OH)D concentrations were entered into the main model to explore the dose-response relation more closely. All analyses were performed with the use of STATA 7.0 (Stata Corp, College Station, TX).

RESULTS

There was a total of 6809 never smokers aged 13 to >90 years with 25(OH)D serum concentrations and periodontal data available with complete data on covariates. Of them, 109 subjects had no teeth with attachment loss ≤ 2 mm. Thus, the final sample consisted of 77 503 mesiobuccal sites (teeth) in 6700 subjects (2448 men and 4252 women). Women are overrepresented in this sample because of the restriction to never smokers.
Demographic characteristics of the sample and distribution of covariates by quintiles of serum concentrations of 25(OH)D are given in Table 1. Men, non-Hispanic whites, and current users of oral contraceptives or hormones among women, on average, higher concentrations of 25(OH)D. Furthermore, subjects with higher serum concentrations of 25(OH)D were more likely to have higher incomes and to visit their dentist or dental hygienist more frequently. No significant interactions were observed with race-ethnicity, age, sex, or effect estimates were similar across strata.

We found a strong negative association between serum concentrations of 25(OH)D and prevalence of bleeding on probing (Table 2). In the model controlling for age, sex, race-ethnicity, dental examiner, and survey phase, the odds ratio for bleeding for the highest (median: 99.6 nmol/L) compared with lowest (median: 32.4 nmol/L) 25(OH)D quintile was 0.74 (95% CI: 0.64, 0.86). Full adjustment for all other covariates resulted in an attenuation of the association. Compared with the lowest 25(OH)D quintile, sites in subjects in the highest 25(OH)D quintile had 20% (95% CI: 8%, 31%) lower odds of bleeding on probing. An increase in serum concentration of 25(OH)D of 30 nmol/L was associated with sites having 10% (95% CI: 5%, 14%) lower odds of bleeding. The association appeared to be linear over the entire 25(OH)D range (Figure 1). Finally, the odds ratio estimates were similar among users of multivitamin and mineral supplements and nonusers.

**DISCUSSION**

In the present study, we found an inverse association between serum concentrations of 25(OH)D and chronic gingivitis as measured by bleeding on probing among participants who never smoked aged 13 to >90 y. The association was independent of age, sex, income, BMI, intake of vitamin C, full crown coverage, calculus, frequency of dental visits, diabetes, use of oral contraceptives and hormone replacement therapy among women, number of missing teeth, and diabetes. The association was consistent across racial or ethnic groups and appeared linear over the entire 25(OH)D range.
considerable evidence is available for the antiinflammatory  
effects of vitamin D from in vitro studies. 1,25-(OH)2D3 has been  
shown to inhibit antigen-induced T cell proliferation and cytokine  
production, specifically interleukin 2 and interferon-γ (1, 2, 25, 26).  
Furthermore, vitamin D has marked effects on antigen-presenting  
cells (27–31). In addition, beneficial effects of 1,25-(OH)2D3 and its  
analogues have been shown in animal models of autoimmunity and  
transplantation. However, evidence from clinical human studies is  
scarce. In a cross-sectional study of 116 subjects, serum concentra-  	ions of 25(OH)D were negatively correlated with serum concentra- 

tions of C-reactive protein. In a subsample of 24 patients from that  
study, vitamin D supplementation significantly reduced serum concentra- 
tions of C-reactive protein by 23% (7). In a small randomized clinical  
trial of critically ill patients in the intensive care unit, supplementation  
with 500 IU parenteral vitamin D significantly decreased circulating  
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tation with 500 IU parenteral vitamin D significantly decreased circulating  
congenital hip dysplasia (11, 47). Interven- 
ation reported here can be explained by a variation in plaque  
levels. However, we cannot rule out residual confounding by  
plaque levels. 

Furthermore, this is a cross-sectional study, and a causal effect  
of 25(OH)D concentrations on gingival inflammation cannot be  
established on the basis of these data. However, the concomitant  
assessment of 25(OH)D concentrations and gingival inflammha- 
tion is not likely to be an important problem, because gingival  
flammation develops and resolves rapidly (11, 47). Interven- 
tion studies will be necessary to establish whether increased  
intake of vitamin D can reduce gingivitis susceptibility. We  
believe that marginal gingivitis may be a useful model to study the  
antiinflammatory effects of vitamin D in humans.

**TABLE 2**  
Odds ratios (ORs) and 95% CIs for prevalence of bleeding on probing by  
25-hydroxyvitamin D [25(OH)D]  

<table>
<thead>
<tr>
<th>Quintile of 25(OH)D</th>
<th>n</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 32.4 nmol/L</td>
<td>1347</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>2: 47.4 nmol/L</td>
<td>1351</td>
<td>0.95 (0.84, 1.07)</td>
<td>0.98 (0.87, 1.11)</td>
</tr>
<tr>
<td>3: 60.7 nmol/L</td>
<td>1332</td>
<td>0.87 (0.75, 0.98)</td>
<td>0.90 (0.80, 1.02)</td>
</tr>
<tr>
<td>4: 75.6 nmol/L</td>
<td>1340</td>
<td>0.86 (0.64, 0.86)</td>
<td>0.88 (0.77, 1.01)</td>
</tr>
<tr>
<td>5: 99.6 nmol/L</td>
<td>1330</td>
<td>0.74 (0.64, 0.86)</td>
<td>0.80 (0.69, 0.92)</td>
</tr>
<tr>
<td>Trend</td>
<td>—</td>
<td>0.88&lt;sup&gt;4,5&lt;/sup&gt; (0.84, 0.93)</td>
<td>0.90&lt;sup&gt;4,5&lt;/sup&gt; (0.86, 0.95)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Median 25(OH)D concentration in quintile.  
<sup>2</sup> Adjusted for age, sex, race-ethnicity, dental examiner, and survey phase.  
<sup>3</sup> Adjusted for age, sex, race-ethnicity, BMI, vitamin C intake, income,  
oral contraceptive use and hormone replacement therapy among women,  
diabetes, missing teeth, full crown coverage, calculus, frequency of dental  
visits, and dental examiner and survey phase.  
<sup>4</sup> OR for increase in 25(OH)D serum concentration by 30 nmol/L.  
<sup>5</sup> P for trend < 0.001.
The results of the present study suggest that increased serum concentrations of vitamin D may be beneficial in regard to gingivitis susceptibility. This inverse association may be due to the antiinflammatory effect of vitamin D, which may be present in serum concentrations of 25(OH)D ≥ 90–100 nmol/L.

The analysis was planned by TD. TD conducted the analysis with contributions by MN. All authors evaluated the results and contributed to their interpretation. TD wrote the manuscript with input from all other authors. None of the authors had a conflict of interest.

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