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Joint effects of serum vitamin D insufficiency and periodontitis on insulin resistance, pre-diabetes, and type 2 diabetes: results from the National Health and Nutrition Examination Survey (NHANES) 2009–2010

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ABSTRACT

Objective Periodontitis is strongly associated with diabetes and is increasingly shown to be associated with other glycemic abnormalities. Vitamin D is postulated to have both anti-inflammatory and antimicrobial activity. Therefore, our aim was to investigate the joint effects of both serum 25-hydroxyvitamin D_3 and total 25-hydroxyvitamin D with periodontitis on homeostatic model assessment for insulin resistance (HOMA-IR), pre-diabetes, and type 2 diabetes.

Research design and methods Using data from the 2009–2010 National Health and Nutrition Examination Survey, the sample was restricted to adults over 30 years of age, who were eligible for oral health examination, and had vitamin D, fasting glucose and insulin measures. The analytic sample includes those with (n=1631) and without (n=1369) type 2 diabetes. Using survey logistic multivariable regression analysis, we examined the following joint effects: (1) vitamin D insufficiency (<50 nmol/L) and moderate to severe periodontitis (VD+PD+); (2) vitamin D insufficiency and mild to no periodontitis (VD+PD−); and (3) vitamin D sufficiency) (>50 nmol/L) and periodontitis (VD−PD+), and compared these groups with the doubly unexposed reference group (VD−PD−).

Results Consistently, the joint effects of vitamin D_3 insufficiency and total vitamin D insufficiency with periodontitis (VD+PD+) were significantly associated with diabetes: OR=2.83 (95% CI 1.34 to 5.96) and OR=1.98 (95% CI 1.04 to 3.76), respectively. However, the joint effects of vitamin D_3 insufficiency and periodontitis were attenuated for HOMA-IR 4.17: OR=1.57 (95% CI 0.97 to 2.55). Pre-diabetes was not associated with either joint effects.

Conclusion In this cross-sectional, nationally representative sample, the joint effects of vitamin D and periodontitis appear to differ for HOMA-IR, pre-diabetes and diabetes.

Significance of this study

What is already known about this subject?

- Using data from the cross-sectional National Health and Nutrition Examination Survey of the US population, this is the first study to assess the joint effects between serum vitamin D and periodontitis on homeostatic model assessment for insulin resistance (HOMA-IR), pre-diabetes, and type 2 diabetes.

What are the new findings?

- Joint effects between vitamin D and periodontitis appear to differ for HOMA-IR, pre-diabetes, and type 2 diabetes.

How might these results change the focus of research or clinical practice?

- Results from this cross-sectional study highlight the need for additional studies that assess possible synergistic effects between serum 25-hydroxyvitamin D_{33} and periodontitis on type 2 diabetes.

Periodontitis is a highly prevalent,[1] biofilm-induced, chronic inflammatory condition characterized by loss of connective tissue support and alveolar bone.[2 3] Periodontitis has been consistently linked with extraoral inflammatory conditions, including type 2 diabetes[2 4] and cardiovascular disease.[5] One possible mechanism underlying these associations is a chronic low-grade inflammatory response to periodontal microbiota in susceptible hosts.[3 6 7] This subclinical inflammatory response, in turn, has been hypothesized as a mediator linking periodontitis, a surrogate for dysbiotic oral microbial communities,

to the development of insulin resistance[8 9] and type 2 diabetes among adults.[4 10 11] However, most effect sizes are modest, and not all individuals are likely susceptible to inflammation secondary to oral microbial exposures. Limited data are available regarding the identification of intermediates that might interact with subgingival dysbiosis to produce an inflammatory response, necessary for periodontal microbiota to influence extraoral outcomes.

Vitamin D is essential for maintaining healthy bones and calcium homeostasis[12] but has been shown to have antimicrobial and anti-inflammatory effects that suppress proinflammatory cytokines.[13] Vitamin D also supports immune regulation and function by controlling over 200 genes that are responsible for cellular proliferation, differentiation, and apoptosis.[14] Vitamin D receptors (VDRs) located in a number of cells function as biological mediators,[15] and recently VDR genes have been shown to be associated with periodontitis.[16] Vitamin D and chronic periodontitis have a close relationship with bone metabolism, inflammation, and immunity. Genetic polymorphisms cause changes in the VDR genes, which may contribute to the development of periodontitis by altering the following four specific gene loci: *Fok-l*, *Bsm-I*, *Apa-I* and *Taq-I* on chromosome 12q12-14.[17] In a meta-analysis, mutated alleles t and F of the Taq-I and Fok-l loci, respectively, show a protective association for chronic periodontitis among Asians but not Caucasians; in contrast Fok-I polymorphisms were shown to be a risk factor for aggressive periodontitis but not chronic periodontitis; and other loci Bsm-I and Apa-I were not associated with disease susceptibility.[17] However, inconsistent findings from other studies are reported for gene loci among ethnic-specific populations.[18–21] VDR polymorphisms and subsequent mediated signaling pathways of 1,25(OH)2D in the susceptibility of periodontitis are unclear.[17 22–24] Vitamin D metabolites, total vitamin D (25-hydroxyvitamin D (25(OH)D)), vitamin D_3 (25-hydroxyvitamin D_3 ($25(OH)D_3$)), and calcitriol, the biologically active form (1,25(OH)D2D), have different half-life ranges of ≈2–3 weeks, ≈15 days, and ≈4–21 hours, respectively.[12 25–27] The VDR binds to calcitriol, the biologically active vitamin D metabolite (1,25(OH)D2D), but $25(OH)D_3$ despite being an inactive metabolite is also reported to have an affinity to VDR.[26 28 29] Considering the short half-life, calcitriol is not an adequate biomarker; hence, total vitamin D (25(OH)D) is clinically relevant for assessing overall vitamin D status.[12] Accordingly, vitamin D may modulate the inflammatory effects of oral microbes that contribute to periodontitis; thus, a synergistic effect could be observed between low serum vitamin D levels and periodontitis. In a large multicenter study, elevated serum vitamin D (25(OH)D) was associated with lower prevalence of periodontal disease[30]; results from nationally representative survey show that low serum vitamin D_3 ($25(OH)D_3$) is associated with periodontal attachment loss among adults over 50 years of age, but higher levels of 25(OH)D decrease gingival inflammation.[31] Further, a recent consensus report from the joint European Federation of Periodontology and European Organisation for Caries Research acknowledges the importance of vitamin D on periodontal health.[32] There is mixed evidence that vitamin D affects glucose homeostasis; systematic reviews and meta-analyses suggest insufficient evidence that vitamin D supplementation benefits glucose metabolism,[33] with weaker evidence from trials that vitamin D supplementation improves insulin resistance.[34] Although both vitamin D and periodontitis are related to glycemic outcomes, studies on the interaction are limited. Therefore, we sought to examine the joint effects of periodontitis and serum $25(OH)D_3$, including total vitamin D (25(OH)D), on insulin resistance, pre-diabetes, and type 2 diabetes in a nationally representative sample.

RESEARCH DESIGN AND METHODS

Data source

We used data from the National Health and Nutrition Examination Survey (NHANES) 2009–2010 cycle. Full details about the NHANES survey are provided elsewhere.[35] In brief, the NHANES uses a complex, multistage probability sample design to examine a nationally representative sample of about 5000 non-institutionalized US civilians annually. Highly trained personnel collect demographic, socioeconomic, and health-related information through questionnaires, physical examinations, and laboratory assessments. Health interviews and physical measurements were conducted inhome and at a mobile examination center (MEC), respectively. Prior to participation written informed consent was obtained from the participants.

Study population

Among n=10 537 NHANES 2009–2010 participants, our analysis included adults ≥30 years who (1) underwent both the interview and MEC examination; (2) were eligible for oral health examination; (3) had measured serum vitamin D 25(OH)D and $25(OH)D_3$ concentrations; and (4) had fasting glucose and insulin levels measured following an overnight fasting state. We excluded adults with type 1 diabetes, which was ascertained by self-report of the following diabetes-related questions[36]: (1) a previous diagnosis of diabetes by a physician or health professional and (2) currently only using insulin medication (n=27).

Respondents without a complete periodontal examination and those who were missing all periodontal attachment loss and pocket depth measures were excluded. The final analytic sample includes those with (n=1631) and without (n=1369) type 2 diabetes. The flow chart of the analytic sample is presented in online supplementary figure S1.

Main outcomes

The HOMA-IR

Fasting insulin and glucose were measured using the Mercodia Insulin ELISA enzyme immunoassay and

hexokinase enzymatic glucose method, respectively. The Fairview Medical Center Laboratory at the University of Minnesota, Minneapolis, Minnesota, processed and analyzed the blood specimens.[37] The homeostatic model assessment for insulin in resistance (HOMA-IR) was calculated according to the formula: fasting insulin (µU/mL) x fasting glucose (mmol/L) / 22.5.[38] Estimates derived from the 'gold standard' euglycemic clamp technique for insulin resistance are shown to correlate well with the validated HOMA-IR model.[39] We used the population-specific 75th percentile as a cut-off level for HOMA-IR, classifying individuals without type 2 diabetes as insulin-resistant or insulin-sensitive, HOMA-IR 4.17 (75th percentile) or HOMA-IR 75th, respectively.

Pre-diabetes and type 2 diabetes

Pre-diabetes and type 2 diabetes were defined according to the American Diabetes Association criteria.[40] The following are the recommended laboratory values for the diagnosis of pre-diabetes and type 2 diabetes, respectively: fasting plasma glucose (FPG) ≥100 to 125 mg/dL (5.6– 6.9 mmol/L) or hemoglobin A1c (HbA1c) 5.7%–6.4% (39–47 mmol/mol); and for type 2 diabetes, FPG ≥126 mg/dL (7.0 mmol/L) or HbA1c ≥6.5% (48 mmol/mol). Based on self-report, adults who answered 'yes' to the following questions were also ascertained as having pre-diabetes or type 2 diabetes, respectively: (1) 'has a doctor or other health professional told you have prediabetes, impaired fasting glucose, impaired glucose tolerance, borderline diabetes?'; and (2) 'has a doctor or other health professional told you have diabetes or sugar diabetes?'[36]

Exposure variables

Oral examination

A detailed description of the NHANES clinical examination guidelines and oral health data collection protocols is found elsewhere.[41 42] Briefly, in 2009–2010 survey cycle, a full-mouth periodontal examination of all four quadrants (excluding third molars) was conducted on adults over 30 years of age. Trained dental examiners used the HU-Friedy periodontal probe (Hu-Friedy Mfg, Chicago, Illinois, USA), which has color-coded graduations at 2, 4, 6, 8, 10, and 12 mm. Gingival margin (GM) level and pocket depth (PD) were measured at the following six sites per tooth: distofacial, mid-facial, mesio-facial, disto-lingual, mid-lingual, and mesio-lingual sites. GM and PD measurements are used to determine clinical attachment levels, and each measurement was rounded to the lower whole millimeter. Overall, the oral health reliability for the 2009–2010 cross-sections is considered 'very good'; however, there were differences in the examiner reliability statistic. Details about interexaminer agreement are provided by Dye *et al*.[42]

Periodontitis

A full-mouth periodontal examination measuring six sites per tooth, excluding third molars, is considered the 'gold standard' for assessing periodontitis in population-based studies.[43 44] Periodontitis was defined using the Centers for Disease Control and Prevention (CDC) and the American Academy of Periodontology definition, a standardized clinical case definition for periodontitis developed for population-based studies.[44]

Serum vitamin D (25(OH)D)

Laboratory specimens collected during the MEC examinations were processed and frozen at −30°C until they are shipped to the CDC Environmental Health Laboratory in Atlanta, Georgia. Serum 25(OH)D_3 and total serum 25(OH)D, the major circulating forms of vitamin D and the best measures of vitamin D status in humans,[12] were analyzed using ultra-high-performance liquid chromatography tandem mass spectrometric method (UHPLC-MS/MS). Laboratory Procedures Manual is provided elsewhere.[45] The Institute of Medicine, Food and Nutrition Board outlines the reference values for serum 25(OH)D insufficiency and sufficiency, respectively, as 25(OH)D <50 nmol/L (<20 ng/mL) and 25(OH)D ≥50 nmol/L.[46]

Risk factor assessment

Demographic information and health-related risk factor variables were self-reported. Age was categorized into the following groups: 30–44, 45–64, and ≥65 years; and race/ethnicity was reported as non-Hispanic white, non-Hispanic black, and all Hispanic, including multiracial. Educational attainment was defined as those who completed less than high school, high school, and some or more college. Health insurance or healthcare coverage (ie, employer, private, or government programs) is reported as 'yes or no' response. Poverty income ratio (PIR), the ratio of family income to poverty threshold, is calculated by dividing the family income by poverty guidelines set forth by the US Department of Health and Human Services, specific to family size, year and state. The PIR values <1.30, 1.30≤PIR<3.50 and PIR≥3.50 represent 'low-income', 'middle-income', and 'high-income', respectively. Any missing values for PIR were coded as a separate category. The season of blood draw is defined according to when the NHANES examinations took place, and blood sampling during winter months (November–April) was carried out in lower latitude regions and during summer months (May–October) in higher latitude regions. For behavioral risk factors such as smoking habits, respondents are described as current smokers if they self-reported smoking at least 100 cigarettes in their entire life and currently smoke cigarettes every day or some days; adults who report they no longer smoke are former smokers; and respondents who never smoked 100 cigarettes in their entire life are never smokers. Current smokers were compared with never/former smokers. Physical activity (low, moderate-to-high intensity) was based on both occupational and recreational-related activities. Health status was assessed by physical examination, and measures include standing

height and body weight; body mass index (BMI) values were classified as normal weight (BMI <25 kg/m^2), overweight (BMI 25 to <30 kg/m^2), or obese (BMI ≥30 kg/m^2).

Statistical analysis

All statistical analyses were carried out using SAS V.9.4 software. To accommodate the complex sample survey design of the NHANES, we used specific survey procedures that incorporate design strata, cluster, and sampling weights to obtain unbiased population estimates. Variance estimates were calculated using Taylor series linearization within the SAS survey procedures. Rao-Scott *F* adjusted χ^2 statistic rather than the Wald χ^2 assessed the differences between categorical variables and glycemic outcomes, providing a more conservative interpretation.[47] Statistical significance was fixed at p value <0.05.

PROC SURVEYLOGISTIC procedures fit the logistic regression models, testing independent associations and joint effects between vitamin D insufficiency for serum 25(OH)D_3, 25(OH)D, and periodontitis on the following outcomes: HOMA-IR ≥4.17, pre-diabetes, and type 2 diabetes. ORs with corresponding 95% CIs were obtained. Multivariable models were adjusted for the following covariates: age, sex, race/ethnicity (non-Hispanic white, non-Hispanic black, total Hispanic including multiracial), education (less than high school, high school graduate, some college or higher), health insurance coverage (yes or no), season of examination (winter or summer), smoking status (never, former and current), physical activity (vigorous-to-moderate, sedentary) and BMI (<25 kg/m^2, 25 to <30 kg/m^2, or ≥30 kg/m^2). In the full cohort, the following variables were missing: data for education (n=5, 0.1%) and BMI (n=6, 0.31%). Poverty (PIR) was missing in n=157 (7.4%) respondents, and this value was coded as a separate category and included in the regression models.

Joint effects analysis was carried out by coding three separate OR variables, OR11, OR10, OR01, and OR00, respectively, which are defined as (1) vitamin D insufficiency (25(OH)D_3 <50 nmol/L) and moderate-to-severe periodontitis (VD+PD+); (2) vitamin D insufficiency (25(OH)D_3 <50 nmol/L) and mild-to-no periodontitis (VD+PD−); and (3) vitamin D sufficiency (25(OH)D_3 >50 nmol/L) and moderate-to-severe periodontitis (VD−PD+), and comparing these groups with the doubly unexposed reference group (VD−PD−). Similarly, separate OR variables were created for total vitamin D (25(OH)D).

Statistical interaction was assessed on the multiplicative and additive scale. Cross-product interaction terms were included in the models to assess multiplicative interaction between serum vitamin D insufficiency and periodontitis. Positive multiplicative interaction was given by the following equation: OR11/(OR10)×(OR01)>1; alternatively, negative multiplicative interaction occurs when OR11/(OR10)×(OR01)<1, respectively, and the effect of both exposures together is greater than (or less than) the product of both exposures considered separately.[48 49]

The presence of additive interaction can be assessed by comparing the observed and the expected joint effects; positive interaction (synergism) is when the observed joint effect of both exposures is greater than the expected sum of the individual effects; alternatively, negative interaction (antagonism) occurs when the observed joint effects are less than the expected sum of the individual effects.[48] We used the methods described by Knol and VanderWeele[49] to estimate the additive interaction using relative excess risk due to interaction (RERI), and attributable proportion (AP) from ORs and 95% CIs were obtained by delta method. The following formulae estimate RERI and AP, respectively: RERI=(OR11–OR10 –OR01+1); AP=(OR 11–OR10–OR01 +1)/OR11.[48] Departures from additivity are present if RERI and AP are not equal to 0; additive interaction can be either positive (RERI>0), negative (RERI<0), or zero (RERI=0). Kalilani and Atashili[50] validated the use of ORs in place of relative risks when assessing measures of additive interaction. Based on the authors' recommendations, the following were considered to avoid misleading conclusions: baseline risk (R00) in the doubly unexposed group, the measure of interaction (ie, RERI and AP), and lastly the direction of interaction (ie, positive or negative). Briefly, in the presence of positive interaction, without substantially strong additive effects, it is suggested that baseline risk in the doubly unexposed group be less than 10%; however, for AP this difference is evident when the baseline risk is approximately 15%.[50]

Adiposity is strongly associated with glycemic abnormalities[51] and vitamin D insufficiency.[52] Therefore, we conducted additional sensitivity analyses among overweight and obese adults, minimizing the potential concern of residual confounding. Subgroup analyses are provided in the online supplementary material.

RESULTS

General characteristics

Baseline participant characteristics are presented table 1 and online supplementary table S1. The full unweighted analytic sample consisted of 1631 respondents, representing 136 081 781 US adults. The prevalence of type 2 diabetes using FPG, HbA1, and self-report was 12.57% (95% CI 10.25 to 14.88). In the subsample of adults, excluding those with type 2 diabetes, the prevalence of pre-diabetes and HOMA-IR ≥4.17 was 54.9% (95% CI 51.24 to 58.59) and 23.6% (95% CI 20.44 to 26.69), respectively.

Overall, in both analytic samples, about half of the adults were middle-aged, with slightly more women (52%). Predominantly, respondents identified as 'non-Hispanic white' and reported having attained some or more college (61%). Less than 20% of adults, in both samples, self-reported having 'no health insurance' coverage. Over two-thirds of adults were described as overweight or obese in our sample, with sufficient levels of serum 25(OH)D. However, comparing those with

Table 1 Participant characteristics of adult participants over 30 years of age by HOMA-IR and type 2 diabetes from the 2009–2010 NHANES*

			Full analytic sample 1				Analytic subsample 2			
			Type 2 diabetes§				HOMA-IR¶			
	Total		Present		Absent		HOMA-IR ≥75th		Insulin-sensitive	
Characteristics	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Unweighted sample	**1631**		**262**	**12.57**	**1369**	**87.43**	**342**	**23.57**	**1027**	**76.43**
Weighted sample	**136 081 781**		**17 101 218**		**118 980 564**		**28 039 126**		**90 941 437**	
Age (years) (%)										
≥30 to <45 (R)	563	37.6	33	12.4	530	41.2	141	43.5	389	40.5
≥45 to <65	710	45.0	126	51.8	584	44.0	145	43.6	439	44.0
≥65	358	17.4	103	35.9	255	14.8	56	12.9	199	15.4
Sex (%)										
Male (R)	788	48.6	151	57.2	637	47.3	185	55.8	452	44.7
Female	843	51.4	111	42.8	732	52.7	157	44.2	575	55.3
Race/ethnicity (%)										
Non-Hispanic white (R)	760	68.9	96	59.3	664	70.3	153	66.2	511	71.6
Non-Hispanic black	275	10.8	52	13.2	223	10.5	67	14.4	156	9.3
Total Hispanic including multiracial	596	20.3	114	27.5	482	19.2	122	19.4	360	19.2
Education (%)										
Some college or higher (R)	829	61.4	113	55.6	716	62.2	156	52.6	560	65.2
High school diploma	364	22.3	63	23.4	301	22.1	87	28.5	214	20.1
Less than high school	433	16.4	85	21.0	348	15.7	98	18.9	250	14.7
Health insurance (%)										
Yes (R)	1225	81.3	215	85.3	1010	80.7	246	79.0	764	81.3
No	406	18.7	47	14.7	359	19.3	96	21.0	263	18.7
PIR (%)										
PIR≥350%= PIR≥3.50 (R)	480	42.8	61	32.9	419	44.3	91	35.7	328	46.9
130%≤PIR< 350%=1.30≤ PIR<3.50	549	33.6	97	38.7	452	32.8	104	33.8	348	32.5
PIR<130%= PIR<1.30	445	16.2	72	18.2	373	15.9	117	21.9	256	14.0
Did not answer (missing)	157	7.4	32	10.2	125	7.0	30	8.5	95	6.5
BMI (%)										
Normal <25 kg/m^2 (R)	405	27.8	35	12.2	370	30.1	17	5.7	353	37.6
Overweight ≥25 to <30 kg/m^2	580	34.0	74	23.8	506	35.5	93	23.9	413	39.1
Obese ≥30 kg/m^2	640	38.2	152	64.0	488	34.4	231	70.4	257	23.3

Continued

Table 1 Continued

			Full analytic sample 1				Analytic subsample 2			
			Type 2 diabetes§				HOMA-IR¶			
	Total		Present		Absent		HOMA-IR ≥75th		Insulin-sensitive	
Characteristics	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Unweighted sample	**1631**		**262**	**12.57**	**1369**	**87.43**	**342**	**23.57**	**1027**	**76.43**
Weighted sample	**136 081 781**		**17 101 218**		**118 980 564**		**28 039 126**		**90 941 437**	
Physical activity (%)										
Active, vigorous-to-moderate (R)	920	62.4	116	51.9	804	63.9	186	56.4	618	66.0
Sedentary	711	37.6	146	48.1	565	36.1	156	43.6	409	33.8
Smoking status (%)										
Former or never smoker (R)	1338	84.1	224	87.8	1114	83.5	271	82.4	843	83.9
Current smoker	293	15.9	38	12.2	255	16.5	71	17.6	184	16.1
Serum vitamin D_3 ($25(OH)D_3$) status (%)†										
Sufficient levels (R)	1039	70.5	135	58.8	904	72.1	200	60.6	704	75.7
Insufficient levels (<50 nmol/L)	592	29.5	127	41.3	465	27.9	142	39.4	232	24.3
Serum vitamin (25(OH)D) status (%)†										
Sufficient levels (R)	1125	75.9	165	71.1	960	76.6	217	66.2	743	79.8
Insufficient levels (<50 nmol/L)	506	24.1	97	28.9	409	23.4	125	33.8	284	20.2
Season of examination (%)†										
Summer, May 1–October 3 (R)	903	61.1	143	61.4	760	61.1	173	57.3	587	62.3
Winter, November 1–April 30	728	38.9	119	38.6	609	38.9	169	42.7	440	37.8
Periodontitis (%)‡										
None or mild (R)	872	60.5	90	38.9	782	63.6	191	61.4	591	64.3
Moderate or severe	759	39.5	172	61.1	587	36.4	151	38.6	436	35.7

Missing values for analytic samples 1 and 2, respectively: education: n=5 (0.1%) and n=4 (0.14%); BMI: n=6 (0.3%) and n=5 (0.32%).
*NHANES survey analytic guidelines recommend the relative SE is not larger than 30%, all estimates for each subgroup were less than 30%.
†Serum vitamin D (25(OH)D) and vitamin D_3 ($25(OH)D_3$) insufficiency is defined as levels <50 nmol/L or <20 ng/mL.
‡Case definitions for periodontitis based on the definition from the Division of Oral Health at the Centers for Disease Control and Prevention, in collaboration with the American Academy of Periodontology.
§Type 2 diabetes based on self-report of a previous diagnosis by a physician or other health professionals, or based on the level of hemoglobin A1c of 6.5% (48 mmol/mol) or greater and fasting plasma glucose level of 126 mg/dL or greater.
¶HOMA-IR ≥4.17 (population-specific 75th percentile) established using fasting glucose and insulin levels by the following formula: HOMA-IR=[glucose (mmol/L)×insulin (μU/mL) / 22.5]. HOMA-IR excluded those with diagnosed and undiagnosed type 2 diabetes.
25(OH)D, 25-hydroxyvitamin D; $25(OH)D_3$, 25-hydroxyvitamin D_3; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; NHANES, National Health and Nutrition Examination Survey; PIR, poverty income ratio; R, reference category.

Table 2 Adjusted logistic regression models for periodontitis and vitamin D exposures associated with HOMA-IR, pre-diabetes, and type 2 diabetes among adults over 30 years of age from the 2009–2010 NHANES

		Point estimate (95% CI)		
Outcomes	**Exposures**	**Unadjusted models**	**Minimally adjusted models***	**Fully adjusted models†**
HOMA-IR‡	Periodontitis§	1.31 (0.85 to 1.50)	1.00 (0.67 to 1.49)	1.09 (0.70 to 1.71)
	Vitamin D_3 insufficiency¶	**2.03 (1.62 to 2.53)**	**1.86 (1.44 to 2.39)****	**1.40 (1.06 to 1.84)****
	Vitamin D insufficiency	**2.02 (1.51 to 2.69)**	**1.80 (1.29 to 2.51)****	1.34 (0.95 to 1.89)
Pre-diabetes††	Periodontitis§	**1.98 (1.5 to 2.62)**	1.18 (0.85 to 1.63)	1.13 (0.80 to 1.61)
	Vitamin D_3 insufficiency¶	1.17 (0.88 to 1.54)	1.04 (0.74 to 1.47)	0.89 (0.64 to 1.23)
	Vitamin D insufficiency	**1.42 (1.08 to 1.86)**	1.24 (0.84 to 1.83)	1.06 (0.72 to 1.55)
Type 2 diabetes‡‡	Periodontitis§	**2.74 (1.65 to 4.54)****	1.51 (0.89 to 2.55)	**1.65 (1.02 to 2.70)****
	Vitamin D_3 insufficiency¶	**1.82 (1.09 to 3.05)****	**1.84 (1.07 to 3.15)****	1.60 (0.97 to 2.63)
	Vitamin D insufficiency	1.33 (0.85 to 2.09)	1.24 (0.74 to 2.07)	1.12 (0.70 to 1.80)

*Minimally adjusted model, age, sex, race/ethnicity, poverty income ratio, season, smoking, and physical activity.
†Fully adjusted model, age, sex, race/ethnicity, education, insurance, season, smoking, physical activity, and body mass index.
‡HOMA-IR ≥4.17 (population-specific 75th percentile) established using fasting glucose and insulin levels by the following formula: HOMA-IR=[glucose (mmol/L)×insulin (μU/mL) / 22.5]. HOMA-IR excluded those with diagnosed and undiagnosed type 2 diabetes.
§Case definitions for periodontitis based on the definition from the Division of Oral Health at the Centers for Disease Control and Prevention, in collaboration with the American Academy of Periodontology.
¶Serum vitamin D (25(OH)D) and vitamin D_3 (25(OH)D_3) insufficiency is defined as levels <50 nmol/L or <20 ng/mL.
**Significance at p<0.05. Statistically significant values are shown in bold.
††Pre-diabetes based on the level of hemoglobin A1c of 5.7%–6.4% (39–47 mmol/mol), fasting plasma glucose level of 100–125 mg/dL, or adults who reported having been told by a health professional that they have any of the following: pre-diabetes, impaired fasting glucose, impaired glucose tolerance, or borderline diabetes. Pre-diabetes excluded those with diagnosed and undiagnosed type 2 diabetes.
‡‡Type 2 diabetes based on self-report of a previous diagnosis by a physician or other health professionals, or based on the level of hemoglobin A1c of 6.5% or greater and fasting plasma glucose level of 126 mg/dL or greater.
25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxyvitamin D_3; HOMA-IR, homeostatic model assessment for insulin resistance; NHANES, National Health and Nutrition Examination Survey.

defined outcomes, 25(OH)D_3 insufficiency was highest among adults with type 2 diabetes (41.3%), followed by HOMA-IR ≥4.17 (39.4%) and pre-diabetes (29.3%). Periodontitis (severe-to-moderate) was also higher among those with type 2 diabetes and pre-diabetes: 61.1% and 43.4%, respectively.

Main effects of associations between vitamin D insufficiency and periodontitis on HOMA-IR, pre-diabetes, and type 2 diabetes

The results from multivariable logistic regression analysis are presented in table 2. Relative to sufficient vitamin D levels, serum 25(OH)D_3 insufficiency was consistently associated with HOMA-IR ≥4.17 (OR=1.40 (95% CI 1.06 to 1.84)) in the fully adjusted model. Serum 25(OH)D_3 insufficiency remained significantly associated with type 2 diabetes (OR=1.84 (95% CI 1.07 to 3.15)) in the minimally adjusted model, and was attenuated in the fully adjusted model (OR=1.60 (95% CI 0.97 to 2.63)). The odds of type 2 diabetes among adults with periodontitis (moderate-to-severe) compared with mild-to-no periodontitis was 1.65 (95% CI 1.02 to 2.70) after full adjustment; however, periodontitis was statistically significant with respect to HOMA-IR ≥4.17.

Joint effect models between vitamin D status and periodontitis on HOMA-IR, pre-diabetes, and type 2 diabetes

Joint effects models between periodontitis and vitamin D, both 25(OH)D_3 and 25(OH)D, on HOMA-IR ≥4.17, pre-diabetes, and type 2 diabetes are presented in tables 3 and 4.

When compared with the doubly unexposed group (ie, vitamin D sufficiency and mild-to-no periodontitis (VD−PD−)), the minimally adjusted joint effect model of serum vitamin D_3 insufficiency without periodontitis (VD+PD−) was significantly associated with HOMA-IR ≥4.17 (OR=1.87 (95% CI 1.40 to 2.49)), but not statistically significantly associated with pre-diabetes or type 2 diabetes. Conversely, vitamin D_3 sufficiency with periodontitis (VD−PD+) was not associated with HOMA-IR ≥4.17, pre-diabetes or type 2 diabetes. The joint effects of both exposures together (VD+PD+) compared with the doubly unexposed group (VD−PD−) increase the odds of HOMA-IR ≥4.17 and type 2 diabetes: OR=1.90 (95% CI 1.46 to 3.16) and OR=3.02 (95% CI 1.30 to 6.98), respectively. However, after full adjustment, the joint effects of both exposures together (VD+PD+) compared with the doubly unexposed group remained associated

Table 3 Logistic regression models assessing the joint effect of serum vitamin D3 (25(OH)D3) levels and periodontitis with HOMA-IR, pre-diabetes, and type 2 diabetes among adults over 30 years of age from the 2009–2010 NHANES

Outcomes		HOMA-IR*		Pre-diabetes†		Type 2 diabetes‡	
Exposures		**With/without outcome§ (n)**	**OR (95% CI)**	**With/without outcome§ (n)**	**OR (95% CI)**	**With/without outcome§ (n)**	**OR (95% CI)**
G=0 (periodontitis none or mild)	E=0 $25(OH)D_3$ sufficiency	112/406	Reference (1.00)	261/257	Reference (1.00)	48/518	Reference (1.00)
	E=1 $25(OH)D_3$ insufficiency¶	79/185	**1.87 (1.40 to 2.49)****	146/118	1.06 (0.71 to 1.60)	42/264	1.37 (0.58 to 3.33)
G=1 (periodontitis moderate or severe) ††	E=0 $25(OH)D_3$ sufficiency	88/298	1.03 (0.70 to 1.59)	269/117	1.19 (0.80 to 1.78)	87/386	1.26 (0.68 to 2.35)
	E=1 $25(OH)D_3$ insufficiency¶	63/138	**1.90 (1.46 to 3.16)****	141/60	1.23 (0.69 to 2.18)	85/201	**3.02 (1.30 to 6.98)****
P values‡‡			0.96		0.91		0.21
		With/without outcome§§ (n)		**With/without outcome§§ (n)**		**With/without outcome§§ (n)**	
G=0 (periodontitis none or mild)	E=0 $25(OH)D_3$ sufficiency	112/406	Reference (1.00)	261/257	Reference (1.00)	48/518	Reference (1.00)
	E=1 $25(OH)D_3$ insufficiency¶	79/185	1.36 (0.94 to 1.95)	146/118	0.90 (0.61 to 1.34)	42/264	1.07 (0.48 to 2.38)
G=1 (periodontitis moderate or severe)††	E=0 $25(OH)D_3$ sufficiency	88/298	1.06 (0.62 to 1.83)	269/117	1.14 (0.73 to 1.77)	87/386	1.27 (0.70 to 2.28)
	E=1 $25(OH)D_3$ insufficiency¶	63/138	1.57 (0.97 to 2.55)	141/60	0.99 (0.58 to 1.68)	85/201	**2.83 (1.34 to 5.96)****
P values‡‡			0.75		0.90		0.10

*HOMA-IR ≥4.17 (population-specific 75th percentile) established using fasting glucose and insulin levels by the following formula: HOMA-IR=[glucose (mmol/L)×insulin (μU/mL) / 22.5]. HOMA-IR excluded those with diagnosed and undiagnosed type 2 diabetes.
†Pre-diabetes based on the level of HbA1c of 5.7%–6.4% (39–47 mmol/mol), fasting plasma glucose level of 100–125 mg/dL, or adults who reported having been told by a health professional that they have any of the following: pre-diabetes, impaired fasting glucose, impaired glucose tolerance, or borderline diabetes. Pre-diabetes excluded those with diagnosed and undiagnosed type 2 diabetes.
‡Type 2 diabetes based on self-report of a previous diagnosis by a physician or other health professionals, or based on the level of HbA1c of 6.5% (48 mmol/mol) or greater and fasting plasma glucose level of 126 mg/dL or greater.
§Minimally adjusted model for age, sex, race/ethnicity, PIR, season, smoking, and physical activity.
¶Serum vitamin D_3 ($25(OH)D_3$) insufficiency defined as levels <50 nmol/L or <20 ng/mL.
**Significance at $p<0.05$. Statistically significant values are shown in bold.
††Case definitions for periodontitis based on the definition from the Division of Oral Health at the Centers for Disease Control and Prevention, in collaboration with the American Academy of Periodontology.
‡‡Represents multiplicative interaction p value results from adjusted survey logistic models.
§§Fully adjusted model for age, sex, race/ethnicity, education, insurance, season, smoking, physical activity, and body mass index.
25(OH)D3, 25-hydroxyvitamin D3; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; NHANES, National Health and Nutrition Examination Survey.

Table 4 Logistic regression models assessing the joint effects of serum vitamin D (25(OH)D) levels and periodontitis with HOMA-IR, pre-diabetes, and type 2 diabetes among adults over 30 years of age from the 2009–2010 NHANES

Outcomes		HOMA-IR*		Pre-diabetes†		Type 2 diabetes‡	
Exposures		**With/without outcome§ (n)**	**OR (95% CI)**	**With/without outcome§ (n)**	**OR (95% CI)**	**With/without outcome§ (n)**	**OR (95% CI)**
G=0 (periodontitis none or mild)	E=0 25(OH)D sufficiency	124/434	Reference (1.00)	281/277	Reference (1.00)	56/558	Reference (1.00)
	E=1 25(OH)D insufficiency¶	68/156	**1.74 (1.18 to 2.57)****	126/98	1.26 (0.77 to 2.06)	34/224	1.02 (0.42 to 2.48)
G=1 (periodontitis moderate or severe)††	E=0 25(OH)D sufficiency	94/308	0.98 (0.66 to 1.45)	279/123	1.19 (0.80 to 1.77)	109/402	1.38 (0.76 to 2.51)
	E=1 25(OH)D insufficiency¶	57/128	**1.86 (1.10 to 3.17)****	131/54	1.46 (0.80 to 2.64)	63/185	**2.02 (0.98 to 4.16)****
P values‡‡			0.69		0.92		0.50
		With/without outcome§§ (n)		**With/without outcome§§ (n)**		**With/without outcome§§ (n)**	
G=0 (periodontitis none or mild)	E=0 25(OH)D sufficiency	124/434	Reference (1.00)	281/277	Reference (1.00)	56/558	Reference (1.00)
	E=1 25(OH)D insufficiency¶	68/156	1.23 (0.79 to 1.91)	126/98	1.07 (0.64 to 1.77)	34/224	0.85 (0.36 to 2.01)
G=1 (periodontitis moderate or severe)††	E=0 25(OH)D sufficiency	94/308	1.02 (0.61 to 1.70)	279/123	1.14 (0.74 to 1.75)	109/402	1.45 (0.83 to 2.53)
	E=1 25(OH)D insufficiency ¶	57/128	1.56 (0.88 to 2.77)	131/54	1.19 (0.68 to 2.09)	63/185	**1.98 (1.04 to 3.76)****
P values‡‡			0.44		0.96		0.37

*HOMA-IR ≥4.17 (population-specific 75th percentile) established using fasting glucose and insulin levels by the following formula: HOMA-IR=[glucose (mmol/L)×insulin (μU/mL) / 22.5]. HOMA-IR excluded those with diagnosed and undiagnosed type 2 diabetes.
†Pre-diabetes based on the level of HbA1c of 5.7%–6.4% (39–47 mmol/mol), fasting plasma glucose level of 100–125 mg/dL, or adults who reported having been told by a health professional that they have any of the following: pre-diabetes, impaired fasting glucose, impaired glucose tolerance, or borderline diabetes. Pre-diabetes excluded those with diagnosed and undiagnosed type 2 diabetes.
‡Type 2 diabetes based on self-report of a previous diagnosis by a physician or other health professionals, or based on the level of HbA1c of 6.5% (48 mmol/mol) or greater and fasting plasma glucose level of 126 mg/dL or greater.
§Minimally adjusted model for age, sex, race/ethnicity, PIR, season, smoking, and physical activity.
¶Serum vitamin D_3 (25(OH)D) insufficiency defined as levels <50 nmol/L or <20 ng/mL.
**Significance at $p<0.05$. Statistically significant values are shown in bold.
††Case definitions for periodontitis based on the definition from the Division of Oral Health at the Centers for Disease Control and Prevention, in collaboration with the American Academy of Periodontology.
‡‡Represents multiplicative interaction p value results from adjusted survey logistic models.
§§Fully adjusted model for age, sex, race/ethnicity, education, insurance, season, smoking, physical activity, and body mass index.
25(OH)D, 25-hydroxyvitamin D; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; NHANES, National Health and Nutrition Examination Survey.

with type 2 diabetes: OR=2.83 (95% CI 1.34 to 5.96). When examining the joint effects of total serum 25(OH)D and periodontitis on type 2 diabetes, the strength of the association was attenuated but with greater precision: OR=1.98 (95% CI 1.04 to 3.76) (table 4). Additional sensitivity analyses among overweight or obese adults are provided in online supplementary tables S3 and S4.

Interactions between vitamin D status and periodontitis on HOMA-IR, pre-diabetes, and type 2 diabetes

Statistical interaction on the multiplicative scale was not evident for any outcomes (p≥0.10). For type 2 diabetes, there was a significant positive additive interaction between 25(OH)D_3 insufficiency and periodontitis in the fully adjusted models: RERI=1.49 (95% CI 0.07 to 2.91); the AP due to this interaction was 0.53 (95% CI 0.19 to 0.86). However, there was no evidence of additive interaction between 25(OH)D insufficiency and periodontitis: RERI=0.68 (95% CI −0.54 to 1.90) and AP=0.34 (95% CI −0.19 to 0.88) (online supplementary table S5).

CONCLUSIONS

In this nationally representative, cross-sectional survey of adults over 30 years of age, our findings show that 25(OH)D_3 insufficiency and periodontitis are associated with type 2 diabetes independently and when both exposures are considered jointly after multivariable adjustment. The results suggest positive additive interaction (synergism) for type 2 diabetes, which indicates that 25(OH)D_3 insufficiency and periodontitis are greater than the sum of the individual effects.

This is the first study to date to examine the joint effects of vitamin D insufficiency and periodontitis on insulin resistance, pre-diabetes, and type 2 diabetes. Previously, only one study assessed the joint effects of obesity and vitamin D insufficiency on insulin resistance and type 2 diabetes. Similarly, the authors report no multiplicative interaction, but positive (synergistic) interaction for type 2 diabetes, although not statistically significant.[53] The direction not the magnitude has important public health implications[48]; however, further studies are needed to assess the significance of this synergistic interaction between serum 25(OH)D_3 and periodontitis on glycemia.

The nature of the cross-sectional study design prevents us from making inferences about temporality and causation. However, it is biologically plausible that higher serum vitamin D concentrations attenuate the inflammatory response resulting from periodontal infections,[31 54] which have been shown to contribute to insulin resistance and diabetes. Several other studies have provided plausible biological basis for the observed association. Recently, in vitro, vitamin D downmodulated cytokines released from cells infected with *Porphyromonas gingivalis*,[55] a Gram-negative bacterium associated with periodontitis. Pathogenic oral microbiota such as *P. gingivalis* are also shown to be strongly associated with pre-diabetes prevalence.[56] Chronic gingivitis, bleeding on probing, and tooth loss are reduced with increased serum 25(OH)D concentrations.[57 58] In a large national study, periodontal attachment loss was inversely associated with 25(OH)D_3[31]; similarly, women with adequate 25(OH)D levels had one-third lower odds of periodontitis.[30]

Further, the literature supports a positive and bidirectional relationship between periodontitis and diabetes.[2] Poorly controlled diabetes worsens periodontal disease.[59] Prospectively, periodontal disease is shown to be associated with incident type 2 diabetes[4] and is associated with higher HbA1c levels over time compared with periodontally healthy adults.[11]

Conversely, fewer studies have examined this relationship with pre-diabetes and insulin resistance, but show conflicting results. Choi *et al*[60] suggest that impaired fasting glucose was associated with higher levels of periodontal attachment loss. However, Arora *et al*[61] indicate that impaired glucose tolerance but not impaired fasting glucose was associated with moderate-to-severe periodontitis; similarly, Zuk *et al*[62] report that after controlling for income and education, periodontitis was not associated with impaired fasting glucose.

Our study has several strengths, which include the use of a nationally representative survey of US adults. Exposures were assessed through objective measurements; UHPLC-MS/MS was described as an accurate and precise method to measure 25(OH)D and 25(OH)D_3; and participants also underwent a comprehensive full-mouth periodontal examination.[42 43] In addition, this is the first analysis, to our knowledge, that assessed the joint effects of vitamin D and periodontitis on HOMA-IR, pre-diabetes, and type 2 diabetes. There are, however, several key limitations. First, the NHANES is a cross-sectional, national survey; thus, temporality to support causal mechanistic interactions cannot be confirmed.[48] Second, our analysis was limited to a single cross-section (2009–2010) where both periodontal health and laboratory data for serum vitamin D measures were available. Third, although we adjusted for season, which affects vitamin D exposure, we were unable to adjust for geographic locations that reflect latitude and longitude as the data were not publicly available. However, in previous studies, latitude was not found to be associated with vitamin D deficiency, and single blood draws in either season may potentially, adequately reflect average vitamin D concentrations over the year.[63] Lastly, residual confounding by smoking is a potential concern given that current smoking is an important modifiable risk factor for periodontitis,[64] and serum 25(OH)D was previously reported to be lower among smokers[65]; however, the rates of current smoking have dropped considerably in the USA,[66] but we continue to see a rising trend of diabetes and high prevalence of periodontitis among American adults.

In conclusion, the joint effects of vitamin D levels and periodontitis appear to differ for HOMA-IR, pre-diabetes, and type 2 diabetes. The findings of joint effects for type 2 diabetes and the direction of additive interaction in the absence of multiplicative interaction have

clinical significance and important implications for future diabetes research; additional studies are needed, however, to confirm synergistic effects.

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