

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

Aleksandra M Zuk,¹ Carlos R Quiñonez,² Olli Saarela,³ Ryan T Demmer,⁴ Laura C Rosella^{1,5,6}

To cite: Zuk AM, Quiñonez CR, Saarela O, *et al.* 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. *BMJ Open Diab Res Care* 2018;**6**:e000535. doi:10.1136/bmjdr-2018-000535

The abstract was previously published as a late breaking abstract and assigned poster presentation (237-LB) in the category 16-F Epidemiology-Other at the American Diabetes Association's 77th Scientific Sessions.

Received 19 March 2018
Revised 24 May 2018
Accepted 2 June 2018



For numbered affiliations see end of article.

Correspondence to
Aleksandra M Zuk;
aleksandra.zuk@mail.utoronto.ca

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 D₃ 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 D₃ 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 D₃ 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 D₃ 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 homeostasis¹² 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-I*, *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-I* 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 D₃ (25-hydroxyvitamin D₃ (25(OH)D₃)), and calcitriol, the biologically active form (1,25(OH)2D), 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)2D), but 25(OH)D₃ 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 disease³⁰; results from nationally representative survey show that low serum vitamin D₃ (25(OH)D₃) 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)D₃, 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, multi-stage 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)D₃ 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 questions³⁶: (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 resistance (HOMA-IR) was calculated according to the formula: fasting insulin ($\mu\text{U/mL}$) \times 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 $\geq 6.5\%$ (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, distolingual, 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)D₃ 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 multi-racial. 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 , $1.30 \leq \text{PIR} < 3.50$ and $\text{PIR} \geq 3.50$ 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/m²), overweight (BMI 25 to <30 kg/m²), or obese (BMI ≥30 kg/m²).

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)D₃, 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/m², 25 to <30 kg/m², or ≥30 kg/m²). 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)D₃ <50 nmol/L) and moderate-to-severe periodontitis (VD+PD+); (2) vitamin D insufficiency (25(OH)D₃ <50 nmol/L) and mild-to-no periodontitis (VD+PD-); and (3) vitamin D sufficiency (25(OH)D₃ >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 VanderWeele⁴⁹ 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=(OR11-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 Atashili⁵⁰ 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 abnormalities⁵¹ 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, HbA1c, 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*

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

Continued

Table 1 Continued

Characteristics	Full analytic sample 1				Analytic subsample 2				
	Type 2 diabetes§				HOMA-IR¶				
	Total	Present	Absent		HOMA-IR	HOMA-IR	Insulin-sensitive		
n	n	n	%	n	n	n	%	n	%
Unweighted sample	1631	262	1369	12.57	1369	87.43	23.57	1027	76.43
Weighted sample	136 081 781	17 101 218	118 980 564		118 980 564	87.43	23.57	90 941 437	
Physical activity (%)									
Active, vigorous-to-moderate (R)	920	116	804	51.9	804	63.9	56.4	618	66.0
Sedentary	711	146	565	48.1	565	36.1	43.6	409	33.8
Smoking status (%)									
Former or never smoker (R)	1338	224	1114	87.8	1114	83.5	82.4	843	83.9
Current smoker	293	38	255	12.2	255	16.5	17.6	184	16.1
Serum vitamin D ₃ (25(OH)D ₃) status (%)*†									
Sufficient levels (R)	1039	135	904	58.8	904	72.1	60.6	704	75.7
Insufficient levels (<50 nmol/L)	592	127	465	41.3	465	27.9	39.4	232	24.3
Serum vitamin (25(OH)D) status (%)*†									
Sufficient levels (R)	1125	165	960	71.1	960	76.6	66.2	743	79.8
Insufficient levels (<50 nmol/L)	506	97	409	28.9	409	23.4	33.8	284	20.2
Season of examination (%)*‡									
Summer, May 1–October 3 (R)	903	143	760	61.4	760	61.1	57.3	587	62.3
Winter, November 1–April 30	728	119	609	38.6	609	38.9	42.7	440	37.8
Periodontitis (%)*‡									
None or mild (R)	872	90	782	38.9	782	63.6	61.4	591	64.3
Moderate or severe	759	172	587	61.1	587	36.4	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 D₃ (25(OH)D₃) 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)D₃, 25-hydroxyvitamin D₃; 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

Outcomes	Exposures	Point estimate (95% CI)		
		Unadjusted models	Minimally adjusted models*	Fully adjusted model†
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: $HOMA-IR = [glucose (mmol/L) \times insulin (\mu U/mL) / 22.5]$. 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 D₃ (25(OH)D₃) 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)D₃, 25-hydroxyvitamin D₃; HOMA-IR, homeostatic model assessment for insulin resistance; NHANES, National Health and Nutrition Examination Survey.

defined outcomes, 25(OH)D₃ 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)D₃ 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)D₃ 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)D₃ 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 D₃ 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 D₃ 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-diabetest		Type 2 diabetes†	
	With/without outcomes§ (n)	OR (95% CI)	With/without outcomes§ (n)	OR (95% CI)	With/without outcomes§§ (n)	OR (95% CI)
Exposures						
	G=0 (periodontitis none or mild)	E=0 25(OH)D ₃ sufficiency	Reference (1.00)	261/257	Reference (1.00)	48/518
	E=1 25(OH)D ₃ insufficiency¶	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	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¶	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
G=0 (periodontitis none or mild)	E=0 25(OH)D ₃ sufficiency	Reference (1.00)	261/257	Reference (1.00)	48/518	Reference (1.00)
	E=1 25(OH)D ₃ insufficiency¶	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	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¶	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 D₃ (25(OH)D₃) 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‡		
	With/without outcome§ (n)	OR (95% CI)	With/without outcome§ (n)	OR (95% CI)	With/without outcome§ (n)	OR (95% CI)	
Exposures							
	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	
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: $\text{HOMA-IR} = \frac{\text{glucose (mmol/L)} \times \text{insulin } (\mu\text{U/mL})}{22.5}$. 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 D₃ (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 ($p \geq 0.10$). For type 2 diabetes, there was a significant positive additive interaction between 25(OH)D₃ 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)D₃ 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)D₃ 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 implications⁴⁸; however, further studies are needed to assess the significance of this synergistic interaction between serum 25(OH)D₃ 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)D₃³¹; 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 diabetes⁴ 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)D₃; 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 smokers⁶⁵; 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.

Author affiliations

¹Division of Epidemiology, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada

²Discipline of Dental Public Health, Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada

³Division of Biostatistics, Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada

⁴Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA

⁵Institute for Clinical Evaluative Sciences, Toronto, Ontario, Canada

⁶Public Health Ontario, Toronto, Ontario, Canada

Contributors AMZ identified the research question, obtained the data, conducted the analysis, interpreted the data, drafted the manuscript, and reviewed and edited the manuscript. AMZ and LCR contributed to the analytic plan. OS provided statistical analysis support. CRQ, OS, RTD, and LCR took part in the conceptualization, provided methodological and content feedback, contributed to discussion, and reviewed and edited the final manuscript.

Funding This research was funded by a Connaught New Researcher Award held by LCR at the University of Toronto (207656).

Competing interests None declared.

Patient consent Not required.

Ethics approval The survey protocol for the NHANES was approved by CDC's National Center for Health Statistics Institutional Research Ethics Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement NHANES data sets are publicly available through the Centers for Disease Control and Prevention website at <https://www.cdc.gov/nchs/nhanes/Default.aspx>.

Open access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

© Article author(s) (or their employer(s) unless otherwise stated in the text of the article) 2018. All rights reserved. No commercial use is permitted unless otherwise expressly granted.

REFERENCES

- Eke PI, Dye BA, Wei L, *et al*. Update on prevalence of periodontitis in adults in the United States: NHANES 2009 to 2012. *J Periodontol* 2015;86:611–22.
- Lalla E, Papapanou PN. Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. *Nat Rev Endocrinol* 2011;7:738–48.
- Hajishengallis G. The inflammophilic character of the periodontitis-associated microbiota. *Mol Oral Microbiol* 2014;29:248–57.
- Demmer RT, Jacobs DR, Desvarieux M. Periodontal disease and incident type 2 diabetes: results from the First National Health and Nutrition Examination Survey and its epidemiologic follow-up study. *Diabetes Care* 2008;31:1373–9.
- Friedewald VE, Kornman KS, Beck JD, *et al*. The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease. *J Periodontol* 2009;80:1021–32.
- Cekici A, Kantarci A, Hasturk H, *et al*. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol* 2014;64:57–80.
- Hasturk H, Kantarci A, Van Dyke TE. Oral inflammatory diseases and systemic inflammation: role of the macrophage. *Front Immunol* 2012;3:118.
- Demmer RT, Breskin A, Rosenbaum M, *et al*. The subgingival microbiome, systemic inflammation and insulin resistance: The Oral Infections, Glucose Intolerance and Insulin Resistance Study. *J Clin Periodontol* 2017;44:255–65.
- Demmer RT, Squillaro A, Papapanou PN, *et al*. Periodontal infection, systemic inflammation, and insulin resistance: results from the continuous National Health and Nutrition Examination Survey (NHANES) 1999–2004. *Diabetes Care* 2012;35:2235–42.
- Mealey BL, Oates TW, American Academy of Periodontology. Diabetes mellitus and periodontal diseases. *J Periodontol* 2006;77:1289–303.
- Demmer RT, Desvarieux M, Holtfreter B, *et al*. Periodontal status and A1C change: longitudinal results from the study of health in Pomerania (SHIP). *Diabetes Care* 2010;33:1037–43.
- Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 2009;19:73–8.
- Adams JS, Hewison M. Update in vitamin D. *J Clin Endocrinol Metab* 2010;95:471–8.
- Holick MF, deficiency VD. Vitamin D deficiency. *N Engl J Med* 2007;357:266–81.
- Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? *Arch Biochem Biophys* 2012;523:123–33.
- Nibali L, Di Iorio A, Tu YK, *et al*. Host genetics role in the pathogenesis of periodontal disease and caries. *J Clin Periodontol* 2017;44(Suppl 18):S52–S78.
- Chen LL, Li H, Zhang PP, *et al*. Association between vitamin D receptor polymorphisms and periodontitis: a meta-analysis. *J Periodontol* 2012;83:1095–103.
- Sun JL, Meng HX, Cao CF, *et al*. Relationship between vitamin D receptor gene polymorphism and periodontitis. *J Periodontol Res* 2002;37:263–7.
- Tachi Y, Shimpuku H, Nosaka Y, *et al*. Vitamin D receptor gene polymorphism is associated with chronic periodontitis. *Life Sci* 2003;73:3313–21.
- Brett PM, Zygogianni P, Griffiths GS, *et al*. Functional gene polymorphisms in aggressive and chronic periodontitis. *J Dent Res* 2005;84:1149–53.
- Gunes S, Sumer AP, Keles GC, *et al*. Analysis of vitamin D receptor gene polymorphisms in patients with chronic periodontitis. *Indian J Med Res* 2008;127:58–64.
- Nibali L, Parkar M, D'Aiuto F, *et al*. Vitamin D receptor polymorphism (-1056 Taq-I) interacts with smoking for the presence and progression of periodontitis. *J Clin Periodontol* 2008;35:561–7.
- Laine ML, Crielaard W, Loos BG. Genetic susceptibility to periodontitis. *Periodontol* 2000 2012;58:37–68.
- Martelli FS, Martelli M, Rosati C, *et al*. Vitamin D: relevance in dental practice. *Clin Cases Miner Bone Metab* 2014;11:15.
- Zerwekh JE. Blood biomarkers of vitamin D status. *Am J Clin Nutr* 2008;87:1087S–91.
- Jones G. Pharmacokinetics of vitamin D toxicity. *Am J Clin Nutr* 2008;88:582S–6.
- Jukic AMZ, Hoofnagle AN, Lutsey PL. Measurement of Vitamin D for Epidemiologic and Clinical Research: Shining Light on a Complex Decision. *Am J Epidemiol* 2017.
- Lou YR, Molnár F, Peräkylä M, *et al*. 25-Hydroxyvitamin D(3) is an agonistic vitamin D receptor ligand. *J Steroid Biochem Mol Biol* 2010;118:162–70.
- Huet T, Laverny G, Ciesielski F, *et al*. A vitamin D receptor selectively activated by gemini analogs reveals ligand dependent and independent effects. *Cell Rep* 2015;10:516–26.
- Millen AE, Hovey KM, LaMonte MJ, *et al*. Plasma 25-hydroxyvitamin D concentrations and periodontal disease in postmenopausal women. *J Periodontol* 2013;84:1243–56.
- Dietrich T, Joshipura KJ, Dawson-Hughes B, *et al*. Association between serum concentrations of 25-hydroxyvitamin D3 and periodontal disease in the US population. *Am J Clin Nutr* 2004;80:108–13.
- Chapple IL, Bouchard P, Cagetti MG, *et al*. Interaction of lifestyle, behaviour or systemic diseases with dental caries and periodontal diseases: consensus report of group 2 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases. *J Clin Periodontol* 2017;44(Suppl 18):S39–S51.
- Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. *Eur J Clin Nutr* 2011;65:1005–15.
- George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabet Med* 2012;29:e142–e150.
- Zipf G, Chiappa M, Porter KS, *et al*. National health and nutrition examination survey: plan and operations, 1999–2010. *Vital Health Stat* 1 2013;56:1–37.
- Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition

- Examination Survey <https://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Questionnaire&CycleBeginYear=2009> (accessed 2 Feb 2018).
37. Centers for Disease Control and Prevention, National Center for Health Statistics. *National Health and Nutrition Examination Laboratory Protocol [Internet]*. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. <https://wwwn.cdc.gov/nchs/nhanes/Search/DataPage.aspx?Component=Laboratory&CycleBeginYear=2009> (accessed 2 Feb 2018).
 38. Matthews DR, Hosker JP, Rudenski AS, *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9.
 39. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487–95.
 40. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: *Standards of Medical Care in Diabetes-2018*. *Diabetes Care* 2018;41(Suppl 1):S13–s27.
 41. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS). *National Health and Nutrition Examination 2009-2010 Survey Operations Manuals*. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. <https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?BeginYear=2009> (accessed 2 Feb 2018).
 42. Dye BA, Li X, Lewis BG, *et al*. Overview and quality assurance for the oral health component of the National Health and Nutrition Examination Survey (NHANES), 2009-2010. *J Public Health Dent* 2014;74:248–56.
 43. Eke PI, Thornton-Evans GO, Wei L, *et al*. Accuracy of NHANES periodontal examination protocols. *J Dent Res* 2010;89:1208–13.
 44. Eke PI, Page RC, Wei L, *et al*. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol* 2012;83:1449–54.
 45. Centers for Disease Control and Prevention. Analytical Note for 25-Hydroxyvitamin D Data Analysis using NHANES III (1988–1994), NHANES 2001–2006, and NHANES 2007–2010. http://wwwn.cdc.gov/Nchs/Nhanes/VitaminD/AnalyticalNote.aspx?h=http%3A%2F%2Fwwwn.cdc.gov%2Fnchs%2Fdata%2Fnhanes%2Fnhanes_09_10%2FVID_F_met_Vitamin_D.pdf&t=2009-2010%20Vitamin%20D%20Lab%20Method (accessed 2 Feb 2018).
 46. Ross AC, Manson JE, Abrams SA, *et al*. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011;96:53–8.
 47. Johnson CL, Paulose-Ram R, Ogden CL, *et al*. National health and nutrition examination survey. Analytic guidelines, 1999-2010. 2013.
 48. VanderWeele TJ, Knol MJ. A Tutorial on Interaction. *Epidemiol Method* 2014;3:33–72.
 49. Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. *Int J Epidemiol* 2012;41:514–20.
 50. Kalilani L, Atashili J. Measuring additive interaction using odds ratios. *Epidemiol Perspect Innov* 2006;3:5.
 51. Mokdad AH, Ford ES, Bowman BA, *et al*. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 2003;289:76–9.
 52. Wortsman J, Matsuoka LY, Chen TC, *et al*. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72:690–3.
 53. Khabazi SM, Lee BK, Liu L. Joint effects of obesity and vitamin D insufficiency on insulin resistance and type 2 diabetes: results from the NHANES 2001–2006. *Diabetes Care* 2012;35:2048–54.
 54. Garcia MN, Hildebolt CF, Miley DD, *et al*. One-year effects of vitamin D and calcium supplementation on chronic periodontitis. *J Periodontol* 2011;82:25–32.
 55. De Filippis A, Fiorentino M, Guida L, *et al*. Vitamin D reduces the inflammatory response by Porphyromonas gingivalis infection by modulating human β -defensin-3 in human gingival epithelium and periodontal ligament cells. *Int Immunopharmacol* 2017;47:106–17.
 56. Demmer RT, Jacobs DR, Singh R, *et al*. Periodontal Bacteria and Prediabetes Prevalence in ORIGINS: The Oral Infections, Glucose Intolerance, and Insulin Resistance Study. *J Dent Res* 2015;94(9 Suppl):201s–11.
 57. Dietrich T, Nunn M, Dawson-Hughes B, *et al*. Association between serum concentrations of 25-hydroxyvitamin D and gingival inflammation. *Am J Clin Nutr* 2005;82:575–80.
 58. Zhan Y, Samietz S, Holtfreter B, *et al*. Prospective Study of Serum 25-hydroxy Vitamin D and Tooth Loss. *J Dent Res* 2014;93:639–44.
 59. Chapple IL, Genco R. Working group 2 of joint EFP/AAP workshop. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Clin Periodontol* 2013;40(Suppl 14):S106–S112.
 60. Choi YH, McKeown RE, Mayer-Davis EJ, *et al*. Association between periodontitis and impaired fasting glucose and diabetes. *Diabetes Care* 2011;34:381–6.
 61. Arora N, Papapanou PN, Rosenbaum M, *et al*. Periodontal infection, impaired fasting glucose and impaired glucose tolerance: results from the Continuous National Health and Nutrition Examination Survey 2009-2010. *J Clin Periodontol* 2014;41:643–52.
 62. Zuk A, Quiñonez C, Lebenbaum M, *et al*. The association between undiagnosed glycaemic abnormalities and cardiometabolic risk factors with periodontitis: results from 2007-2009 Canadian Health Measures Survey. *J Clin Periodontol* 2017;44:132–41.
 63. Major JM, Graubard BI, Dodd KW, *et al*. Variability and reproducibility of circulating vitamin D in a nationwide U.S. population. *J Clin Endocrinol Metab* 2013;98:97–104.
 64. Eke PI, Wei L, Thornton-Evans GO, *et al*. Risk Indicators for Periodontitis in US Adults: NHANES 2009 to 2012. *J Periodontol* 2016;87:1174–85.
 65. Kassi EN, Stavropoulos S, Kokkoris P, *et al*. Smoking is a significant determinant of low serum vitamin D in young and middle-aged healthy males. *Hormones* 2015;14:245–50.
 66. Jamal A, King BA, Neff LJ, *et al*. Current Cigarette Smoking Among Adults - United States, 2005-2015. *MMWR Morb Mortal Wkly Rep* 2016;65:1205–11.
 67. Ding C, Wilding JP, Bing C. 1,25-dihydroxyvitamin D3 protects against macrophage-induced activation of NF κ B and MAPK signalling and chemokine release in human adipocytes. *PLoS One* 2013;8:e61707.