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ABSTRACT

We conducted a cross-sectional analysis to evaluate the relationship between serum antibody titers against 19 selected oral microorganisms and measures of hyperglycemia in a large, nationally representative data set. The study population consisted of 7,848 participants from the National Health and Nutrition Examination Survey III (1988-1994) who were at least 40 yrs old, with complete serum IgG antibody data against 19 oral microorganisms. The 19 antibody titers were grouped into 4 categories via cluster analysis—orange-red, yellow-orange, orange-blue, and red-green—named to reflect predominant antibody titers against microorganisms in Socransky's classification scheme for oral microbes. Linear regression models weighted for complex survey design were used in which fasting blood glucose, fasting insulin, and HbA1c were outcomes and antibody cluster scores were exposures, adjusting for potential confounders. Higher orange-red cluster scores were associated with increased hyperglycemia, while higher orange-blue cluster scores were related with decreased hyperglycemia. A 1-unit-higher orange-red cluster score was associated with 0.46 mg/dL higher fasting blood glucose ($p = .0038$), and a 1-unit-higher orange-blue cluster score was associated with 0.34% lower HbA1c ($p = .0257$). Groups of antibody titers against periodontal microorganisms were associated with hyperglycemia independent of known risk factors.

KEY WORDS: periodontal microorganisms, antibody titer, periodontal diseases, diabetes, prediabetic state, HbA1c.

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Association between Serum Antibodies to Oral Microorganisms and Hyperglycemia in Adults

INTRODUCTION

Periodontal infection is associated with hyperglycemia and diabetes (Demmer *et al.*, 2012; Borgnakke *et al.*, 2013). Approximately 11% of U.S. adults have diabetes, and 39% have moderate to severe periodontal disease (Eke *et al.*, 2012). Diabetes prevalence is approximately 4 times higher in people with the most severe periodontal damage (Choi *et al.*, 2011), and periodontal treatment in diabetes generally improves glycemic control (Engelbreton and Kocher, 2013); however, a recent clinical trial of nonsurgical periodontal treatment showed otherwise (Engelbreton *et al.*, 2013).

There is emerging evidence to support the hypothesis that specific groups of periodontal microorganisms are associated with systemic outcomes. In a longitudinal analysis, carotid intimal medial thickness increase was attenuated with decreased counts of a selected group of oral microorganisms (Desvarieux *et al.*, 2013). Some studies have reported differences in the oral microorganism profile of individuals with and without type 2 diabetes (Ebersole *et al.*, 2008; Makiura *et al.*, 2008), while others have not (Yuan *et al.*, 2001; da Cruz *et al.*, 2008; Field *et al.*, 2012). *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella* spp., *Fusobacterium* spp., and a few other organisms have been associated with type 2 diabetes (Ebersole *et al.*, 2008; Makiura *et al.*, 2008; Casarin *et al.*, 2013; Zhou *et al.*, 2013). The studies evaluating this relationship had small sample sizes and were heterogeneous with respect to method of microbial assessment, age, severity of periodontal disease, degree of diabetes control, and treatment of both periodontal disease and diabetes.

IgG antibody titers are produced in response to infections and remain elevated over a period of 30 mo even after periodontal treatment (Papapanou *et al.*, 2004), and *P. gingivalis* and *A. actinomycetemcomitans* were found stable over 15 yrs among individuals who had periodontitis (Lakio *et al.*, 2009). IgG antibody titers against oral microorganisms may therefore represent a cumulative measure of periodontal infection. In 2008, the Centers for Disease Control and Prevention released IgG antibody titer values for oral microorganisms (Vlachojannis *et al.*, 2010) from analysis of stored serum samples of individuals who participated in the National Health and Nutrition Examination Survey III (NHANES III), conducted between 1988 and 1994. The analyses in this report are based on those data. The aim of this study was to evaluate the relationship between serum antibody titers against 19 selected microorganisms and measures of hyperglycemia in a large, nationally representative sample of U.S. adults.

MATERIALS & METHODS

Data Source

We conducted a cross-sectional analysis of the NHANES III data, a nationally representative sample of the noninstitutionalized U.S. population, collected through multistage probability cluster sampling. The data included information on a variety of health risks and behaviors, diabetes, periodontal disease, and clinical and antibody information related to periodontal disease (Ezzati *et al.*, 1992).

The study population consisted of NHANES III participants who were at least 40 yrs old, with complete serum IgG antibody data against 19 oral microorganisms. Records of these individuals were linked with relevant dental, medical, socioeconomic, anthropometric, laboratory, and nutritional information available in NHANES III. All data for this report are available at <http://www.cdc.gov/nchs/nhanes/nh3data.htm>.

Study Population

Of 33,994 participants, 14,464 were at least 40 yrs old; 11,448 completed interviews; 9,379 underwent examinations; and 8,153 provided blood samples, which were analyzed for IgG antibody titer levels against a broad panel of 19 periodontal bacteria (Vlachojannis *et al.*, 2010). Individuals were excluded if they reported taking insulin (mostly type 1 diabetes) or had gestational diabetes, leaving 7,848 participants in the final sample.

IgG Assay for Periodontal Bacteria

Sera from 8,153 NHANES III participants aged 40+ yrs were tested for IgG antibodies against 19 oral bacterial species via the “checkerboard” immunoassay technique at the Oral and Diagnostic Sciences Laboratory, Columbia University College of Dental Medicine; results were reported in gravimetric units. Details are described in NHANES III documentation (Centers for Disease Control and Prevention, 2008). The strains used to prepare whole cell antigenic extracts for checkerboard immunoassay are provided in the Appendix.

Cluster Formation and Naming the Clusters

Serum IgG antibody titer values for each bacterial species were natural log transformed and standardized by dividing by the log-transformed population standard deviation. To form antibody clusters, we used data from individuals with HbA1c ≥ 5.7 ($n = 4,424$). Standardized z scores of IgG antibodies against 19 periodontal bacteria were included in cluster analysis to derive 4 mutually distinct groups of IgG antibodies against periodontal bacteria, as described in the Figure. After deriving the clusters in this group, we calculated cluster scores for everybody in the population (healthy, prediabetes, diabetes) by summing z scores for each antibody titer ($n = 7,874$). For instance, one cluster consisted of *P. melaninogenica*, *P. intermedia*, *P. nigrescens*, and *P. gingivalis*. The z scores of these antibody titers were summed to obtain a score for that cluster.

Figure. Composition of 4 mutually exclusive clusters formed via cluster analysis of z scores of serum antibody titers against 19 periodontal bacteria. Naming of clusters was done through Socranksy's microbial color complexes, shown in parentheses.

To name the clusters, we adapted Socranksy's color coding for periodontal bacteria as follows (Socranksy and Haffajee, 2002, 2005): Cluster 1 included antibodies against 4 organisms, of which 3 were from Socranksy's orange complex and one was from the red complex; we named that cluster orange-red. Based on this approach, the 4 clusters were named orange-red, red-green, yellow-orange, and orange-blue, as shown in the Figure.

In Socranksy's scheme, organisms in the red and orange clusters are related to periodontal disease; yellow and purple cluster organisms are associated with a healthy periodontal state; blue cluster organisms (including the *Actinomyces* sp.) are found in both periodontal disease and healthy states; and green complex organisms are weakly related to periodontal disease (Socranksy and Haffajee, 2002, 2005).

Exposure Measures

Cluster scores were computed for each individual in the study by summing the standardized z scores of IgG titers against oral microorganisms in each of the 4 clusters. These 4 cluster scores were the main exposure variables used in this analysis.

Outcome Measures

The 3 outcomes, which were log transformed, were fasting plasma insulin ($n = 7,812$), fasting plasma glucose ($n = 7,812$), and HbA1c percentage ($n = 7,833$).

Covariate Information

Covariates included age, sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American and others), income-to-poverty ratio (≤ 1.5 , >1.5 to ≤ 3.0 , and >3.0), years of formal education (≤ 6 yrs, 7 to 12 yrs, ≥ 13 yrs), smoking and alcohol intake (current, past, never), physical activity metabolic equivalents (active, ≥ 6 ; moderate, ≥ 4 to <6 ; less active, <4), central adiposity (≥ 101.6 cm for males and ≥ 88.9 cm for females) (Choi *et al.*, 2011), and diet (daily intake of calories; grams of carbohydrate, protein, fat, and fiber). Body mass index was computed from measured height and weight by dividing weight in kilograms by height in meters squared. Annual visits to the dentist was a binary variable (yes/no).

Table 1. Characteristics of Study Population: National Health and Nutrition Examination Survey III (1988-1994)

Characteristic	Healthy (n = 3,424)	Prediabetes (n = 3,334)	Diabetes (n = 1,090)
Age, yrs	53.85 (0.3)	59.5 (0.6)	61.7 (0.6)
Sex			
Male	42.7	50.2	46.8
Female	57.3	49.8	53.2
Race/ethnicity			
Non-Hispanic whites	85.3	76.9	71.6
Non-Hispanic blacks	6.1	11.3	14.6
Mexican American	2.6	3.9	6.0
Others	5.9	7.9	7.7
Education, yrs			
≤6	4.7	8.5	12.3
7-12	50.4	57.6	62.2
≥13	44.9	33.9	25.4
Income:poverty ratio			
Lower, ≤1.5	15.0	22.0	30.2
Middle, ≤3.0	28.6	30.5	31.5
Higher, >3.0	56.4	47.4	38.3
Smoking			
Current	41.6	35.1	37.2
Past	41.7	46.4	47.3
Never	16.7	18.5	15.5
Alcohol intake			
Current	13.9	15.3	20.0
Past	34.0	42.2	48.9
Never	52.0	42.5	31.1
Physical activity			
Sedentary	54.8	49.3	45.6
Moderately active	12.3	13.4	11.1
Vigorously active	8.0	7.4	6.7
Missing	24.9	29.8	36.6
Missing teeth			
0	53.1	51.7	50.5
1-5	37.1	34.7	29.7
6-10	7.7	9.3	12.1
>10	2.1	4.3	7.8
Annual visits to dentist			
Yes	59.1	43.9	38.4
No	40.9	56.1	61.6
Waist circumference			
Normal	60.0	45.2	23.8
Elevated	40.0	54.8	76.2
Nutrition, g/d			
Dietary fibers	17.2 (0.3)	16.4 (0.3)	16.2 (0.5)
Total carbohydrate	250.5 (4.1)	243.9 (4.1)	222.7 (5.1)
Proteins	76.6 (1.0)	77.8 (1.3)	75.0 (2.2)
Total fats	77.0 (1.3)	75.7 (1.6)	70.4 (2.8)
Body mass index, kg/m ²	26.4 (0.1)	27.8 (0.2)	30.1 (0.3)
Score			
Orange-red	-0.5 (0.1)	-0.3 (0.1)	0.1 (0.2)
Red-green	-0.1 (0.3)	0.02 (0.3)	-0.2 (0.5)
Yellow-orange	-0.01 (0.2)	0.004 (0.2)	0.06 (0.3)
Orange-blue	0.2 (0.1)	0.07 (0.1)	-0.04 (0.1)

Values with parentheses indicate mean (SE). Stand-alone values indicate percentages.

Table 2. Association between Cluster Scores and Fasting Blood Glucose, Glycemic Hemoglobin, and Fasting Insulin

	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue
	$e^{\beta} - 1$ (95% CI)	$e^{\beta} - 1$ (95% CI)	$e^{\beta} - 1$ (95% CI)	$e^{\beta} - 1$ (95% CI)
Fasting blood glucose				
Model 1	0.60 (0.32, 0.88)	0.02 (−0.29, 0.33)	−0.21 (−0.54, 0.12)	−0.72 (−1.09, −0.35)
Model 2	0.51 (0.22, 0.81)	−0.08 (−0.38, 0.23)	−0.10 (−0.42, 0.23)	−0.49 (−0.87, −0.11)
Model 3	0.43 (0.14, 0.72)	−0.04 (−0.34, 0.25)	−0.07 (−0.40, 0.25)	−0.38 (−0.81, 0.06)
Model 4	0.46 (0.15, 0.76)	−0.08 (−0.38, 0.22)	−0.02 (−0.36, 0.31)	−0.35 (−0.79, 0.10)
HbA1c				
Model 1	0.34 (0.15, 0.55)	0.03 (−0.16, 0.22)	−0.05 (−0.27, 0.17)	−0.68 (−0.98, −0.39)
Model 2	0.51 (0.22, 0.81)	−0.08 (−0.38, 0.23)	−0.10 (−0.42, 0.23)	−0.49 (−0.87, −0.11)
Model 3	0.17 (−0.06, 0.39)	−0.04 (−0.19, 0.13)	0.11 (−0.09, 0.31)	−0.30 (−0.59, −0.01)
Model 4	0.17 (−0.07, 0.40)	−0.02 (−0.18, 0.15)	0.09 (−0.10, 0.29)	−0.34 (−0.64, −0.04)
Fasting insulin				
Model 1	1.34 (0.40, 2.29)	0.61 (−0.09, 1.31)	−1.03 (−1.98, −0.07)	−0.89 (−2.06, 0.29)
Model 2	1.21 (0.27, 2.16)	0.51 (−0.19, 1.21)	−0.89 (−1.84, 0.07)	−0.67 (−0.82, 0.48)
Model 3	0.60 (−0.05, 1.25)	0.61 (−0.09, 1.31)	−0.77 (−1.61, 0.08)	−0.82 (−1.90, 0.27)
Model 4	0.69 (0.002, 1.39)	0.47 (−0.26, 1.22)	−0.58 (−1.48, 0.34)	−0.69 (−0.80, 0.43)

Back-transformed to natural units by exponentiation log-transformed estimates. CI, confidence interval. Bold indicates $p < .05$.

Orange-red: *P. melaninogenica*, *P. intermedia*, *P. nigrescens*, *P. gingivalis*. Red-green: *T. forsythia*, *T. denticola*, *A. actinomycetemcomitans*, *E. corrodens*, *S. noxia*, *V. parvula*, *C. rectus*. Yellow-orange: *S. intermedius*, *S. oralis*, *S. mutans*, *F. nucleatum*, *P. micra* (previously *M. micros*), *C. ochracea*. Orange-blue: *E. nodatum*, *A. naeslundii*.

Model 1: Crude association. Model 2: Age and sex adjusted. Model 3: Adjusted further for age, sex, education, race/ethnicity, income:poverty ratio, smoking, alcohol, physical activity, fiber intake, dentist visits, body mass index, and waist circumference. Model 4: Adjusted further for protein intake, carbohydrate intake, total fat, total calories, fiber intake, and dentist visits, including the variables in model 3.

Statistical Methods

SAS 9.3 (SAS Institute, Cary, NC) was used for data management and statistical analyses. SAS survey procedures were used with sample weights, cluster, and strata variables provided by the Center for Disease Control and Prevention to account for the complex sampling design. The threshold for statistical significance was 0.05.

Descriptive statistics were estimated via procedures for complex surveys in SAS (proc surveymeans and proc surveyfreq) (Table 1). Linear regression models were run with log-transformed fasting plasma glucose, HbA1c, and fasting insulin as outcomes and with the 4 z score clusters as the predictors. The second model was adjusted for age and sex. The third model was further adjusted for race and ethnicity, income:poverty ratio, smoking, drinking alcohol, physical activity, fiber intake (continuous), dentist visits, waist circumference, and body mass index (continuous) (Table 2). These results were further stratified by teeth present (dentate and edentulous) (Table 3) and periodontal status, as defined by Eke *et al.* (2012): (1) no or mild periodontal destruction or (2) moderate or severe periodontal destruction (Table 4).

RESULTS

In this sample, 9.6% of individuals had diabetes, and 36.6% had prediabetes. Individuals with prediabetes or diabetes were more likely to be older, male, nonwhite, with <12 yrs of formal education, belonging to the lower and middle-income categories;

they were more likely to be past or current smokers and alcohol drinkers, have higher adiposity measures, consume less fiber, miss >6 teeth, and be less likely to visit a dentist yearly (Table 1).

Antibody clusters were associated with measures of hyperglycemia. A 1-unit-higher orange-red cluster score corresponded with 0.46 mg/dL higher fasting plasma glucose ($p = .0038$), and 1.69 μ U/mL higher fasting insulin ($p = .0494$) after multivariable adjustment (Table 2). In contrast, a 1-unit-higher orange-blue cluster score was associated with 0.34% lower HbA1c ($p = .0257$). Red-green and yellow-orange clusters were not associated with any of the 3 hyperglycemia outcome measures (Table 2). Among the dentate population, the orange-blue cluster remained inversely associated with HbA1c and the orange-red cluster positively associated with fasting insulin. The association between the orange-red cluster and fasting glucose was positive in the edentulous population and positive but nonsignificant in the dentate population (Table 3). Among individuals with moderate or severe periodontal destruction, the orange-blue cluster remained inversely associated with HbA1c and the orange-red cluster positively associated with fasting insulin. These associations were qualitatively similar among individuals without periodontal disease, albeit they were not statistically significant (Table 4).

DISCUSSION

Antibody clusters were associated with measures of hyperglycemia independent of other risk factors. Orange-red antibody cluster scores were positively correlated with fasting plasma

Table 3. Association between Cluster Scores and Fasting Blood Glucose, Glycemic Hemoglobin, and Fasting Insulin in the Dentate and Edentulous Population

	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue
	e ^β - 1 (95% CI)	e ^β - 1 (95% CI)	e ^β - 1 (95% CI)	e ^β - 1 (95% CI)
Dentate population (n = 5,430)				
Fasting blood glucose				
Model 1	0.53 (0.27, 0.80)	0.16 (-0.19, 0.50)	-0.31 (-0.66, 0.05)	-0.97 (-1.42, -0.51)
Model 2	0.37 (0.09, 0.64)	0.05 (-0.27, 0.37)	-0.16 (-0.49, 0.17)	-0.71 (-1.18, -0.24)
Model 3	0.22 (-0.07, 0.51)	0.10 (-0.23, 0.43)	-0.17 (-0.52, 0.18)	-0.47 (-1.00, 0.06)
Model 4	0.26 (-0.04, 0.56)	0.07 (-0.26, 0.39)	-0.12 (-0.48, 0.24)	-0.48 (-1.01, 0.06)
HbA1c				
Model 1	0.38 (0.19, 0.57)	0.10 (-0.10, 0.30)	-0.10 (-0.35, 0.14)	-0.81 (-1.20, -0.44)
Model 2	0.26 (0.08, 0.44)	0.02 (-0.15, 0.20)	-0.004 (-0.24, 0.24)	-0.56 (-0.92, -0.20)
Model 3	0.02 (-0.20, 0.24)	-0.01 (-0.18, 0.17)	0.12 (-0.11, 0.40)	-0.30 (-0.65, -0.42)
Model 4	0.01 (-0.21, 0.24)	0.01 (-0.16, 0.18)	0.11 (-0.12, 0.35)	-0.37 (-0.73, -0.01)
Fasting insulin				
Model 1	1.37 (0.49, 2.26)	0.86 (0.13, 1.60)	-1.31 (-2.23, -0.38)	-1.58 (-2.93, -0.20)
Model 2	1.10 (0.22, 2.0)	0.71 (-0.005, 1.44)	-1.08 (-2.0, -0.16)	-1.30 (-2.67, 0.04)
Model 3	0.33 (-0.34, 1.0)	0.85 (0.09, 1.61)	-0.93 (-1.80, -0.06)	-1.01 (-2.26, 0.25)
Model 4	0.48 (-0.25, 1.21)	0.70 (-0.18, 1.5)	-0.71 (-1.64, 0.22)	-0.90 (-2.18, 0.40)
Edentulous population (n = 1,484)				
Fasting blood glucose				
Model 1	1.15 (0.49, 1.81)	-0.59 (-1.11, -0.07)	0.04 (-0.65, 0.74)	0.06 (-0.63, 0.75)
Model 2	1.11 (0.47, 1.76)	-0.69 (-1.21, -0.17)	0.18 (-0.52, 0.87)	0.14 (-0.54, 0.82)
Model 3	0.97 (0.45, 1.50)	-0.66 (-1.10, -0.23)	0.45 (-0.07, 0.97)	0.11 (-0.50, 0.71)
Model 4	0.98 (0.46, 1.51)	-0.68 (-1.11, -0.25)	0.47 (-0.06, 1.00)	0.13 (-0.48, 0.74)
HbA1c				
Model 1	0.59 (0.08, 1.09)	-0.09 (-0.44, 0.25)	-0.12 (-0.64, 0.40)	-0.28 (-0.85, 0.30)
Model 2	0.57 (0.06, 1.08)	-0.15 (-0.50, 0.21)	-0.05 (-0.57, 0.47)	-0.24 (-0.81, 0.34)
Model 3	0.38 (-0.04, 0.80)	-0.14 (-0.48, 0.21)	0.17 (-0.26, 0.60)	-0.30 (-0.81, 0.20)
Model 4	0.35 (-0.06, 0.77)	-0.07 (-0.40, 0.26)	0.11 (-0.30, 0.52)	-0.25 (-0.74, 0.24)
Fasting insulin				
Model 1	1.52 (-0.60, 3.70)	-0.18 (-2.02, 1.70)	-0.48 (-2.83, 1.93)	2.93 (0.93, 4.98)
Model 2	1.67 (-0.46, 3.84)	-0.09 (-1.99, 1.84)	-0.61 (-2.97, 1.82)	2.88 (0.82, 4.98)
Model 3	1.07 (-0.45, 2.61)	-0.46 (-1.76, 0.86)	0.31 (-1.61, 2.26)	1.11 (-0.81, 3.07)
Model 4	1.03 (-0.55, 2.62)	-0.43 (-1.77, 0.93)	0.26 (-1.69, 2.25)	1.33 (-0.69, 3.39)

Back-transformed to natural units by exponentiation log transformed estimates. CI, confidence interval. Bold indicates $p < .05$.

Orange-red: *P. melaninogenica*, *P. intermedia*, *P. nigrescens*, *P. gingivalis*. Red-green: *T. forsythia*, *T. denticola*, *A. actinomycetemcomitans*, *E. corrodens*, *S. noxia*, *V. parvula*, *C. rectus*. Yellow-orange: *S. intermedius*, *S. oralis*, *S. mutans*, *F. nucleatum*, *P. micra* (previously *M. micros*), *C. ochracea*. Orange-blue: *E. nodatum*, *A. naeslundii*.

Model 1: Crude association. Model 2: Age and sex adjusted. Model 3: Adjusted further for age, sex, education, race/ethnicity, income:poverty ratio, smoking, alcohol, physical activity, dentist visits, body mass index, and waist circumference. Model 4: Adjusted further for protein intake, carbohydrate intake, total fat, total calories, fiber intake, and dentist visits, including the variables in model 3.

glucose and fasting insulin, and orange-blue antibody cluster scores were negatively correlated with HbA1c, after accounting for other risk factors in multivariable analyses.

The orange-red cluster of antibodies included *P. gingivalis*, which has been found to be increased among individuals with diabetes (Ebersole *et al.*, 2008; Makiura *et al.*, 2008). IgG antibody levels against *P. gingivalis* are highest among individuals with periodontal disease. The IgG levels decline following periodontal treatment; however, they level out but still are higher than levels among individuals who never had periodontal disease (Kudo *et al.*, 2012). IgG antibodies against *P. gingivalis* are a marker of periodontal disease among individuals without diabetes (Kudo *et al.*, 2012) and may also be an indicator of periodontal disease in people with diabetes. Another explanation of

these findings may be that as *P. gingivalis* is linked with systemic inflammation (Hayashi *et al.*, 2010), which can impair insulin action and lead to hyperglycemia (Gregor and Hotamisligil, 2011). Periodontal treatment has been shown to reduce antibody titers against *P. gingivalis* (Okada *et al.*, 2013) and improve glycemic control in people with diabetes (Sgolastra *et al.*, 2013). In hyperglycemia, advanced glycation end products are deposited in the periodontal tissues (Lalla and Papapanou, 2011), which can lead to increased inflammation and periodontal destruction and possibly increased levels of *P. gingivalis* and its corresponding antibody titers.

The orange-blue cluster includes *A. naeslundii* and *E. nodatum* antibodies; *A. naeslundii* has been associated with a healthy state in oral (Socransky and Haffajee, 2002, 2005) and systemic

Table 4. Association between Cluster Scores and Fasting Blood Glucose, Glycemic Hemoglobin, and Fasting Insulin Stratified by Periodontal Destruction in Dentate Population

	Orange-Red	Red-Green	Yellow-Orange	Orange-Blue
	$e^{\beta} - 1$ (95% CI)	$e^{\beta} - 1$ (95% CI)	$e^{\beta} - 1$ (95% CI)	$e^{\beta} - 1$ (95% CI)
No periodontal destruction ($n = 3,059$)				
Fasting blood glucose				
Model 1	0.39 (0.03, 0.75)	0.07 (-0.38, 0.52)	-0.22 (-0.61, 0.18)	-0.89 (-1.44, -0.33)
Model 2	0.22 (-0.14, 0.58)	-0.00 (-0.42, 0.42)	-0.11 (-0.47, 0.26)	-0.58 (-1.17, 0.02)
Model 3	0.08 (-0.23, 0.40)	0.04 (-0.35, 0.43)	-0.13 (-0.49, 0.22)	-0.24 (-0.84, 0.36)
Model 4	0.10 (-0.24, 0.44)	0.04 (-0.37, 0.45)	-0.10 (-0.47, 0.27)	-0.22 (-0.82, 0.38)
HbA1c				
Model 1	0.32 (0.09, 0.55)	-0.006 (-0.26, 0.25)	-0.002 (-0.33, 0.32)	-0.69 (-1.13, -0.25)
Model 2	0.21 (-0.01, 0.43)	-0.04 (-0.26, 0.18)	0.05 (-0.24, 0.35)	-0.39 (-0.80, 0.02)
Model 3	-0.02 (-0.28, 0.23)	-0.05 (-0.25, 0.15)	0.15 (-0.11, 0.40)	-0.09 (-0.49, 0.31)
Model 4	-0.07 (-0.31, 0.17)	-0.01 (-0.22, 0.20)	0.12 (-0.14, 0.38)	-0.17 (-0.58, 0.25)
Fasting insulin				
Model 1	1.45 (-0.34, 3.26)	0.50 (-0.27, 1.28)	-0.74 (-1.74, 0.27)	-1.88 (-3.52, -0.21)
Model 2	1.17 (-0.59, 2.96)	0.39 (-0.40, 1.18)	-0.55 (-1.56, 0.47)	-1.49 (-3.13, 0.17)
Model 3	0.80 (-0.15, 1.77)	0.62 (-0.06, 1.31)	-0.73 (-1.69, 0.25)	-0.91 (-2.34, 0.54)
Model 4	0.90 (-0.03, 1.83)	0.46 (-0.27, 1.19)	-0.46 (-1.46, 0.56)	-0.57 (-2.11, 1.0)
Moderate or severe periodontal destruction ($n = 2,371$)				
Fasting blood glucose				
Model 1	0.39 (0.03, 0.75)	0.07 (-0.38, 0.52)	-0.22 (-0.61, 0.18)	-0.89 (-1.44, -0.33)
Model 2	0.59 (0.21, 0.97)	0.19 (-0.43, 0.82)	-0.31 (-1.05, 0.44)	-0.85 (-1.53, -0.16)
Model 3	0.46 (0.03, 0.89)	0.23 (-0.49, 0.96)	-0.27 (-1.14, 0.62)	-0.92 (-1.72, -0.11)
Model 4	0.48 (-0.01, 0.97)	0.17 (-0.54, 0.88)	-0.18 (-1.04, 0.69)	-0.92 (-1.77, -0.06)
HbA1c				
Model 1	0.39 (0.07, 0.70)	0.26 (-0.16, 0.68)	-0.25 (-0.74, 0.25)	-0.75 (-1.32, -0.18)
Model 2	0.33 (-0.006, 0.66)	0.18 (-0.23, 0.60)	-0.15 (-0.64, 0.34)	-0.69 (-1.25, -0.12)
Model 3	0.13 (-0.24, 0.51)	0.13 (-0.36, 0.62)	0.01 (-0.53, 0.56)	-0.67 (-1.26, -0.08)
Model 4	0.16 (-0.26, 0.59)	0.11 (-0.38, 0.60)	0.05 (-0.51, 0.61)	-0.69 (-1.33, -0.05)
Fasting insulin				
Model 1	1.06 (-0.48, 2.63)	1.67 (-0.10, 3.48)	-2.57 (-4.73, -0.37)	-0.26 (-2.33, 1.86)
Model 2	0.95 (-0.61, 2.52)	1.69 (-0.02, 3.43)	-2.54 (-4.62, -0.41)	-0.43 (-2.52, 1.71)
Model 3	-0.37 (-1.73, 1.002)	1.14 (-0.57, 2.88)	-1.29 (-3.42, 0.90)	-1.03 (-2.88, 0.86)
Model 4	-0.04 (-1.43, 1.38)	1.02 (-0.65, 2.72)	-1.21 (-3.31, 0.95)	-1.28 (-3.18, 0.64)

Back-transformed to natural units by exponentiation log-transformed estimates. CI, confidence interval. Bold indicates $p < .05$.

Orange-red: *P. melaninogenica*, *P. intermedia*, *P. nigrescens*, *P. gingivalis*. Red-green: *T. forsythia*, *T. denticola*, *A. actinomycetemcomitans*, *E. corrodens*, *S. noxia*, *V. parvula*, *C. rectus*. Yellow-orange: *S. intermedius*, *S. oralis*, *S. mutans*, *F. nucleatum*, *P. micra* [previously *M. micros*], *C. ochracea*. Orange-blue: *E. nodatum*, *A. naeslundii*.

Model 1: crude association. Model 2: Age and sex adjusted. Model 3: Adjusted further for age, sex, education, race/ethnicity, income:poverty ratio, smoking, alcohol, physical activity, dentist visits, body mass index, and waist circumference. Model 4: Adjusted further for protein intake, carbohydrate intake, total fat, total calories, fiber intake, and dentist visits including the variables in model 3.

conditions (Desvarieux *et al.*, 2013), albeit through incompletely understood mechanisms. Even though the presence of *E. nodatum* and *T. denticola* in the mouth has been associated with periodontal disease (Haffajee *et al.*, 2006), IgG antibodies against *E. nodatum* (orange complex), *F. nucleatum* (orange complex), and *T. denticola* (red complex) were higher in controls as compared with cases of periodontal disease (Papapanou *et al.*, 2000). Higher antibody levels against these organisms may prevent subsequent colonization of these organisms in the mouth and thus be protective against new periodontal lesions, which may explain this finding.

This study has limitations. First, because of the cross-sectional design it was not possible to determine whether

antibody titers contributed to hyperglycemia or were a consequence of it. For the same reason, the individuals in the study are prone to survivorship bias because those with more serious disease either died or did not participate in the survey. If the relationship between oral microorganisms and hyperglycemia were indeed causal, this bias would attenuate the associations that we detected. Second, we did not adjust for clinically defined periodontal disease (*e.g.*, clinical attachment loss), because proliferation of periodontal microorganisms precedes periodontal destruction. Clinically defined, periodontal disease is thus on the causal pathway between oral microorganisms and the outcomes, and adjusting for it would cause bias (VanderWeele, 2009). The results were qualitatively similar after stratification

by periodontal status. Moreover, we previously reported that clinical attachment loss and periodontal pocket depth were positively associated with diabetes and impaired fasting glucose in these data (Choi *et al.*, 2011). Third, we were able to evaluate only 19 of the many oral microorganisms in the mouth. Fourth, we included edentulous individuals in the main analysis because they have elevated IgG antibodies as a result of long-term prior exposure to oral microorganisms. Excluding edentulous individuals did not materially alter the results (Table 3). Fifth, although the data were not recent (*i.e.*, 1988-1994) and prevalence of disease has changed (Eke *et al.*, 2012), this should not influence the relation between serum antibodies and measures of hyperglycemia. Last, because this is an observational study, unmeasured confounding is possible, even though we accounted for a number of factors in the analyses.

The strengths of this study were as follows: First, this was a large representative population-based sample, reducing the likelihood of false-negative results. To our knowledge, this is the largest study to evaluate the relation between titers against oral microorganisms and hyperglycemia. Second, the antibody titers (Papapanou *et al.*, 2001; Centers for Disease Control and Prevention, 2008) and the outcomes were measured in a standardized way. Third, we adjusted for a number of potential confounders, thereby reducing the chances of bias; moreover, the results were consistent in all analyses. Fourth, we grouped the antibody titers against oral microorganisms via cluster analysis. This method uses the correlations among different variables to form mutually exclusive groups. This was an appropriate method to classify antibodies against the 19 oral microorganisms because the microorganisms are correlated and their roles not yet clearly defined. We obtained cluster scores by summing *z* scores of the titers. We did this because we were interested in evaluating the relative (rather than absolute) contributions of the titers to the overall score. Forming the clusters also reduced the data, thereby minimizing the chance of spurious results from multiple testing (Wang *et al.*, 2007). Finally, in the multivariable models, we evaluated the clusters together. Thus, a 1-unit increase in the orange-red titer score represented a corresponding change in a measure of hyperglycemia, holding the other clusters constant. With this approach, we were able to evaluate the composition antibody titers in relation to the outcome. This measure is biologically important because oral microorganisms are ubiquitous and changes in microbial composition occur relative to other organisms in the mouth (Desvarieux *et al.*, 2013; Socransky & Haffajee, 2002, 2005).

Groups of antibody titers against periodontal microorganisms were associated with hyperglycemia independent of known risk factors. Antibody titers against periodontal microorganisms may characterize the relation between periodontal disease and hyperglycemia.

AUTHOR CONTRIBUTIONS

A.T.M. conceived the idea, wrote the first draft, directed the analyses, interpreted the results, and revised the manuscript. D.S. analyzed the data, interpreted the results, and revised the

manuscript. C.C., Y.C., L.H., and J.Z. participated in analyses, helped with interpretation, and revised the manuscript.

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