

SYNERGISM BETWEEN OBESITY AND POOR ORAL HEALTH ASSOCIATED WITH DIABETES IN AN ELDERLY HUMAN POPULATION

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Abstract. We investigated associations between type 2 diabetes (DM) and several variables, including poor oral health and overweight/obesity, among a group of elderly Hmong subjects (60 years and older) who emigrated to the United States following the Vietnam conflict. Each subject was interviewed and their weight, height and waist circumference were measured. Each subject had an oral health examination. Each subject's saliva was analyzed for seven components related to inflammation. The presence of DM was correlated with poor oral health (POH) and overweight/obesity (OW) separately. There was a strong correlation between concurrent POH and OW and the presence of DM: all subjects with both POH and OW had DM. Logistic multivariate analysis of OW, POH, age, years of residence in California, and stress level revealed a significant association between the presence of DM and concurrent OW and POH. A change in diet after immigration was excluded as an explanatory variable. Subjects with DM and concurrent OW and POH had significantly elevated salivary levels of five analytes related to chronic inflammation. The association between POH and OW and the presence of DM needs further study.

Keywords: oral health, obesity, type 2 diabetes, elderly population

INTRODUCTION

There is a strong link between obesity and the chronic inflammatory diseases (CIDs) (Despres and Lemieux, 2006; Hotamisligil, 2006). The CIDs are more frequent among the elderly in whom they are more difficult and more expensive to treat (Albright and Albright, 2003; Naughton *et al*,

2006; Thorpe and Philyaw, 2012). There is a link between disorders, such as Type 2 diabetes (DM), cardiovascular disorders, rheumatoid arthritis, and the presence of poor oral health (POH), especially periodontitis (Pussinen *et al*, 2007; Pischon *et al*, 2008; Inaba and Amano, 2010). The link between POH and DM is well known and has received much attention (Lamster *et al*, 2008) The emphasis has centered on exacerbation of POH by the presence of DM (Loe, 1993). The existence of a "two-way connection" between POH and DM

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has been proposed (Grossi and Genco, 1998; Taylor, 2001; Demmer *et al*, 2008). DM is associated with disorders which have inflammation as a component.

Relatively little attention has been given to POH among the elderly. The prevalence of POH among economically and socially disadvantaged elderly in the United States is much higher than it should be; a report by the US Department of Health and Human Services (2000) revealed the prevalence of severe periodontal disease was 23% among adults aged 65-74 years and the frequency of edentulousness was 24%. Four years later, those numbers had not changed much; for example, edentulousness among those aged 65-74 years was approximately 21% (CDC, 2004). The poverty rate remained flat over that period (CDC, 2007). Worldwide, POH among people of all ages has been largely ignored. In some developing countries the concentration of dentists in the population is less than one per hundred thousand population and in some countries there are fewer than 10 dentists for the entire population, and the dentists who are practicing in those countries are nearly all located in large cities (WHO, 2009). People in the rural and semirural areas are unlikely to have ever been examined by a dentist. Edentulousness and oral pain are considered an inevitable consequence of growing old. In both developed and some developing countries the proportion of the population in the older age group is increasing (UNPD, 2010). The World Health Organization Global Oral Health Programme reported: "Globally, the burden of oral disease is particularly high among older people and has a negative effect on their quality of life" (Petersen and Yamamoto, 2005). DM and cardiovascular disorders have been linked to excess body weight

(overweight, OW) and POH (Campus *et al*, 2005; Hotamisligil, 2006; Demmer *et al*, 2008; Inaba and Amano, 2010). POH can be a major contributing factor to OW (Reeves *et al*, 2006; Goodson *et al*, 2009). The likelihood that OW and POH may interact in synergistic fashion in the pathogenesis of CIDs has been proposed, and a supporting model presented (Genco *et al*, 2005). To date, evidence in support of synergism has been limited (Albright, 2010; Lamster *et al*, 2008). In a previous report (Albright, 2010) we presented evidence of synergism between OW and POH and the pathogenesis of diabetes among elderly, immigrant Hmong people located in the Central Valley of California. In this study, we conducted oral examinations in that population and analyzed the saliva for the presence of seven substances likely to be related to chronic inflammation.

MATERIALS AND METHODS

Study population

The subjects enrolled in this study were taken from a large group of elderly Hmong immigrants involved in an earlier survey (Albright, 2010). The previous study included 495 elderly Hmong living in Merced County, California, USA. Each of those subjects filled out a questionnaire used in the previous study. From those 495 questionnaires, 240 were selected at random. Using the questionnaires, we divided the 240 subjects into two subgroups: those with DM (146) and those without DM (94). The availability of each prospective participant was then determined. Some were deceased, some were ill, others could not be located and a few were unwilling to participate. The final study group comprised of 88 subjects, 46 of whom were female (mean age 68.8 years) and 42 were male (mean

age 68.9 years). The subject age range was 60-92 years. In our previous survey the oral health status of the subjects was self-reported. In the present study, we conducted oral examinations of the subjects and determined the levels of seven salivary components listed below.

Interviews and data from questionnaires

The study protocol and questionnaire utilized in this study were reviewed and approved by a human subjects review committee (Ethical Review Committee, Inc., Independence, MO, USA).

On a prearranged day, groups of 10-15 subjects each were transported from their homes to the examination site. The purpose and procedure of the study were explained and each subject gave written informed consent prior to participation in the study. During a brief interview, we confirmed information they had provided in the previously administered questionnaire, including current age, years of residence in California, whether or not a physician had diagnosed them with DM and selected questions concerning their routine diets and whether or not they were experiencing stress. Each subject was weighed and their height and waist circumference were measured. Finally, an oral examination was conducted by professional dental personnel and salivary specimens were collected.

Body mass index (BMI) and risk categories

We adopted the scheme recommended by a World Health Organization Consultation Group for Asian populations to categorize the BMI data of subjects at risk for type 2 DM (WHO Expert Consultation, 2004). That scheme differs slightly from the WHO international classification scheme which is now recommended for all ethnic groups (<http://www.who.int/features/factfiles/obesity/facts/en>). The

data we obtained from the elderly Hmong in this study indicate DM was associated with BMI values below a BMI of 25kg/m². Similar findings have been reported by others (Stommel and Schoenborn, 2010). A BMI of 18.5-23.0 kg/m² was rated as normal (acceptable risk, A); a BMI of 23.1-27.5 was rated as overweight (elevated risk, E); and a BMI > 27.5 was rated as obese (high risk, H). We found it useful to split the elevated risk range into two categories: El (23.1-25.0) and Eh (25.1-27.5).

Oral health evaluation

The dental examiners questioned the subjects about dental prostheses, determined missing teeth, the presence and location of cavities, the condition of the gingiva and existence of gingival pockets, the mobility of and results of percussion of teeth and the presence of halitosis or xerostomia. The examination did not include probing gingival pockets or evaluation of gingival bleeding. The oral examinations were performed without any invasive procedures.

The scheme used for evaluating oral health was based on a blend of criteria from the "periodontal index" (<http://www.enotes.com/dental-indices-reference/dental-indices>) and the "decayed, missing, filled teeth index" (<http://www.enotes.com/dental-indices-reference/dental-indices>) (Table 1).

Using the 2 indices above a score was given to each subject. A score of greater than 3 was considered indicative of poor oral health.

Analysis of saliva

Each subject rinsed their mouth with water prior to examination. Approximately 5 minutes later, the subject expectorated saliva into a sterile, cold plastic cup with a cap. One point five milliliters of well mixed saliva was immediately

transferred to a sterile microfuge tube bearing an ID number for the subject and placed in dry ice for transport. The samples were shipped overnight in a container packed with dry ice to BioLegend Inc in San Diego, California, USA, where they were stored at -70C until analyzed. Eighty-eight specimens, one from each subject in the study, were analyzed for seven components: endotoxin (ET), adiponectin (ADN), leptin (Lep), C-reactive protein (CRP), TNF α , IL-6 and soluble IL-6 receptor (sIL-6R). All analytes (except for ET) were analyzed with a multiplex assay panel based on Luminex beads (BioLegend, San Diego, CA). Briefly, the saliva samples were placed in a 96-well filter plate and incubated for 2 hours at room temperature with a mixture of the bead population to which specific capture antibodies were covalently conjugated. The wells were washed twice and beads containing the captured target molecules were subsequently incubated for 1 hour at room temperature with a cocktail of detection antibodies specific for the targets. Streptavidin-phycoerythrin was then added to the wells and the plate was further incubated for 30 minutes at room temperature. Finally, the beads in the well were resuspended with sheath fluid and read on a Luminex¹⁰⁰ instrument. Sample concentrations were calculated from standard curves using data reduction software designed for immune assays with a 5-parameter logistic curve-fitting algorithm (Brendan Scientific, Carlsbad, CA).

ET was determined using a Toxin Sensor Endotoxin Detection System (Gen Script USA, Piscataway, NJ) following the manufacturer's instructions with some slight modifications. Briefly, the saliva samples were diluted in ET-free reagent grade water and, along with the standards and blanks, were mixed with

Limulus amoebocyte lysate and then substrate solution. The reaction was stopped with the provided "stop" solution. After adding color stabilizer, the mixture was transferred to the wells of a 96-well plate. Absorbance of the reaction mixture in each well was measured using an ELISA plate reader at 545 nm. ET concentrations were determined using a reference plot of absorbance standards.

Data analysis

Statistical analyses of the data included chi-square, odds ratio, one-way ANOVA, correlation, multiple logistic regression, 95% confidence interval for log-normal data and the Wilcoxon two sample test. Those analyses were performed by use of the Winks Statistical Data Analysis program (TexaSoft, Winks SDA Software, Sixth Edition, Cedar Hill, TX).

RESULTS

Oral health, BMI and diabetes

Fifty-eight percent of the subjects enrolled in this study had type 2 diabetes (DM) (Table 2). Seventy-five percent of the diabetic subjects were in the elevated risk BMI categories Eh and H. Only 5% of the nondiabetic subjects were in an elevated risk BMI category. Sixty-nine percent of diabetic subjects and 35% of nondiabetic subjects had POH. None of the nondiabetic subjects had concurrent OW and POH; in contrast, 47% of diabetics had both OW and POH. The significance of the association between the presence of DM and concurrent OW and POH was tested. A chi-square test revealed the association between DM and concurrent OW and POH was significantly greater than the association between DM and either OW or POH separately. The frequency of DM in subjects having concurrent OW and POH was significantly greater than the sum of

Table 1
Criteria used for evaluating oral health of subjects, and range of demerits used to characterize degree of imperfection of each criterion.

Criteria	Range of demerits
Number of pairs of opposing molars	0-4
Adequacy of prosthetic devices (if any; fit, degree of pain, frequency of use)	0-3
Cavities (number and severity)	0-2
Number of gingival pockets	0-4
Halitosis (indicative of oral infection)	0-3

Table 2
Distribution of elderly diabetic and nondiabetic subjects by presence of overweight/obesity and poor oral health.

Condition	Diabetic subjects		Nondiabetic subjects	
	Number	Percent of diabetics	Number	Percent of nondiabetics
OW	14	27.5	2	5.4
POH	11	21.6	13	35.1
OW and POH	24	47.1	0	0
Neither OW nor POH	2	3.9	22	59.5

OW, overweight/obesity; POH, poor oral health

subjects who had only one of these two conditions ($p=0.002$; OR 7.2; CL 34.7, 1.5).

Independent variables and frequency of diabetes

Several other variables possibly affecting the prevalence of DM were also studied. These were: age, gender, stress, years of residence in California and diet. The latter was eliminated because 96% of the subjects in the initial study (Albright, 2010) declared their daily diet was composed of green, leafy vegetables, fish, chicken and pork. They shunned simple carbohydrates, fat, and low fiber foods typical in the American diet.

Table 3 shows the results of logistic regression analysis which showed: age of the subject (≥ 60 years), years of residence in California and stress were not significantly associated with the prevalence of DM. BMI and POH were associated with DM. Forward and backward selection of explanatory variables did not change the conclusions.

Salivary analytes of subjects

There is little information in the literature about normal levels of salivary components in elderly humans. The results of our analytic determinations are shown in Table 4. When the data for diabetic and

Table 3
Multiple logistic regression analysis of association between type 2 diabetes and several variables.

Independent variables	Regression coefficient	Standard error	Wald chi-square	<i>p</i> -value	Odds ratio	Wald 95% confidence interval
POH	2.144	0.80	7.10	0.009	8.53	6.96-10.1
BMI	0.853	0.20	19.0	0.001	2.35	1.96-2.73
Age	- 0.016	0.05	0.13	0.723	-	-
Years in CA	0.022	0.04	0.36	0.549	-	-
Stress	0.971	0.75	1.69	0.197	2.64	1.18-4.10

POH, poor oral health; BMI, body mass index; Years in CA, years residence in California

Table 4
Concentrations of salivary analytes in diabetic and nondiabetic elderly Hmong subjects.

Analyte	Concentration in saliva					
	Diabetics			Nondiabetics		
	Number of samples	Concentration (pg/ml)	95% CI	Number of samples	Concentration (pg/ml)	95% CI
ET	49	2,042	646 - 6,457	35	1,862	708 - 4,898
ADN	51	2,570	708 - 9,333	35	2,818	741 - 10,715
CRP	50	55	13 - 229	34	37	8 - 174
sIL-6R	51	43	11 - 166	36	34	10 - 117
IL-6	38	8	3 - 23	32	6	2 - 16
TNF α	20	9	3 - 29	11	8	3 - 19

ET, endotoxin; ADN, adiponectin; CRP, C-reactive protein; sIL-6R, soluble IL-6 receptor, IL-6, interleukin-6; TNF α , tumor necrosis factor-alpha; CI, confidence interval

nondiabetic subjects were compared, not taking into account gender, age, BMI, stress or POH, there were few significant differences (Table 4).

However, when the data were grouped taking into account diabetes, BMI and POH there were important differences (Table 5). Nondiabetic subjects could be divided into two groups: those with an acceptable BMI who had good

oral health and those with an acceptable BMI who had POH. These two groups differed significantly in salivary concentrations of ADN, CRP and sIL-6R, but not in concentrations of ET or IL-6 (Tables 5 and 6). Those data suggest POH in the absence of OW is associated with elevated ADN, CRP, and the inflammatory cytokine receptor, sIL-6R. The effect of POH on these analytes was not seen among

Table 5
Salivary analytes among diabetic and nondiabetic elderly subjects with various risk factors.

Condition	DM	OW	POH	Salivary concentration (pg/ml)					
				ET	ADN	CRP	sIL-6R	IL-6	TNF α
1	0	0	0	3.19	3.07	1.38	1.28	0.71	0.72
				0.32	0.48	0.51	0.34	0.29	0.20
				21	21	20	23	20	8
2	0	0	1	3.32	3.74	1.84	2.06	0.84	0.73
				0.38	0.30	0.31	0.27	0.38	-
				11	10	11	11	8	2
3	1	1	0	3.57	3.24	1.70	1.40	1.05	1.04
				0.41	0.42	0.54	0.41	0.49	0.43
				11	12	11	12	9	7
4	1	0	1	3.25	3.49	1.59	1.66	0.75	1.09
				0.52	0.25	0.42	0.45	0.53	0.48
				12	11	11	11	10	4
5	1	1	1	3.34	3.53	1.84	1.59	0.88	0.98
				0.45	0.58	0.33	0.52	0.37	0.23
				23	24	22	23	19	6

DM, diabetes (0, no; 1, yes); OW, overweight (0, no; 1, yes); POH, poor oral health (0, no; 1, yes)
The upper number for each condition is the geometric mean, the middle number is 1 standard deviation and the lower number is *n*.

diabetic, OW subjects, which suggests that OW and POH influence each other. Associations between DM and OW, and between DM and POH, may be present since OW was associated with slightly elevated salivary CRP, and POH was associated with slightly elevated ADN and sIL-6R; but these associations did not reach statistical significance. Only two subjects in this study were nondiabetic and OW, thus determining the effect of OW on salivary levels in this group was not possible. However, CRP appeared to be slightly elevated in these OW two subjects, but this did not reach significance ($p=0.1$). The concentrations of all of the analytes, except ET, were significantly higher among diabetic subjects with OW

and POH (Tables 5 and 6). Salivary ET was not significantly elevated in any of the groups.

Salivary leptin

Lep was present in a higher proportion of diabetic subjects who were OW than in those who were not (Table 7). Seventy-four percent of subjects (14/19) of both genders with a BMI ≥ 28.5 kg/m² also had Lep in the saliva. Seventy-nine percent of them were female. In contrast, 13% of DM subjects (4/31) with a BMI ≤ 28.5 kg/m² had salivary Lep; there was no gender bias. Lep occurred randomly among 32% of nondiabetic subjects (14/44). Diabetic and nondiabetic subjects did not differ significantly in Lep concen-

Table 6

Table of *p*-values from comparisons of salivary analytes in diabetic and nondiabetic subjects who differed in conditions of body weight and oral health.

Conditions ^a compared	Variable	Analyte concentrations compared (<i>p</i> -values)					
		ET	ADN	CRP	sIL-6R	IL-6	TNF α
0-0-0 and 1-1-1	DM	0.22	0.008	0.003	0.023	0.062	0.039
0-0-0 and 0-0-1	POH	0.32	0.001	0.02	0.001	0.34	-
0-0-1 and 1-0-1	POH	0.71	0.07	0.17	0.03	0.70	-
1-1-0 and 1-1-1	POH	0.18	0.14	0.37	0.31	0.34	-
1-0-1 and 1-1-1	OW	0.61	0.84	0.09	0.71	0.45	0.58

^aFirst digit, DM (0 = no, 1 = yes); second digit, OW (0 = no, 1 = yes); third digit, POH (0 = no, 1 = yes) DM, diabetes mellitus; POH, poor oral health; OW, overweight

Table 7

Presence of leptin in the saliva of elderly diabetic and nondiabetic subjects.

Subjects	BMI	Number of subjects			Number with leptin		Leptin concentration (pg/ml)	
		Total	Female	Male	Female	Male	Mean	95% CI
Diabetic	≥ 28.5	19	15	4	11	3	19	12 - 30
Diabetic	28.5	31	16	15	2	2	26	13 - 53
Non-diabetic	28.5	44	19	25	7	7	14	9 - 20

CI, confidence interval

trations (Table 7). There was no pattern to the distribution of Lep among subjects or of ADN and Lep combined.

DISCUSSION

The subjects in this study avoided a diet high in fat, simple carbohydrates and calories. However, there was a high prevalence of OW and obesity, possibly attributable to a "thrifty genome" (Neel, 1962). Hmong immigrants gain weight quickly after arrival in the US which could support the idea of a thrifty phenotype (Baig *et al.*, 2011). Another possible cause of obesity among Hmong immigrants is social

interactions. Christakis and Fowler (2007) reported that an individual's chances of becoming obese increased significantly if he or she had a friend, sibling, or spouse who became obese as well.

Our previous study found DM was more frequently associated with concurrent OW and POH than with either of these conditions alone. The results of the present study support this conclusion. In addition to age, BMI and POH, several other variables may have affected the prevalence of DM among these Hmong immigrants. The influence of diet after immigration was considered to be of

secondary importance for two reasons: detailed questioning revealed the subjects continued to eat foods they were accustomed to before leaving their native country and no association was seen between BMI and length of residence in California. Other independent variables studied were length of residence in California, age and stress. The latter was considered important because many of the male subjects had participated in the Vietnam conflict and along with their families had become refugees at the end of the conflict, and culturally and economically, the life of an immigrant was difficult. The variables significantly associated with the frequency of DM were BMI, POH and possibly stress. The finding that POH and DM are associated is not new but has generally been considered a one-way relationship with the presence of DM exacerbating POH (Lamster *et al*, 2008). However, there has been interest in the possibility POH (especially periodontitis) may be a cause of metabolic syndrome (D'Aiuto *et al*, 2008; Motita *et al*, 2010), obesity (Saito *et al*, 2001; Reeves *et al*, 2006) cardiovascular disease (Pussinen *et al*, 2007; Hotamisligil, 2006), musculoskeletal disease (Pischon *et al*, 2008) and DM (Canpus *et al*, 2005; Demmer *et al*, 2008).

A study by Bretz *et al* (2005) among elderly subjects (mean age 73 years) found periodontitis was associated with elevated plasma concentrations of IL-6, TNF α and CRP. There was a strong relationship between increased concentrations of those substances (especially CRP) and the presence of oral pathogenic bacteria. Noack *et al* (2001) found an association between serum CRP and BMI among older patients afflicted with severe periodontitis (loss of attachment) and an elevated CRP was correlated with the presence of four species of oral bacteria. An association between

periodontitis, oral pathogens, and serum pentraxin levels (CRP and PTX3) has been observed among middle-age patients of different ethnicities (Pitiphat *et al*, 2008; Pradeep *et al*, 2010).

It might be expected the effective treatment of oral diseases, such as periodontitis, would lessen the severity and/or persistence of inflammatory disorders; however, the results of studies in this area have been mixed (D'Aiuto *et al* 2005; Taylor *et al*, 2006; Behle *et al*, 2009). In one comprehensive study (Behle *et al*, 2009), the effect of thorough surgical and antibiotic therapy resulted in a substantial reduction in inflammation in about one-third of the patients; increased inflammation in one-fourth of patients; and no effect in the remainder.

In our study, ADN and Lep, were not normally distributed in either diabetic or nondiabetic subjects. The salivary content of ADN in elderly subjects (approximately 10-15 ng/ml) was approximately 1,000-fold lower than the blood level (8-11 μ g/ml). ADN levels in blood decline with adiposity, whereas the concentration of Lep rises (Stommel and Schoenborn, 2010; Pham *et al*, 2012). That was true of Lep concentrations in the present study, but not of ADN levels. Higher serum levels of Lep occurred in women in this study which can be attributed to the greater proportion of body fat (Ruhl *et al*, 2004). The total Lep level in the plasma is the sum of "free" leptin and the Lep bound to the soluble receptor, sOb-R (Brabant *et al*, 2000). We cannot exclude the possibility that significant amounts of receptor-bound Lep were not recovered in the specimens of saliva. CPR may increase by 500-fold in inflammation, triggered primarily by IL-6 (Ganapathi *et al*, 1988). IL-6 trans-signaling is involved in the transition from acute to chronic inflammation and in

maintaining chronic inflammatory disease (Jones *et al*, 1999; Rose-John *et al*, 2006). Trans-signaling has been demonstrated in periodontitis (Jones *et al*, 2001; Galicia *et al*, 2006). The salivary levels of CRP and sIL-6R in the saliva of the elderly in this study were considerably less than those reported in normal adult plasma (Lange *et al*, 2006; Behle *et al*, 2009), suggesting they may have been synthesized locally in the mouth rather than being equilibrated with the blood. Both IL-6 and CRP may be produced by cells associated with salivary glands (Wei *et al*, 2001).

ET from oral/gut pathogens have been suggested to initiate or exacerbate obesity, systemic inflammation and inflammatory diseases (Socransky and Haffajee, 2000; Backhed *et al*, 2004; DiBaise *et al*, 2008; Al-Attas *et al*, 2009; Ghoshal *et al*, 2009; Goodson *et al*, 2009). ET was chosen as an analyte for this study because it was considered a possible stimulant of inflammation in subjects with POH and OW.

ET in the saliva is presumably derived from oral microorganisms although it may enter the saliva from the bloodstream after originating from gut microorganisms (Backhed *et al*, 2004, DiBaise *et al*, 2008) and being transported by chylomicrons (Ghoshal *et al*, 2009). The normal ET level in the serum of an adult human is 200 - 600 pg/ml and among diabetic is 800-900 pg/ml (Al-Attas *et al*, 2009; Behle *et al*, 2009). The concentrations of ET we found in the saliva of nondiabetic subjects (1.7 ng/ml) and diabetic subjects (2.4 ng/ml) in this study were not significantly different from each other ($p=0.59$) which argues against the equilibration of ET between the blood and saliva. There is evidence of ET-releasing bacteria in the oral cavity of people with periodontitis (Socransky and Haffajee, 2000; Ledder *et al*, 2007). Because studies have demonstrated a

relationship between POH and the presence of pathogenic organisms in the oral cavity (Paju and Scannapieco, 2007; Darveau, 2009) we were puzzled by the lack of significant differences in ET levels between subjects with and without POH or OW. We propose two explanations for the lack of differences in ET levels: (1) the most likely is the method employed for analysis of ET failed to accurately quantify the endotoxins released by the major pathogenic organisms associated with periodontitis; (2) the second is ET tolerance (Martin *et al*, 2001; Hajishengallis *et al*, 2002). We focused on assessing lipopolysaccharides (LPS) that bind to toll-like receptor 4 (TLR4) because TLR4 seemed likely to be involved in the TLR activation of adipose tissue macrophages (MP) and dendritic cells (DC). LPS derived from *Porphyromonas gingivalis*, a pathogen in human periodontitis, is known to activate monocyte-derived DC via TLR2 (Jotwani *et al*, 2003; Hajishengallis *et al*, 2004). The fimbriae of *P. gingivalis* serve as stimulants of monocyte activation and adhesion by binding to TLR2 (Hajishengallis *et al*, 2006). Hundreds of species of microorganisms have been detected in periodontal disease (Socransky and Haffajee, 2000), so there is much yet to be learned about mechanisms of oral diseases initiated by microbial substances. A single exposure of target cells (monocytes, MP) to *Escherichia coli* LPS render the cells hyporesponsive (tolerant) to a subsequent exposure to LPS (Martin *et al*, 2001). Similarly, exposure of monocytes to *E. coli* LPS renders the monocytes tolerant of *P. gingivalis* LPS (Hajishengallis *et al*, 2002). The reverse, however, is not the case; the exposure of monocytes first to *P. gingivalis* LPS does not render the cells tolerant of *E. coli* LPS (Hajishengallis *et al*, 2002). Oral mucosal cells exist in a state of ET tolerance

(Muthukuru *et al*, 2005); presumably, this is the case among our subjects with POH. Mucosal cells that may vary considerably in their ability to ingest and destroy ambient microorganisms might develop tolerance to ETs. Other mechanisms, probably ecological, might limit the population of pathogens and the concentration of ETs.

In conclusion, our findings suggest synergism between OW and POH that is associated with a higher frequency of DM in the elderly. Further exploration of the treatment of oral pathology on chronic diseases among elderly humans is needed.

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