ORAL DISEASES

Oral Diseases (2010) 16, 96–101. doi:10.1111/j.1601-0825.2009.01619.x

www.wiley.com

ORIGINAL ARTICLE

Relationship between folic acid intake and gingival health in non-smoking adults in Japan

M Esaki[1], M Morita[2], R Akhter[2], K Akino[1], O Honda[1]

[1]*Department of Preventive Dentistry, Division of Oral Health Science, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan;* [2]*Department of Oral Health, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*

OBJECTIVE: To assess the relationship between dietary intake of folate and gingival bleeding in non-smoking adults in Japan.

MATERIALS AND METHODS: Data were obtained from residents who participated in the regional nutrition survey and survey of dental diseases conducted by the administrative office of northernmost prefecture of Japan. Dietitians visited households to collect data on dietary intake. Clinical parameters, including Community Periodontal Index (CPI) and bleeding on probing (BOP), were examined in community centers. Information on smoking habit was obtained from the interview. Then the data from 497 non-smoking adults with 20 teeth or more, aged 18 years or older, were analyzed. The relationship between dietary intake of folic acid and gingival bleeding status was estimated using multivariate analysis.

RESULTS: Pearson's correlation coefficient showed a significant negative correlation between dietary folate level and bleeding on probing. The negative association between folate level and bleeding on probing remained statistically significant in multiple regression analysis (standardized $\beta = -0.204$, $P < 0.001$). However, no significant association was found between CPI scores and folate intake level.

CONCLUSIONS: The results suggest that dietary intake of folic acid, an important indicator of gingival bleeding in adults, may provide an important clinical target for intervention to promote gingival health.

Oral Diseases (2010) **16**, 96–101

Keywords: folic acid; gingivitis; non-smoker; adults

Introduction

Periodontal disease is a multifactorial disease. Manifestation and progression of periodontitis are influenced by a wide variety of determinants, including social, behavioral, systemic, and genetic factors. Recent research has suggested a possible association between oral health and nutritional status in older adults (Clarke *et al*, 2003; Moynihan and Petersen, 2004; Yu *et al*, 2007). It has been shown that antioxidant nutritional elements such as vitamin C and vitamin E are related to periodontal disease (Cohen and Meyer, 1993; Nishida *et al*, 2000; Amarsena *et al*, 2005; Amaliya *et al*, 2007). Folic acid, which belongs to the vitamin B group, is generally known as a hemocytopoietic vitamin and is an essential factor for the growth of animals. Folic acid deficiency markedly affects cell production and mitosis (Fujimoto,1984).

Folic acid deficiency is the most common nutrient deficiency in the world (Krause and Mahan, 1984). However, little is known about its influence on periodontal health. It has been reported that a low serum folate level is associated with periodontal disease in non-institutionalized older adults (Yu *et al*, 2007). Folate deficiency, which is associated with increased oxidative stress, endothelial dysfunction, genomic instability, defective DNA repair, and apoptosis, has been shown to be related to a number of human diseases, including periodontal disease (Yu *et al*, 2007). Another study investigated the relationship between cigarette smoking and serum levels of folic acid in patients with periodontal disease, and found that serum folic acid concentration was significantly lower in smokers with more periodontal destruction than in non-smokers with less periodontal destruction (Erdemir and Bergstrom, 2006).

Based on these findings, it seems reasonable to assume that insufficient nutrition intake will lead to a low serum folate level, followed by deterioration of periodontal health. Clinical research has shown that folic acid supplementation results in a significant reduction in gingival inflammation as determined by gingival redness,

Correspondence: M Morita, DDS, PhD, Professor, Department of Preventive Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8525 Japan. Tel: +81 86-706 6710, Fax: +81 86 235 6714, E-mail: mmorita@md.okayama-u.ac.jp
Received 21 April 2009; revised 22 July 2009; accepted 23 July 2009

bleeding tendency, tenderness, and presence of exudates (Thomson and Pack, 1982; Pack, 1984). So, we focused on dietary folate level instead of folic acid deficiency in serum, and hypothesized that dietary folate level might be associated with gingival health. However, there have been no epidemiologic studies on the relationship between gingival health and dietary intake of folic acid. If folic acid deficiency in diet correlates with the presence of gingivitis or periodontal disease, this might have a great impact on public health.

The purpose of this study was to investigate epidemiologically any correlation of gingival health characterized by gingival bleeding tendency and dietary folate level. Associations of gingival health with intake of other dietary variables, including energy, protein, total fat, calcium, iron, vitamin A, vitamin B1, vitamin B2, vitamin C, and salt, were also investigated.

Subjects and methods

Study populations

This study was performed by the administrative office of Hokkaido from November 1st to 30th in 2004. Hokkaido, the northernmost island of Japan, has a population of 5.7 million in an area of 78 420 km^2. The subjects were selected from the residents of Hokkaido according to the two-stage cluster sampling procedure that was used the same way as in the National Health and Nutrition Survey, Japan. First, 59 areas were selected by the stratified random sampling method. Then, all adults over 18 years old (3474 residents in 1460 households) were selected for this regional survey, and the data from 497 non-smoking adults with 20 teeth or more were used for further analysis.

Questionnaire survey

Questionnaire survey was conducted according to the procedure in the National Health and Nutrition Survey. Dietitians visited individual households to collect data on 1-day dietary intake (24-h). Verbal consent for participation in the study was obtained. Detailed descriptions of all foods, beverages, and supplements consumed during 24-h period before the interview, including the quantity, cooking method, and brand names, were recorded. Household representatives weighed and recorded the quantity of each food item. Dietary records were subsequently processed for nutrition content by computer analysis (nutrient software package which uses the Japan food composition database) with the assistance of a consultant dietitian (Katanoda and Matsumura, 2002; Tanaka *et al*, 2008). We used the Standard Tables of Food Composition in Japan (5th ed.), in which values of folic acid is based on the microbiologic assay using *Lactobacillus casei* (*ssp. rhamnosus*) ATCC 7469 (Japan Department of Science and Technology Agency, 2000).

Information on medication, smoking habit, drinking habit, and various health-related activities including exercise, sleeping, and dental health behavior was obtained from the interview. Information on dental health behavior included information on frequency of use of extra cleaning devices (everyday, sometimes, never) and regular visits to a dentist.

Clinical examination

After the interviews, participants were asked to participate in clinical examinations at a designated regional community center on a specific day. Body weight (kg) and height (m) were measured and body mass index (BMI) was calculated. Trained dentists recorded oral health status in terms of caries, periodontal disease, and prosthetic appliances using a mouth mirror under an artificial light according to the manual of the National Survey of Dental Disease (NSDD). Lost teeth were defined as permanent teeth lost due to extraction. Third molars and implants, if present, were not included in the measurements.

Periodontal status was assessed using a CPI probe (YDM Co., Tokyo, Japan) and standard WHO (World Health Organization, 2005) criteria for the Community Periodontal Index (CPI). The oral cavity was divided into six dental segments (sextants), and 10 index teeth in six sextants were evaluated in each participant. The index teeth were 17, 16, 11, 26, and 27 in the upper jaw, and 37, 36, 31, 46, and 47 in the lower jaw. Six points on each tooth, mesio-buccal, mid-buccal, disto-buccal, and the corresponding lingual sites, were examined. The index teeth were measured and the highest score was recorded in descending order of severity as follows: deep pockets of 6 mm or more (score 4), deep pockets of 4–5 mm (score 3), dental calculus detected by probing (score 2), bleeding after probing (score 1), and healthy (score 0). The highest score in the six sextants was used as individual CPI score.

Generally, only CPI score is recorded in the NSDD. As this system prefers the presence of periodontal pockets or calculus to bleeding after probing, the actual number of sites with bleeding after probing cannot be recorded correctly. Therefore, in this study, the sites with bleeding on probing were recorded separately regardless of the presence of calculus or periodontal pockets.

Statistical analysis

Data from non-smoking adults, aged 18 years or older, with 20 teeth or more were used for analysis. Subjects were excluded if there were oral conditions present that could interfere with examination procedures; if they were pregnant; if they suffered from systemic diseases such as cardiovascular disease, liver dysfunction, hepatitis, or any disease requiring continuous medication; if they had received antibiotic therapy within the past 3 months; or if they had taken non-steroidal anti-inflammatory drugs in the past 6 weeks.

The baseline characteristic results were reported as mean ± s.d. One-way ANOVA or *t*-test was used to compare mean BOP% and CPI scores between various subgroups. Pearson's correlation coefficients between periodontal variables and numeric variables, including nutrient intake, were calculated.

Ordinal stepwise multiple regression analysis was carried out to determine the degrees of association

between periodontal indices and other explanatory variables using age, gender, nutrients, dental visits, and inter-dental cleaning as independent variables. The variables that showed significance in bivariate analysis were considered for further regression analysis. Calculations were carried out using the SPSS software package (SPSS Inc., version 15.0 Family, Chicago, IL, USA). The level of statistical significance was fixed at $P \leq 0.05$.

Results

Baseline characteristics (mean ± s.d.) of the subjects and amounts of nutrition intake are summarized in Table 1. Compared with reference values obtained from the National Health and Nutrition Survey (2006), the subjects took slightly more amount of total protein, calcium, vitamin A, and salt. The number (%) of subjects with folic acid intake less than the mean level set by the National Health and Nutrition Survey (2006) was 302 (60.7%). Mean (s.d.) of bleeding on probing (%) was 23.7 ± 31.0. Percent distribution of subjects according to the CPI code was 19.9%, 14.9%, 34.4%, 24.1%, and 6.6% for CPI codes 0, 1, 2, 3, and 4, respectively. Figure 1 shows the number of subjects according to BOP%. Two-thirds (66%) of the subjects had 20% or less sites of bleeding on probing.

Table 2 shows the mean BOP% and CPI scores according to independent variables including gender, inter-dental cleaning, and regular dental visits. The male subjects had a significantly higher percentage of bleeding (mean = 28.8, s.d. = 33.2) and higher CPI score (mean = 2.08, s.d. = 1.19) than those in the female subjects (mean = 21.1, s.d. = 29.6 for BOP% and mean = 1.79, s.d. = 1.18 for CPI score). The subjects who did not perform inter-dental cleaning had significantly worse CPI scores (mean = 1.98, s.d. = 1.18) than those for subjects who performed it sometimes (mean = 1.61, s.d. = 1.20) or everyday (mean = 1.69, s.d. = 1.17). BOP% and CPI score were significantly higher in subjects who never visited a dentist than that in subjects who visited a dentist regularly.

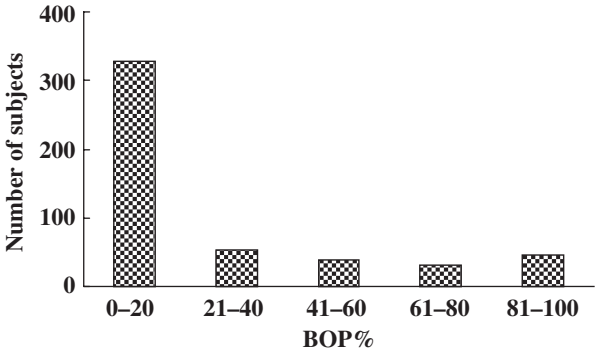

Figure 1 Number of subjects according to BOP%

Table 3 shows the correlation coefficients of BOP% and CPI scores with age, BMI, and intake levels of all dietary nutrients. There were significant negative correlations of calcium, iron, vitamin A, vitamin C, and folic acid with BOP%, and significant positive correlations of age, BMI, total protein, and calcium with CPI score.

All of the independent variables that showed significant effects on BOP% and CPI scores at the bivariate level (age, gender, BMI, inter-dental cleaning, regular dental visits, total protein, calcium, iron, vitamin A, vitamin C, and folic acid) were included in multiple linear regression analysis. The variables that remained significant in the final model are shown in Table 4. Dietary intake of folic acid had shown a significant correlation with BOP% (correlation coefficient = −0.204, $P < 0.001$). Older age, male gender, and irregular dental visits were identified as significant factors for increase in BOP%. Older age, irregular dental visits, and no inter-dental cleaning were identified as important correlates of CPI scores.

Table 1 Descriptive statistics of the sample population

		Mean	*s.d.*
Age (years)		50.2	14.8
BMI (kg m^{-2})		23.8	3.5
Number of teeth present		25.2	2.6
BOP%		23.7	31.0
Nutrition Intake	*Mean*	*s.d.*	*Reference*[a]
Energy (kJ)	8414.0	2661.0	8372.0
Total Protein (g)	79.0	27.0	76.9
Total fat (g)	48.3	21.2	54.6
Calcium (mg)	595.0	287.0	536.0
Iron (mg)	8.6	3.6	8.8
Vitamin A (μgRE[b])	951.0	876.0	912.0
Vitamin B1 (mg)	1.1	0.6	1.5
Vitamin B2 (mg)	1.4	0.7	1.6
Vitamin C (mg)	130.0	83.0	129.0
Salt (g)	12.9	7.5	11.9
Folic acid (μg)	372.5	198.9	400.0

$N = 497$, s.d., standard deviation.
[a]Mean dietary intake (50 to 59-year-old Japanese) obtained from National Health and Nutrition Survey, 2006.
[b]RE, retinol equivalents.

Discussion

To the best of our knowledge, this is the first report based on the epidemiologic study showing an association between dietary folate levels and gingival bleeding in a representative non-smoking adult population. As a result, low folate intake levels were associated with gingival bleeding in a non-smoking adult population. Bleeding on probing (BOP) is generally accepted as an inflammatory parameter. BOP reflects decreased collagen density, increased blood vessel density and fragility, and a reduction of epithelial thickness and epithelial integrity (Nesse *et al*, 2008). More attention to dietary intake of folic acid might lead to effective prevention of gingivitis. On the other hand, the difference of dietary folate level was not big between our result and the National Health and Nutrition Survey. It is also possible that gingival bleeding might be a sensitive sign

Table 2 Mean BOP% and CPI scores according to gender, frequency of inter-dental cleaning, and regular dental visits

		BOP%			*CPI scores*		
	Number	*Mean*	*s.d.*	P-*value* *(2-sided)*	*Mean*	*s.d.*	P-*value* *(2-sided)*
Gender							
Male	170	28.8	33.2	*	2.08	1.19	**
Female	327	21.1	29.6		1.79	1.18	
Inter-dental cleaning							
Everyday	81	20.7	30.8		1.69	1.17	**
Sometimes	152	20.2	29.0		1.61	1.20	
No	260	26.5	32.0		1.98	1.18	
Regular dental visits							
Yes	137	16.5	26.3	***	1.58	1.30	**
No	360	27.1	32.7		1.92	1.14	
Total	481	23.7	31.0		1.82	1.20	

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
One-way ANOVA or *t*-test was conducted to compare mean BOP% and CPI scores between various subgroups.

Table 3 Correlation coefficients (*r*) of age, BMI, and energy and nutrients intake with BOP% and CPI scores

	BOP%		*CPI*	
	r	P-*value* *(2-sided)*	*r*	P-*value* *(2-sided)*
Age	0.106	*	0.341	***
BMI	−0.024		0.107	*
Energy	−0.051		0.044	
Total protein	−0.055		0.113	*
Total fat	−0.077		0.055	
Calcium	−0.092	*	0.143	**
Iron	−0.116	**	0.001	
Vitamin A	−0.096	*	−0.069	
Vitamin B1	−0.041		0.010	
Vitamin B2	−0.087		0.058	
Vitamin C	−0.122	**	−0.032	
Folic acid	−0.147	**	−0.033	
Salt	0.012		0.027	

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

indicating the relatively less folate intake from diet even in a population with adequate dietary habit.

Folate, an essential carrier of 1-carbon moieties such as methyl and formyl groups within the cell, is crucial for *de novo* synthesis of several important building blocks of human macromolecules, including purines, deoxythymidylate monophosphate, and methionine (Babior and Bunn, 2005; Yu *et al*, 2007). As folic acid is important in DNA synthesis, cellular turnover rate is affected by folate level. If folic acid level is reduced, the function of gingival tissue as a barrier against bacterial insults may be reduced (Poppell *et al*, 1991), followed by increased tendency for bleeding on probing. Past clinical trials have also shown that folic acid supplementation results in significant reduction in gingival inflammation as determined by gingival redness, bleeding tendency, tenderness, and exudates (Thomson and Pack, 1982; Pack, 1984), findings that support the results of our study.

This study suggests the importance of sufficient dietary intake of folic acid. A previous study reported that flow of gingival exudates was reduced when subjects were supplemented with 2 mg systemic folate daily (Vogel *et al*, 1976). The effect of local application of folate has also been reported. Improvement in gingivitis had been found following a regime of mouth-rinsing with 0.1% topical folate (George and Pack, 1983). They reported that topical application of folate would reduce the level of antigenic stimulation to which the local immune system would be exposed and thus reduce the

Table 4 Multiple regression analysis of bleeding on probing (BOP)% and community periodontal index (CPI) scores

		Non-standardized		*Standardized*	
Independent variables	*Dependent variables*	*B*	*Standard error*	*β*	P-*value*
BOP%	Folic acid	−0.032	0.007	−0.204	***
	Age	0.311	0.099	0.147	**
	Regular dental visits[a]	9.809	3.060	0.142	**
	Gender[b]	−6.990	2.961	−0.106	*
CPI scores	Age	0.027	0.003	0.338	***
	Regular dental visits	0.279	0.115	0.105	*
	Inter-dental cleaning[c]	0.112	0.047	0.102	*

R^2: 0.082 for BOP% and 0.136 for CPI scores.
$*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
[a]Yes (0), No (1).
[b]Male (0), Female (1).
[c]Everyday (1), Sometimes (2), Never (3).

level of inflammation in gingival tissues (Vogel *et al*, 1976; George and Pack, 1983). It is feasible that topical application of folic acid is effective for improving periodontal health in subjects with impaired digestive function.

In this study, we did not find any correlation between CPI index and folic acid deficiency. The reason may be that the potential of folic acid is directly absorbed by the gingiva rather than periodontal tissues. It is reported that folic acid deficiency occurs only at the end organ level (gingiva), because folic acid supplementation was associated with an apparent decrease in Gingival Index and gingival exudates flow, irrespective of radiographic evidence of bone loss (Vogel *et al*, 1976). On the other hand, a recent study has shown that low serum folate level was independently associated with clinical attachment loss and pocket depth in older adults (Yu *et al*, 2007). It might be feasible that we can find similar results when serum folate level, instead of folate intake estimated using dietary record, was actually determined.

No significant correlation of other micronutrients with gingival bleeding or periodontal disease was found in this study. Previous studies showed that insufficient intake of vitamin C could aggravate the progression of periodontal breakdown (Nishida *et al*, 2000; Amarsena *et al*, 2005). In both of those studies, a significant relationship was found in current and former smokers but not in subjects who had never smoked. These results do not conflict with the results of our study. Lower dietary vitamin C intake could be a reflection of the lifestyle of smokers, as smokers may have a less healthy lifestyle and have less interest in eating healthy food (Nishida *et al*, 2000). It has also been suggested that lower dietary intake of vitamin C causes a less antioxidant situation and that oxidative damage might contribute to an increased risk of periodontitis, especially in smokers who take cigarette smoke-related oxidants (Nishida *et al*, 2000). In our study, calcium deficiency was not selected as a significant factor for periodontal disease. Likewise, another study also reported that calcium deficiency is not a main cause of periodontal disease, as they did not find any changes in marginal bone level or density in radiographic assessment (Uhrbom and Jacobson, 1984). Further studies are needed to determine the possible micronutrients that contribute to gingival or periodontal health.

Dietary intake can be assessed by a variety of means, including 24-h, 3-day, or 7-day diet records, and a variety of structured assessment instruments, most commonly a food frequency questionnaire. Our study, as a part of prefectural survey, assessed dietary intake using a 24-h dietary recall questionnaire on the day of examination according to the procedure used in the Japanese National Health and Nutrition Survey (Ministry of Health, Labor and Welfare of Japan, 2006). The 24-h method has also been used by both the US Department of Health and Human services and the US Department of Agriculture as a key tool for assessing dietary patterns (Alaimo *et al*, 1994; Enns *et al*, 1997). Furthermore, it has been stated that 24-h dietary recall interview is used as the assessment method of choice for large surveys using tools identical to those we employed in this study (Novotny *et al*, 2003).

This study has potential limitations. First, because of the cross-sectional design of the study, causal relationships between dietary folate levels and gingival bleeding cannot be established. Second, we have assessed only 1-day dietary intake, which cannot provide accurate information to assess folic acid intake. In addition, as whole families participated, the folic acid intake would be similar within the families. Third, we mentioned previously that plasma folate level was not measured in our study. Finally, only representative teeth, not all teeth present, were examined clinically. Therefore, it is possible that bleeding tendency was overestimated or underestimated. In addition, the majority of the subjects had relatively lower BOP% (0–20%). The lower prevalence of residents with mild to severe gingivitis might have skewed the result. Further research is required to collect the data of all teeth from patients with different severity of gingivitis.

In conclusion, this investigation demonstrated a significant relationship between folic acid deficiency and gingival bleeding in non-smokers in Japan. This study suggests the importance of nutritional status for oral health.

Author contributions

M.E. – conceived the study, participated in its design and coordination, and drafted the manuscript. M.M. – participated in the design of the study, performed analysis and interpretation of data, and helped in final drafting of the manuscript. R.A. – participated in the design and helped in drafting the manuscript. K.A. – helped to conceive the study and participated in its design and acquisition of data. O.H. – participated in its design and coordination, and revised it critically for important intellectual content. All authors read, commented upon, and approved the final manuscript.

Conflict of interest

None of the authors reported any conflict of interest.

Source of Funding

None.

References

Alaimo K, McDowell MA, Briefel RR *et al* (1994). Dietary intake of vitamins, minerals, and fiber of persons ages 2 months and over in the United States: Third National Health and Nutrition Examination Survey, Phase 1, 1988–91. *Adv Data* **14:** 1–28.

Amaliya, Timmerman MF, Abbas F *et al* (2007). Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis. *J Clin Periodontol* **34:** 299–304.

Amarsena N, Ogawa H, Yoshihara A, Hanada N, Miyazaki H (2005). Serum vitamin C-periodontal relationship in community dwelling elderly Japanese. *J Clin Periodontol* **32:** 93–97.

Babior BM, Bunn HF (2005). Megaloblastic anemias. In: Kasper DL, Braunwald E, Fauci AS *et al*, eds *Harrison's Principles of Internal Medicine*. The McGraw-Hill Companies, Inc: New York, pp. 601–606.

Clarke R, Refsum H, Birks J (2003). Screening for vitamin B12 and folate deficiency in older persons. *Am J Clin Nutr* **77:** 1241–1247.

Cohen ME, Meyer DM (1993). Effect of dietary vitamin E supplementation and rotational stress on alveolar bone loss in rice rats. *Arch Oral Biol* **38:** 601–606.

Enns CW, Goldman JD, Cook A (1997). Trends in food and nutrient intakes by adults: NFCS 1977–78, CSFII 1989–91, and CSFII 1994–95. *Fam Econ Nutr Rev* **10:** 16–31.

Erdemir EO, Bergstrom J (2006). Relationship between smoking and folic acid, vitamin B12 and some haematological variables in patients with chronic periodontal disease. *J Clin Periodontol* **33:** 878–884.

Fujimoto K (1984). Periodontal tissue changes in a recovery experiment using folic acid deficient rats. *J Osaka Dent Univ* **18:** 1–20.

George R, Pack ARC (1983). Inhibition of mitogen-induced lymphoblastic transformation by folate. *J Dent Res* **62:** 404. (abstract).

Japan Department of Science and Technology Agency, Japan. (2000). *Standard Tables of Food Composition in Japan*, 5th revised edn. Japan National Printing Bureau: Tokyo.

Katanoda K, Matsumura Y (2002). National Nutrition Survey in Japan-its methodological transition and current findings. *J Nutr Sci Vitaminol* **48:** 423–432.

Krause M, Mahan L (1984). *Food and Nutrition Diet Therapy*, 7th edn. W.B. Saunders: Philadelphia, PA, pp. 677–679.

Ministry of Health, Labor and Welfare of Japan (2006). *The National Health and Nutrition Survey in Japan 2004*. Daiichi Shuppan Publishing Co., Ltd: Tokyo (in Japanese).

Moynihan P, Petersen PE (2004). Diet, nutrition and the prevention of dental diseases. *Public Health Nutr* **7:** 201–226.

Nesse W, Abbas F, van der Ploeg I, Spijkervet FK, Dijkstra PU, Vissink A (2008). Periodontal inflamed surface area: quantifying inflammatory burden. *J Clin Periodontol* **35:** 668–673.

Nishida M, Grossi SG, Dunford RG, Ho AW, Trevisan M, Genco RJ (2000). Vitamin C and the risk for periodontal disease. *J Periodontol* **71:** 1215–1223.

Novotny JA, Rumpler WV, Riddick H *et al* (2003). Personality characteristics as predictors of underreporting of energy intake on 24-hour dietary recall interviews. *J Am Diet Assoc* **103:** 1146–1151.

Pack ARC (1984). Folate mouthwash: effects on established gingivitis in periodontal patients. *J Clin Periodontol* **11:** 619–628.

Poppell TD, Keeling SD, Collins JF, Hassell TM (1991). Effect of folic acid on recurrence of phenytoin-induced gingival overgrowth following gingivectomy. *J Clin Periodontol* **18:** 134–139.

Tanaka K, Sasaki S, Murakami K *et al* (2008). Relationship between soy and isoflavone intake and periodontal disease: The Freshmen in Dietetic Courses Study II. *BMC Public Health* **8:** 39–47.

Thomson ME, Pack ARC (1982). Effects of extended systemic and topical folate supplementation on gingivitis of pregnancy. *J Clin Periodontol* **9:** 275–280.

Uhrbom E, Jacobson L (1984). Calcium and Periodontitis: clinical effect of calcium medication. *J Clin Periodontol* **11:** 230–241.

Vogel RT, Fink RA, Schneider LC, Frank O, Baker H (1976). The effect of folic acid on gingival health. *J Periodontol* **47:** 667–668.

World Health Organization (2005). *Global Oral Health Data Bank: Periodontal Country Profiles*. WHO: WHO Headquarters, Geneva.

Yu Y, Kuo HK, Lai YL (2007). The association between serum folate levels and periodontal disease in older adults: data from the national health and nutrition examination survey 2001/02. *J Am Geriatr Soc* **55:** 108–113.