J Clin Periodontol 2007; 34: 1074–1081 doi: 10.1111/j.1600-051X.2007.01154.x

Journal of Clinical Periodontology

Effect of smoking on folic acid and vitamin B_{12} after nonsurgical periodontal intervention

Ebru Olgun Erdemir[1] and Jan Bergstrom[2]

[1]Periodontology Department, Faculty of Dentistry, Kirikkale University, Kirikkale, Turkey; [2]Institute of Odontology, Karolinska Institutet, Stockholm, Sweden

Erdemir EO, Bergstrom J. Effect of smoking on folic acid and vitamin B_{12} after nonsurgical periodontal intervention. J Clin Periodontol 2007; 34: 1074–1081. doi: 10.1111/j.1600-051X.2007.01154.x.

Abstract

Aim: The main purpose of this study was to investigate the effect of smoking on the serum levels of folic acid and vitamin B_{12} in smokers and nonsmokers with chronic periodontal disease after nonsurgical intervention.

Material and Methods: The study base consisted of 45 current smokers and 43 nonsmokers. The clinical parameters included plaque index (PI), gingival index (GI), pocket depth (PD), and clinical attachment level (CAL). Folic acid and vitamin B_{12} were determined from peripheral blood samples. Clinical measurements and blood samples were collected at baseline and 1, 3, and 6 months after the intervention.

Results: Mean PI was significantly greater in smokers compared with non-smokers throughout the observation period ($p<0.001$). During the first month, GI levels significantly decreased in both groups. From months 1 through 6, a significant return towards an increased GI level was observed in smokers ($p<0.001$). PD and CAL levels significantly decreased during the first month in both groups. Thereafter, increasing levels of PD and CAL were seen in both groups, although significantly more pronounced in smokers. Throughout the observation period, the mean CAL was significantly greater in smokers relative to nonsmokers ($p<0.001$). In smokers, the mean folic acid level gradually and significantly decreased and a slight and significant decrease in mean vitamin B_{12} levels was observed in both groups over the entire observation period ($p<0.001$).

Conclusion: The clinical response to nonsurgical intervention is impaired by smoking and smoking seems to negatively influence the serum level of folic acid following non-surgical intervention.

Key words: chronic periodontitis; folic acid; smoking; vitamin B_{12}

Accepted for publication 1 September 2007

It is now well established from a large body of epidemiologic evidence that cigarette smoking is the major preventable risk factor in the incidence and progression of periodontal disease (Bergstrom & Eliasson 1987a, b, Bergstrom 1989, 2004, 2006, Bergstrom & Preber 1994, Grossi et al. 1994, 1995, Bergstrom et al. 2000, Hyman & Reid 2003, Borrell & Papapanou 2005, Dye & Selwitz 2005, Heitz-Mayfield 2005, Palmer et al. 2005, Tonetti & Claffey 2005). Besides the adverse effects of smoking on immunology and the host–bacterial interactions, which may account for its deleterious effects on periodontal health (Bouclin et al. 1997, Fredriksson et al. 1999, Apatzidou et al. 2005), smoking also negatively affects vitamin B_{12} and folic acid mechanisms (Piyathilake et al. 1994, Mansoor et al. 1997, O'Callaghan et al. 2002, van Wersch et al. 2002, Ozerol et al. 2004). Organic nitrites, nitrous oxide, cyanates, and isocyanates found in cigarette smoke have been shown to interact with folic acid and vitamin B_{12} co-enzymes, transforming them into biologically inactive compounds (Khaled et al. 1985).

Vitamin B_{12} (cobalamin) is a hydrogen-acceptor co-enzyme involved in the reduction of ribonucleotides. It, therefore, plays an important role in growth and marrow haemopoiesis (Campbell 1995). And it was concluded that vitamin B_{12} deficiency may also be an aetiological factor in recurrent aphthous stomatitis (Piskin et al. 2002).

Folic acid (also known as folate) is an essential vitamin. Folic acid levels have

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.
The study was self-funded by the authors and their institutions.

been implied as important in the pathophysiology of many diseases, including neural tube defects in neonates (Butterworth & Bendich 1996), colorectal cancer and breast cancer (Langman & Boyle 1998, Kim 1999), and atherosclerotic disease in adults (Klor et al. 1997). Vitamin B_{12} and folic acid facilitate steps required for cellular division (Pollack 1979). It has been suggested that folic acid deficiency is the most common nutrient deficiency in the world (Krause & Mahan 1984). Cigarette smoking is one of the factors that affect the levels of vitamin B_{12} and folic acid (Lökk 2004). Active cigarette smokers have lower folic acid levels in their serum, red blood cells, and respiratory tract (Heimburger 1992, Piyathilake et al. 1994, Giles et al. 1998).

A number of studies have focused on the effect of smoking on the serum levels of folic acid and vitamin B_{12} in adults, but no studies have analysed the relation of smoking with folic acid or vitamin B_{12} in patients with chronic periodontal disease. Therefore, we have recently made a research on this subject and reported that among patients with periodontal disease the serum folic acid concentration was lower in smokers compared with non-smokers (Erdemir & Bergstrom 2006). A longitudinal study is likely to provide stronger evidence and, therefore, the main aim of the present investigation was to elucidate the response of smokers and non-smokers to non-surgical intervention in terms of folic acid and vitamin B_{12} during 6 months of follow-up. In addition, the clinical response in terms of gingival inflammation, probing depth, and attachment level was observed.

Material and Methods

Patients

The study population included 88 patients, 45 smokers and 43 non-smokers in the age range of 31–68 years. The patients had chronic periodontal disease as evidenced from a probing depth of 6 mm or more at 80% of the proximal sites and radiographic bone loss of more than 50% (Consensus report 1999). The participants were drawn from the waiting list of patients with untreated disease at the Department of Periodontology, Faculty of Dentistry at Kirikkale University. All subjects had no medical condition that would affect their participation in the study. An extensive medical history was taken both by a written questionnaire and by interview. Exclusion criteria applied were a course of anti-inflammatory or antimicrobial therapy within the previous 3 months, a history of use of vitamin or iron supplementation within the previous 3 months. Pregnant women and individuals who suffered, apart from periodontitis, from any given acute or chronic medical condition, including diabetes, viral, fungal or bacterial infections, or had recent trauma or tooth extractions were also excluded. None of the patients were alcohol consumers or obese. The purpose and nature of the study, including the types of clinical measurements and blood sample collection, were explained to all potential participants. After reading and signing the consent form, the subjects were enrolled into the study. The study was approved by the Medical Ethical Committee of Kirikkale University Faculty of Dentistry.

Smoking habits were recorded for all participants and patients were classified as either current smokers, i.e., regular smokers of 20 cigarettes per day (45 patients), or non-smokers, i.e., individuals who had never smoked tobacco (43 patients).

After baseline measurements all participants received a primary phase of non-surgical treatment including oral hygiene instruction, scaling and root planing. Full-mouth supragingival professional tooth cleaning (scaling and polishing) were performed in a single session for 60 min. and oral hygiene instructions were given at this time. One week later, the subsequent non-surgical treatment consisted of sub-gingival debridement using Gracey curettes (Hu Friedy Instruments, Chicago, IL, USA) under local anaesthesia in one session for 60 min. Clinical data were recorded and blood samples were collected at baseline and the 1st, the 3rd, and the 6th months following the intervention. The treatment and all clinical examinations were performed by one of the authors (EOE). All participants completed the 6 months trial and provided clinical and blood sample data at all time points.

Clinical recordings

Supragingival plaque was scored using the plaque index (PI, Silness & Löe 1964) and gingival inflammation using the gingival index (GI, Löe & Silness 1963). Probing depth (PD) and clinical attachment level (CAL) were measured at six sites per tooth of all teeth using a conventional periodontal probe with 1 mm between increments (Hu-Friedy). The probe was directed parallel to the long axis of the tooth. CAL measurements were made from the cement–enamel junction to the bottom of the periodontal pocket or sulcus. The reliability of the peridontal measurements were not assessed.

Blood analysis

After a 12-h fasting period, venous blood samples were obtained between 08.30 and 11.00 hours by venepuncture in the antecubital fossa without excessive venous stasis. The blood was taken into EDTA containing vacuum tubes (HEMA, Neuss, Germany) at the Department of Biochemistry, Faculty of Medicine, Kirikkale University. The laboratory analysis of blood samples was performed immediately with the Sysmex XT 2000i (Roche, Basel, Switzerland). The levels of folic acid and vitamin B_{12} were calculated according to standardized and automated procedures.

Statistical analysis

The distributions of most variables were normal or approximately normal. Exceptions were GI and CAL, which variables, therefore, were log-transformed to achieve approximate normality. Results were expressed as means and 95% confidence intervals (95% CI). The statistical significance of differences between groups was tested using General Linear Models, repeat measures design. Univariate analysis of co-variance (ANCOVA) with PI and/or GI as continuous co-variable(s) was used to test differences between smokers and non-smokers at specific time points. Multiple linear regression analysis was performed with folic acid as the dependent variable and age, CAL, gender, GI, PI, PD, smoking, and vitamin B_{12} as independent variables. Logistic regression was used to estimate the relative risk expressed as odds ratio (OR) and 95% CI. When estimating the relative risk for a low folic acid level, a cutoff point of 7.0 ng/ml, representing the 25th percentile, was used. Thus, this variable was dichotomized into (1) <7.0 ng/ml or (2) otherwise. Vitamin B_{12} was stratified into three approximately equal strata according to (1) <290 pg/ml

Table 1. Changes in (a) PI and (b) GI over time. Mean and 95% CI according to smoking

	Baseline		6 months		Change		*p*
	mean	95% CI	mean	95% CI	mean	95% CI	
(a) PI							
Smoker	2.06	1.96–2.15	1.73	1.62–1.85	0.32	0.20–0.44	<0.001
Non-smoker	1.89	1.79–1.98	1.15	1.03–1.27	0.74	0.61–0.86	<0.001
Difference	0.17	0.04–0.30	0.58	0.42–0.75	−0.42	−0.59 to −0.24	
p	<0.05		<0.001		<0.001		
(b) GI							
Smoker	1.82	1.74–1.89	1.42	1.35–1.49	0.40	0.30–0.49	<0.001
Non-smoker	1.92	1.84–2.00	1.43	1.36–1.51	0.49	0.40–0.58	<0.001
Difference	−0.10	0.00–0.21	−0.01	−0.09–0.11	−0.09	−0.22–0.03	
p	0.053		>0.05		>0.05		

PI, plaque index; GI, gingival index; CI, confidence interval.

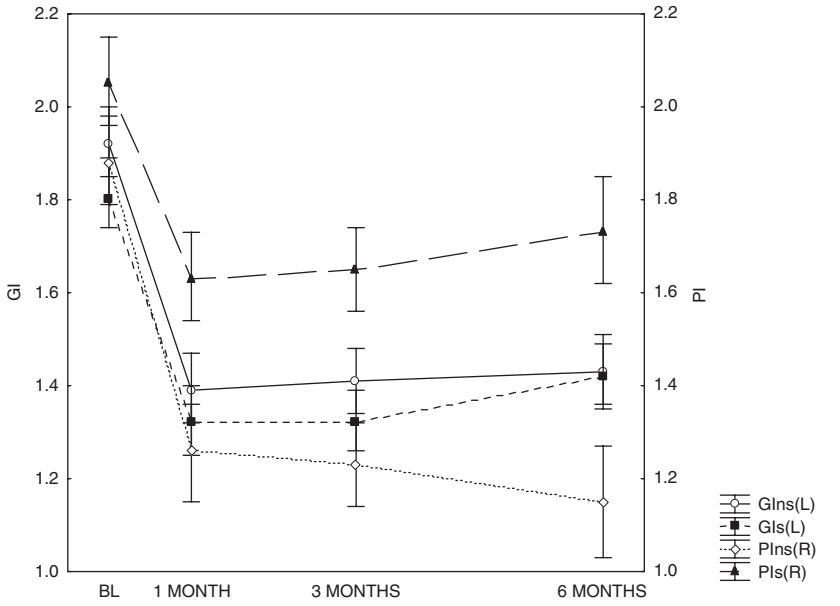

Fig. 1. Plaque index (PI) and gingival index (GI) following non-surgical intervention. Mean PI was significantly higher in smokers ($p<0.001$). Mean GI tended to be lower in smokers ($p=0.053$).

($n=29$), (2) 291–390 pg/ml ($n=30$), and (3) >390 pg/ml ($n=29$). To estimate the risk for relapse CAL, as the dependent variable, was dichotomized into (1) the difference between months 1 and 6 observations >1.5 mm (corresponding to 25th percentile), or (0) otherwise. The STATISTICA (StatSoft Scandinavia, Uppsala, Sweden) software program (version 7.0) was used for the calculations. The null-hypothesis was rejected at $p<0.05$.

Results

In the study, all smokers were cigarette smokers and the mean ± SD number of years smoked was 17.5 ± 1.7. The mean ± SD age of smokers and non-smokers was 45.5 ± 8.5 and 45.9 ± 8.0, respectively. The age difference between groups was not statistically significant ($p>0.05$). Body mass index (BMI) was also recorded and there was no statistically significant difference between groups ($p>0.05$).

Clinical characteristics

Plaque and gingivitis

The mean PI decreased significantly from baseline to the month 1 observation following the intervention in both smokers and non-smokers ($p<0.001$, Table 1a, Fig. 1). The decrease, however, was significantly less pronounced in smokers ($p<0.01$). From months 1 through 6 there was a further decrease in non-smokers ($p<0.05$), whereas a trend towards increasing levels was observed in smokers ($p=0.052$). The difference in PI trend patterns between smokers and non-smokers was statistically significant ($p<0.01$). The interaction between smoking and PI was statistically significant ($p<0.001$), suggesting an influence from smoking on the plaque level. In addition, mean PI remained significantly greater in smokers throughout the observation period ($p<0.001$).

The mean GI significantly decreased during the first month following the intervention in both smokers and non-smokers ($p<0.001$, Table 1b, Fig. 1). From months 1 through 6, a significant return towards an increased GI level was observed in smokers ($p<0.001$) but not in non-smokers ($p>0.05$), the difference between groups being statistically almost significant ($p=0.053$). Controlling for PI by means of univariate ANCOVA, the mean GI level was significantly lower in smokers relative to non-smokers at baseline ($p<0.01$) and month 3 ($p<0.05$).

Pocket depth and clinical attachment level

Significant decreases in mean PD were observed in both smokers and non-smokers from baseline to the month 1 observation following the intervention ($p<0.001$, Table 2a, Fig. 2). Subsequently, a return towards increasing levels was seen in both groups, although significantly more pronounced in smokers ($p<0.05$). Throughout the observation period, the mean PD tended to be greater in smokers than in non-smokers ($p=0.061$). Controlling for GI by means of univariate ANCOVA, the mean PD level was significantly greater in smokers relative to non-smokers at baseline ($p<0.05$), 3 months ($p<0.05$), and 6 months ($p<0.05$). There was, however, no interaction between smoking and PD. Controlling also for PI, the statistical significance was attenuated.

The mean CAL significantly decreased in both smokers and non-smokers from baseline to the month 1 observation ($p<0.001$, Table 2b, Fig. 2). Thereafter, a significant return towards an increasing level was noted in smokers ($p<0.001$), but not in non-smokers ($p>0.05$). The difference in CAL responses was statistically significant ($p<0.05$), suggesting relapse in smokers. Throughout the observation period, the mean CAL was significantly greater in smokers relative to non-smokers ($p<0.001$). Controlling for PI or GI did not influence the result. There was no interaction between smoking and CAL.

Table 2. Changes in (a) PD and (b) CAL over time. Mean and 95% CI according to smoking

	Baseline		6 months		Change		*p*
	mean	95% CI	mean	95% CI	mean	95% CI	
(a) PD							
Smoker	4.05	3.87–4.22	3.08	2.95–3.21	0.97	0.82–1.11	<0.001
Non-smoker	3.85	3.67–4.03	2.88	2.75–3.01	0.97	0.82–1.12	<0.001
Difference	0.20	−0.05–0.45	0.20	0.01–0.38	0.00	−0.20–0.21	
p	>0.05		<0.05		>0.05		
(b) CAL							
Smoker	3.84	3.69–4.00	3.30	3.20–3.41	0.54	0.42–0.65	<0.001
Non-smoker	3.41	3.25–3.57	2.85	2.74–2.96	0.56	0.44–0.68	<0.001
Difference	0.43	0.21–0.66	0.45	0.30–0.61	−0.02	−0.01 to −0.02	
p	<0.001		<0.001		>0.05		

CAL, clinical attachment level; PD, pocket depth; CI, confidence interval.

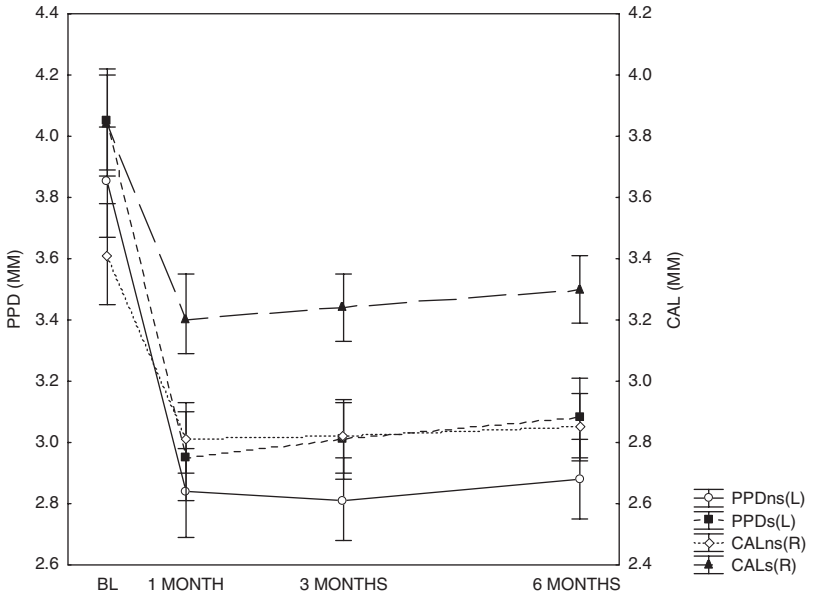

Fig. 2. Pocket depth (PD) and clinical attachment level (CAL) following non-surgical intervention. Means and 95% CIs in smokers (PDs, CALs) and non-smokers (PDns, CALns). Mean PD tended to be greater in smokers ($p = 0.061$). Mean CAL was significantly greater in smokers ($p < 0.001$).

Table 3. Risk assessment. Risk for CAL relapse of 1.5 mm or more from months 1 through 6 of follow-up

	OR	95% CI
Non-smoker		
PI level unchanged	1.00 (reference)	
PI level increased	1.99	1.16–3.42
Smoker		
PI level unchanged	4.00	1.34–11.70
PI level increased	7.91	1.56–40.20

CAL, clinical attachment level; CI, confidence interval; PI, plaque index; OR, odds ratio.

Risk for relapse

The risk for relapse towards an increased CAL (>1.5 mm) from months 1 through 6 during follow-up was 5.4-fold elevated in smokers compared with non-smokers using univariate analysis (OR = 5.4, 95% CI 1.4–21.1; $p < 0.05$). Accounting for PI, a smoker with elevated PI levels during follow-up was at a 7.9-fold greater risk for CAL relapse than a non-smoker with stable PI levels ($p < 0.05$, Table 3). A smoker with stable PI levels during follow-up still ran a four-fold elevated relapse risk.

Blood analysis

Folic acid vitamin B_{12}

The behaviour following the intervention with reference to folic acid was quite different in smokers and non-smokers, suggesting a highly significant interaction effect between smoking and folic acid ($p < 0.001$, Fig. 3). In smokers, a gradual and significant decrease from 8.0 ng/ml (95% CI 7.2–8.8) at baseline to 7.2 ng/ml (95% CI 6.4–7.9) at 6 months was observed ($p < 0.001$), whereas in non-smokers there was a gradual and significant increase from 10.8 ng/ml (95% CI 9.9–11.7) at baseline to 11.7 ng/ml (95% CI 10.9–12.5) at 6 months ($p < 0.001$). The differences between smoking groups were statistically significant at all time points ($p < 0.001$). At month 6, the folic acid level of smokers was significantly reduced as compared with baseline ($p < 0.01$), whereas in non-smokers it remained significantly elevated ($p < 0.001$). At baseline, 11.1% of the smokers and none of the non-smokers displayed a folic acid level below 5.0 ng/ml. The corresponding proportions at the month 6 observation were 15.6% of the smokers and 0% of the non-smokers.

A slight and statistically significant decrease in the vitamin B_{12} level was observed in both smokers and non-smokers over the entire observation period ($p < 0.001$, Fig. 3). The baseline levels were 363.9 pg/ml (95% CI 323.3–404.5) and 363.4 pg/ml (95% CI 321.9–405.0) in smokers and non-smokers, respectively. At the month 6 observation, the vitamin B_{12} level remained significantly decreased as compared with baseline in both smokers (342.1 95% CI 304.1–380.1) and non-smokers (342.8 95% CI 304.0–381.7). There was no interaction between smoking and vitamin B_{12} level.

Multiple linear and logistic regressions

Multiple regression analysis at each time point with folic acid as the dependent variable and age, CAL, gender, GI, PI, PD, smoking, and vitamin B_{12} as independent variables indicated that smoking ($t = -3.4$ to -7.4, $p < 0.001$) and vitamin B_{12} ($t = 2.3$–2.6, $p < 0.05$) were the only statistically significant factors. The fraction of variance explained ranged between $R^2 = 0.27$ and 0.46.

The risk for a low folic acid level (<7.0 ng/ml) was assessed in a model using smoking and vitamin B_{12} as risk factors. The relative risk associated with

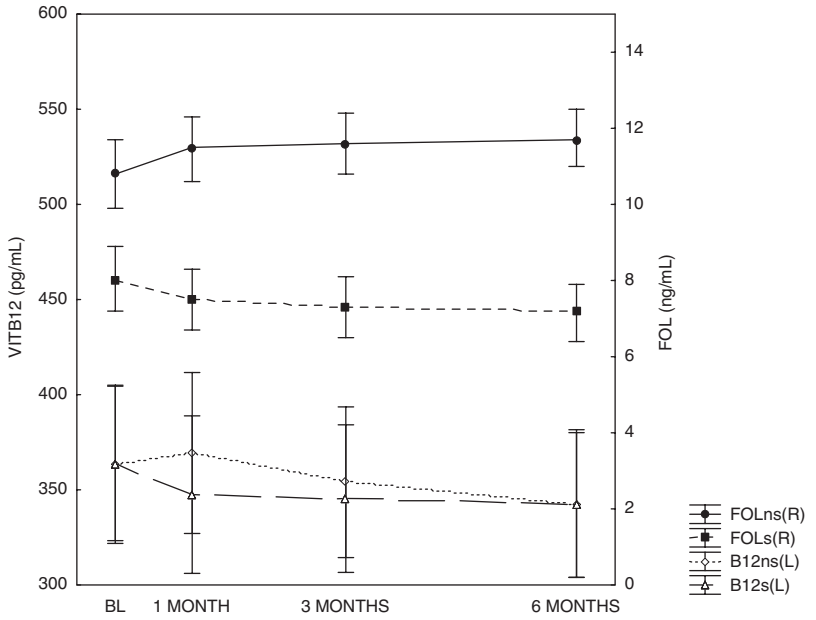

Fig. 3. Folic acid and vitamin B_{12} following non-surgical intervention. Means and 95% CIs in smokers (FOLs, B12s) and non-smokers (FOLns, B12ns). There was a significant interaction effect between smoking and mean folic acid ($p<0.001$). Vitamin B_{12} mean levels significantly decreased in both smokers and non-smokers ($p<0.001$).

smoking ranged between OR = 11.9 (95% CI 2.9–48.4, $p<0.001$) and OR = 44.9 (95% CI 5.5–368.3, $p<0.001$) and the relative risk associated with a low vitamin B_{12} level between OR = 3.0 (95% CI 1.4–6.5, $p<0.01$) and OR = 1.5 (95% CI 0.7–3.0, $p>0.05$) at the different time points.

Discussion

In the present study, the serum levels of folic acid and vitamin B_{12} were explored in patients with chronic periodontal disease in relation to the patients' smoking habits following non-surgical intervention comprising oral hygiene instruction and supra- and sub-gingival scaling. The results confirmed that the clinical response to non-surgical intervention is impaired by smoking. In addition, the observations suggested a negative influence of smoking on the serum level of folic acid following non-surgical intervention in this group of patients. The vitamin B_{12} level decreased regardless of smoking.

In terms of folic acid there was a gradual and significant decrease in smokers, whereas in non-smokers there was a gradual and significant increase resulting in a greater difference between smokers and non-smokers 6 months after the intervention than before. In fact, the proportion of smokers with a suboptimal (although by definition not deficient) level increased during the course of study.

There may be several explanations for our findings of a decreased folic acid level in smokers. Differentiations of dietary intake, residual confounding, or differential absorption remain possible explanations. Other studies have demonstrated that localized deficiencies of folic acid can occur in aero-digestive tissue and are thought to be related to rapid tissue proliferation or repair or to inactivation or alteration of the function of folic acid by external agents such as tobacco (Heimburger 1992, Piyathilake et al. 1992). Ozerol et al. (2004) and de Bree et al. (2001) suggested that nicotine consumption induces an increase of serum homocysteine. Homocysteine is a sensitive marker of vitamin B_{12} and folic acid deficiencies (Ueland et al. 2000). An inverse relationship between low folate levels and high homocysteine levels in smokers is associated with increased risk of cardiovascular disease (O'Callaghan et al. 2002).

Furthermore, cigarette smoke is known to contain nitric oxide (NO), superoxide and other reactive oxygen species, some of the adverse effects of smoking may result from oxidative damage to endothelial cells which results in NO shortage (Ozerol et al. 2004). During inflammatory reactions, where large amounts of NO and superoxide are formed, the combination of both leads to the formation of reactive nitrogen species, such as the peroxynitrite anion, a toxic product of NO when combining with superoxide (Beckman & Koppenol 1996, Szabo 1996). The tissue injury induced by peroxynitrite may lead to an excessive local amplification of the immune response, resulting in migration of inflammatory cells (Leitao et al. 2005). Verhaar et al. (1998) observed in vitro that 5-methyltetrahydrofolic acid, the active form of folic acid, reduced superoxide generation, thus providing antioxidant potential. On the other hand, free radicals and oxidants, which cause DNA and membrane damage, are present in high levels in tobacco smoke and may also play a role in decreases in folic acid levels (Niki et al. 1993, Pryor & Stone 1993). Doshi et al. (2001) suggested that folic acid abolishes the homocysteine-induced increases in endothelial superoxide. In animal models, folic acid deficiency has been shown to increase lipid peroxidation and decrease cellular antioxidant defences (Durand et al. 1996). Another mechanism potentially involved in the beneficial effects of folic acid per se on endothelial function involves its effects on the enzyme endothelial nitric oxide synthase (eNOS). Under certain conditions, eNOS can ''switch'' from chiefly NO synthesis to production of superoxide (Stroes et al. 1998, 2000, Xia et al. 1998). Recently, it was found that a low serum folic acid level was independently associated with periodontal disease in older adults (Yu et al. 2007). The authors suggested that the serum folic acid level might be an important indicator of periodontal disease in older adults and provide a clinical target for intervention to promote oral health. Folic acid supplementation, therefore, might be beneficial in smokers with chronic periodontal disease.

A slight decrease in vitamin B_{12} levels was observed in both smokers and non-smokers over the 6-month study period. There was no interaction between smoking and vitamin B_{12} level. This observation is in line with most previous studies (Piyathilake et al. 1994, O'Callaghan et al. 2002, van Wersch et al. 2002, Ozerol et al. 2004, Erdemir & Bergstrom 2006). There are studies that report lower vitamin B_{12} concentrations in the smokers (McGarry & Andrews 1972, Pagan et al. 2001). Poorer vitamin B_{12} status among smokers is usually attributed to an inactivation of methylcobalamin by cyanide inhaled from cigarette smoke (Preston 1991). On the other hand, a significantly higher

vitamin B_{12} concentration in smokers compared with non-smokers has also been reported (Tungtrongchitr et al. 2003). van Wersch et al. (2002) observed no significant difference in number of patients with decreased vitamin B_{12} levels between the smoking and non-smoking pregnant women during pregnancy. In the present study, although vitamin B_{12} was a significant factor on folic acid, there was a decrease in the vitamin B_{12} levels regardless of smoking in response to the intervention. This study does not provide evidence of circulating vitamin B_{12} decrease that is due to cigarette smoking. Other variables not considered in this study, such as dietary habits may be more important in explaining the variation of vitamin B_{12} concentrations. A common characteristic of the patients in our study was the fact that they had chronic inflammation. What impact inflammation may have on the vitamin B_{12} levels is, however, currently not known.

In terms of plaque reduction over the 6 months duration of the study, the intervention was significantly less effective in smokers. The immediate PI reduction as seen after 1 month was 21% in smokers compared with 33% in non-smokers and at the end of study 16% as against 33%, respectively. Thus, the reduction, however less pronounced in smokers, was rather moderate overall. The inferior plaque reduction in smokers may simply reflect inferior compliance. Even though there is evidence that plaque formation rates are similar in smokers and non-smokers (Bastiaan & Waite 1978, Bergstrom 1981), it cannot be ruled out that smoking to some extent interferes with plaque adhesion properties or with the individual's sensation of oral cleanliness. Smoking seems to promote the formation of supra- as well as subgingival calculus (Bergstrom 1999, 2005).

To what extent differences in plaque levels can explain differences in treatment responses of smokers and non-smokers is likely to be dependent of outcome measure. The response in terms of GI was of the same magnitude in both smokers and non-smokers, suggesting a reduction of gingival inflammation. The GI reduction (22% in smokers and 26% in non-smokers) was modest, however, and notably a GI level below 1.0 was reached in none of the patients at the end of the 6-month observation period. Despite a smaller plaque reduction, resulting in a comparably elevated mean plaque level, smokers throughout exhibited a lower mean GI level than non-smokers. This outcome is predictable and agrees with previous observations (Bergstrom & Flodérus-Myrhed 1983, Preber & Bergstrom 1985, 1986, Bergstrom & Preber 1986, Bergstrom 1990, Bergstrom & Bostrom 2001, Apatzidou et al. 2005, Darby et al. 2005, Shimazaki et al. 2006). The reason for this suppressive effect of smoking on clinical signs of gingival inflammation remains elusive.

The overall response in terms of PD or CAL was a reduction of similar magnitude in both smokers and non-smokers during the first month, followed by a subsequent return towards increasing levels, significant in smokers but not in non-smokers, suggesting a relapse trend. The PD and CAL reductions after 6 months were 24% and 14%, respectively, in smokers and 25% and 16%, respectively, in non-smokers, again suggesting a modest outcome. Earlier observations by several research groups indicate that the response to non-surgical intervention is inferior in smokers when compared with non-smokers (Preber & Bergstrom 1985, Preber et al. 1995, Palmer et al. 1999, Ryder et al. 1999, Apatzidou et al. 2005, Darby et al. 2005, Heasman et al 2006, Hughes et al. 2006). Some other studies suggest a similar 6-month outcome following scaling and root planing in smokers and non-smokers (Preshaw et al. 1999).

The present observations further suggest that all measures of clinical morbidity, although reduced immediately following the non-surgical intervention, tended to recur during the subsequent observation period regardless of smoking. This relapse trend was particularly evident in terms of clinical attachment level and on the part of smokers. A relapse trend observed as early as 6 months following treatment seems to forebode a greatly inferior response for smokers in the long-run as suggested by studies over longer periods of follow-up (Ah et al. 1994, Loesche et al. 2002, Carnevale et al. 2007) and the remarkably predominant relapse rate observed among smokers (Bergstrom & Blomlof 1992, MacFarlane et al. 1992, Magnusson et al. 1994). It may be questioned, therefore, at least regarding patients who smoke, whether the non-surgical intervention is an effective means of stopping the progression of the disease in a long run perspective. It should not come as a surprise that plaque elimination, which is commonly considered an anti-infectious strategy, is inefficient in smokers, because there is no other smoking associated disease that has been successfully treated with anti-infectious strategies. For a better understanding of the long-term outcome of non-surgical intervention further longitudinal investigations are warranted.

A limitation of the present study was the fact that the smoking habits of participants relied on self report, which may have caused some misclassification. Further, the clinical assessments were performed with the observer unmasked with regard to smoking, which may have influenced the methodological objectivity. Although sample size was reasonably great, these shortcomings might reduce the power of the study to detect minor differences between smoking groups were there any. The socio-economic standard of participants was not investigated and, in particular, their dietary habits during the course of the study were unknown. This may have resulted in incomplete adjustment for potential confounding.

In conclusion, the present observations confirm the understanding that smoking exerts a negative influence on the response to non-surgical periodontal intervention. Intriguingly, the intervention was associated with a gradually lowered folic acid concentration in smoker patients, whereas in non-smoker patients the level gradually increased. Regardless of smoking, vitamin B_{12} levels remained decreased still after 6 months. Diets rich in folic acid and vitamin B_{12} may be beneficial in this group of patients. In future, RCTs are required to investigate the possible effects of dietary supplementation of folic acid and vitamin B_{12} on the periodontal tissues in smoking and non-smoking patients with chronic periodontal disease.

References

Ah, M. K., Johnson, G. K., Kaldahl, W. B., Patil, K. D. & Kalkwarf, K. L. (1994) The effect of smoking on the response to periodontal therapy. *Journal of Clinical Periodontology* **21**, 91–97.

Apatzidou, D. A., Riggio, M. P. & Kinane, D. F. (2005) Impact of smoking on the clinical, microbiological and immunological parameters of adult patients with periodontitis. *Journal of Clinical Periodontology* **32**, 973–983.

Bastiaan, R. I. & Waite, I. M. (1978) Effects of tobacco smoking on plaque development and gingivitis. *Journal of Periodontology* **49**, 480–482.

Beckman, J. S. & Koppenol, W. H. (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *American Journal of Physiology* **271**, 1424–1437.

Bergstrom, J. (1981) Short-term investigation on the influence of cigarette smoking upon plaque accumulation. *Scandinavian Journal of Dental Research* **89**, 235–238.

Bergstrom, J. (1989) Cigarette smoking as risk factor in chronic periodontal disease. *Community Dentistry and Oral Epidemiology* **17**, 245–247.

Bergstrom, J. (1990) Oral hygiene compliance and gingivitis expression in cigarette smokers. *Scandinavian Journal of Dental Research* **98**, 497–503.

Bergstrom, J. (1999) Tobacco smoking and supragingival dental calculus. *Journal of Clinical Periodontology* **26**, 541–547.

Bergstrom, J. (2004) Tobacco smoking and chronic destructive periodontal disease. *Odontology* **92**, 1–8.

Bergstrom, J. (2005) Tobacco smoking and subgingival dental calculus. *Journal of Clinical Periodontology* **32**, 81–88.

Bergstrom, J. (2006) Periodontitis and smoking: an evidence-based appraisal. *Journal of Evidence-Based Dental Practice* **6**, 33–41.

Bergstrom, J. & Blomlof, L. (1992) Tobacco smoking major risk factor associated with refractory periodontal disease. *Journal of Dental Research* **71**, 297.

Bergstrom, J. & Bostrom, L. (2001) Tobacco smoking and periodontal hemorrhagic responsiveness. *Journal of Clinical Periodontology* **28**, 680–685.

Bergstrom, J. & Eliasson, S. (1987a) Cigarette smoking and alveolar bone height in subjects with high standard of oral hygiene. *Journal of Clinical Periodontology* **14**, 466–469.

Bergstrom, J. & Eliasson, S. (1987b) Noxious effect of cigarette smoking on periodontal health. *Journal of Periodontal Research* **22**, 513–517.

Bergstrom, J., Eliasson, S. & Dock, J. (2000) A 10-year prospective study of tobacco smoking and periodontal health. *Journal of Periodontology* **71**, 1338–1347.

Bergstrom, J. & Flodérus-Myrhed, B. (1983) Co-twin control study of the relationship between smoking and some periodontal disease factors. *Community Dentistry and Oral Epidemiology* **11**, 113–116.

Bergstrom, J. & Preber, H. (1986) Influence of cigarette smoking on the development of experimental gingivitis. *Journal of Periodontal Research* **21**, 668–676.

Bergstrom, J. & Preber, H. (1994) Tobacco use as a risk factor. *Journal of Periodontology* **65**, 545–550.

Borrell, L. N. & Papapanou, P. N. (2005) Analytical epidemiology of periodontitis. *Journal of Clinical Periodontology* **32** (Suppl. 6), 132–158.

Bouclin, R., Landry, R. G. & Noreau, G. (1997) The effects of smoking on periodontal structures: a literature review. *Canadian Dental Association* **63**, 356–363.

Butterworth, C. E. J. & Bendich, A. (1996) Folic acid and the prevention of birth defects. *Annual Review of Nutrition* **16**, 73–97.

Campbell, B. A. (1995) Megaloblastic anemia in pregnancy. *Clinical Obstetrics and Gynecology* **38**, 455–462.

Carnevale, G., Cairo, F. & Tonetti, M. S. (2007) Long-term effects of supportive therapy in periodontal patients treated with fibre retention osseous resective surgery. 1: recurrence of pockets, bleeding on probing and tooth loss. *Journal of Clinical Periodontology* **34**, 334–341.

Consensus Report (1999) Chronic periodontitis. *Annals of Periodontology* **4**, 38–39.

Darby, I. B., Hodge, P. J., Riggio, M. P. & Kinane, D. F. (2005) Clinical and microbiological effect of scaling and root planing in smoker and non-smoker chronic and aggressive periodontitis patients. *Journal of Clinical Periodontology* **32**, 200–206.

De Bree, A., Verschuren, W. M., Blom, H. J. & Kromhout, D. (2001) Lifestyle factors and plasma homocysteine concentrations in general population sample. *American Journal of Epidemiology* **154**, 150–154.

Doshi, S. N., McDowell, I. F., Moat, S. J., Lang, D., Newcombe, R. G., Kredan, M. B., Lewis, M. J. & Goodfellow, J. (2001) Folate improves endothelial function in coronary heart disease: an effect mediated by reduction of intracellular superoxide? *Arteriosclerosis Thrombosis & Vascular Biology* **21**, 1196–1202.

Durand, P., Prost, M. & Blache, D. (1996) Prothrombotic effects of a folic acid deficient diet in rat platelets and macrophages related to elevated homocysteine and decreased n-3 polyunsaturated fatty acids. *Atherosclerosis* **121**, 231–243.

Dye, B. A. & Selwitz, R. H. (2005) The relationship between selected measures of periodontal status and demographic and behavioural risk factors. *Journal of Clinical Periodontology* **32**, 798–808.

Erdemir, E. O. & Bergstrom, J. (2006) Relationship between smoking and folic acid, vitamin B12 and some haematological variables in patients with chronic periodontal disease. *Jounal of Clinical Periodontology* **33**, 878–884.

Fredriksson, M. I., Figueredo, C. M. S., Gustafsson, A., Bergström, K. G. & Asman, B. (1999) Effect of periodontitis and smoking on blood leukocytes and acute-phase proteins. *Journal of Periodontology* **70**, 1355–1360.

Giles, W. H., Kittner, S. J., Croft, J. B., Anda, R. F., Casper, M. L. & Ford, E. S. (1998) Serum folate and risk for coronary heart disease: results from a cohort of US adults. *Annals of Epidemiology* **8**, 490–496.

Grossi, S. G., Genco, R. J., Machtei, E. E., Ho, A. W., Koch, G., Dunford, R., Zambon, J. J. & Hausmann, E. (1995) Assessment of risk for periodontal disease. II. Risk indicators for alveoler bone loss. *Journal of Periodontology* **66**, 23–29.

Grossi, S. G., Zambon, J. J., Ho, A. W., Koch, G., Dunford, R., Machtei, E., Norderyd, O. & Genco, R. J. (1994) Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *Journal of Periodontology* **65**, 260–267.

Heasman, L., Stacey, F., Preshaw, P. M., McCracken, G. I., Hepburn, S. & Heasman, P. A. (2006) The effect of smoking on periodontal treatment response: a review of clinical evidence. *Journal of Clinical Periodontology* **33**, 241–253.

Heimburger, D. C. (1992) Localized deficiencies of folic acid in aerodigestive tissues. *Annals of the New York Academy of Sciences* **669**, 87–95.

Heitz-Mayfield, L. J. A. (2005) Disease progression: identification of high-risk groups and individuals for periodontitis. *Journal of Clinical Periodontology* **32** (Suppl. 6), 196–209.

Hughes, F. J., Syed, M., Koshy, B., Bostaci, N., McKay, I. J., Curtis, M. A., Marcenes, W. & Croucher, R. E. (2006) Prognostic factors in the treatment of generalized aggressive periodontitis: II. Effects of smoking on initial outcome. *Journal of Clinical Periodontology* **33**, 671–676.

Hyman, J. J. & Reid, B. C. (2003) Epidemiologic risk factors for periodontal attachment loss among adults in United States. *Journal of Clinical Periodontology* **30**, 230–237.

Khaled, M. A., Watkins, C. L. & Krumdieck, C. L. (1985) Inactivation of B12 and folate coenzymes by butyl nitrite as observed by NMR: implications on one-carbon transfer mechanism. *Biochemical and Biophysical Research Communications* **130**, 1273–1280.

Kim, Y. I. (1999) Folate and cancer prevention: a new medical application of folate beyond hyperhomocysteinemia and neural tube defects. *Nutrition Reviews* **57**, 314–321.

Klor, H. U., Hauenschild, A., Holbach, I., Schnell-Kretschmer, H. & Stroh, S. (1997) Nutrition and cardiovascular disease. *European Journal of Medical Research* **2**, 243–257.

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

Langman, M. & Boyle, P. (1998) Chemoprevention of colorectal cancer. *Gut* **43**, 578–585.

Leitao, R. F. C., Riberio, R. A., Chaves, H. V., Rocha, F. A. C., Lima, V. & Brito, G. A. C. (2005) Niric oxide synthase inhibition prevents alveolar bone resorbtion in experimental periodontitis in rats. *Journal of Periodontology* **76**, 956–963.

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* **21**, 533–551.

Loesche, W. J., Giordano, J. R., Soehren, S. & Kaciroti, N. (2002) The non-surgical treatment of patients with periodontal disease: results after five years. *Journal of the American Dental Association* **133**, 311–320.

Lökk, J. (2004) Association of vitamin B_{12}, folate, homocysteine and cognition in the elderly. *Scandinavian Journal of Nutrition* **47**, 132–138.

MacFarlane, G. D., Herzberg, M. C., Wolff, L. F. & Hardie, N. A. (1992) Refractory periodontitis associated with abnormal polymorphonuclear leukocyte phagocytosis and cigarette smoking. *Journal of Periodontology* **63**, 908–913.

Magnusson, I., Low, S. B., McArthur, W. P., Marks, R. G., Walker, C. B., Maruniak, J., Taylor, M., Padgett, P., Jung, J. & Clark, W. B. (1994) Treatment of subjects with refractory periodontal disease. *Journal of Clinical Periodontology* **21**, 628–637.

Mansoor, M., Kristensen, O., Hervig, T., Drablos, P. A, Jacob, A., Stakkestad, J. A., Woie, L., Hetland, Q. & Osland, A. (1997) Low concentrations of folate in serum and erythrocytes of smokers: Methionine loading decreases folate concentrations in serum of

smokers and non-smokers. *Clinical Chemistry* **43**, 2192–2194.
McGarry, J. M. & Andrews, J. (1972) Smoking in pregnancy and vitamin B12 metabolism. *British Medical Journal* **805**, 74–77.
Niki, E., Minamisawa, S., Oikawa, M. & Komuro, E. (1993) Membrane damage from lipid oxidation induced by free radicals and cigarette smoke. *Annals of the New York Academy of Sciences* **686**, 29–37.
O'Callaghan, P., Meleady, R., Fitzgerald, T. & Graham, I. (2002) Smoking and plasma homocysteine. *European Heart Journal* **23**, 1580–1586.
Ozerol, E., Ozerol, I., Gokdeniz, R., Temel, I. & Akyol, O. (2004) Effect of smoking on serum concentrations of total homocysteine, folate, vitamin B12, and nitric oxide in pregnancy: a preliminary study. *Fetal Diagnosis and Therapy* **19**, 145–148.
Pagan, K., Hou, J., Goldenberg, R. L., Cliver, S. P. & Tamura, T. (2001) Effect of smoking on serum concentrations of total homocysteine and B vitamins in mid-pregnancy. *Clinica Chimica Acta* **306**, 103–109.
Palmer, R. M., Matthews, J. P. & Wilson, R. F. (1999) Non-surgical periodontal treatment with and without adjunctive metronidazole in smokers and non-smokers. *Journal of Clinical Periodontology* **26**, 158–163.
Palmer, R. M., Wilson, R. F., Hasan, A. S. & Scott, D. (2005) Mechanism of action of environmental factors. Tobacco smoking. *Journal of Clinical Periodontology* **32** (Suppl. 6), 180–195.
Piskin, S., Sayan, C., Durukan, N. & Senol, M. (2002) Serum iron, ferritin, folic acid, and vitamin B_{12} levels in recurrent aphthous stomatitis. *Journal of European Academy of Dermatology and Venereology* **16**, 66–67.
Piyathilake, C. J., Hine, R. J., Dasanayake, A. P., Richards, E. W., Freeberg, L. E., Vaughn, W. H. & Krumdieck, C. L. (1992) Effect on smoking on folate levels in buccal mucosal cells. *International Journal of Cancer* **52**, 566–569.
Piyathilake, C. J., Macaluso, M., Hine, R. J., Richards, E. W. & Krumdieck, C. L. (1994) Local and systemic effects of cigarette smoking on folate and vitamin B-12. *American Journal of Clinical Nutrition* **60**, 559–566.
Pollack, S. V. (1979) Wound healing: a review. III. Nutritional factors affecting wound healing. *Journal of Dermatology and Surgical Oncology* **5**, 615–619.
Preber, H. & Bergstrom, J. (1985) The effect of non-surgical treatment on periodontal pockets in smokers and non-smokers. *Journal of Clinical Periodontology* **13**, 319–323.
Preber, H. & Bergstrom, J. (1986) Effect of non-surgical periodontal treatment on gingival bleeding in smokers and non-smokers. *Acta Odontologica Scandinavica* **44**, 85–90.
Preber, H., Linder, L. E. & Bergstrom, J. (1995) Periodontal healing and periopathogenic microflora in smokers and nonsmokers. *Journal of Clinical Periodontology* **22**, 946–952.
Preshaw, P. M., Lauffart, B., Zak, E., Jeffcoat, M. K., Barton, I. & Heasman, P. A. (1999) Progression and treatment of chronic adult periodontitis. *Journal of Periodontology* **70**, 1209–1220.
Preston, A. M. (1991) Cigarette smoking- nutritional implications. *Progress in Food & Nutrition Science* **15**, 183–217.
Pryor, W. & Stone, K. (1993) Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Annals of the New York Academy of Sciences* **686**, 12–27.
Ryder, M. I., Pons, B., Adams, D., Beiswanger, B., Blanco, V., Bogle, G., Donly, K., Hallmon, W., Hancock, E. B., Hanes, P., Hawley, C., Johnson, L., Wang, H. L., Wolinsky, L., Yukna, R., Polson, A., Carron, G. & Garrett, S. (1999) Effects of smoking on local delivery of controlled-release doxycycline as compared to scaling and root planing. *Journal of Clinical Periodontology* **26**, 683–691.
Shimazaki, Y., Toshiyuki, S., Kiyohara, Y., Kato, I., Kubo, M., Iida, M. & Yamashita, Y. (2006) The influence of current and former smoking on gingival bleeding: the Hisayama study. *Journal of Periodontology* **77**, 1430–1435.
Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* **22**, 121–135.
Stroes, E., Hijmering, M., van Zandvoort, M., Wever, R., Rabelink, T. J. & van Faassen, E. E. (1998) Origin of superoxide production by endothelial nitric oxide synthase. *FEBS Letters* **438**, 161–164.
Stroes, E. S. G., van Faessen, E. E. & Yo, M. (2000) Folic acid reverts dysfunction of endothelial nitric oxide synthase. *Circulation Research* **86**, 1129–1134.
Szabo, C. (1996) The role of peroxynitrite in the pathophysilogy of shock, inflammation and ischemia-reperfusion injury. *Shock* **6**, 79–88.
Tonetti, M. S. & Claffey, N. (2005) Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C Consensus report of the 5th European workshop in periodontology. *Journal of Clinical Periodontology* **32** (Suppl. 6), 210–213.
Tungtrongchitr, R., Pongpaew, P., Soonthornruengyot, M., Viroonudomphol, D., Vudhivai, N., Tungtrongchitr, A., Phonrat, B., Pooudong, S. & Schelp, F. P. (2003) Relationship of tobacco smoking with serum vitamin B12, folic acid and haematological indices in healthy adults. *Public Health Nutrition* **6**, 675–681.
Ueland, P. M., Refsum, H., Beresford, S. A. & Vollset, S. E. (2000) The controversy over homocysteine and cardiovascular risk. *American Journal of Clinical Nutrition* **72**, 324–332.
van Wersch, J. W., Janssens, Y. & Zandvoort, J. A. (2002) Folic acid, Vitamin B(12), and homocysteine in smoking and non-smoking pregnant women. *European Journal of Obstetrics & Gynecology and Reproductive Biology* **103**, 18–21.
Verhaar, M. C., Wever, R. M. F., Kastelein, J. J. P., van Dam, T., Koomans, H. A. & Rabelink, T. J. (1998) 5-Methyltetrahydrofolate, the active form of folic acid, improves endothelial function in familial hypercholesterolemia. *Circulation* **97**, 237–241.
Xia, Y., Tsai, A. L., Berka, V. & Zweier, J. L. (1998) Superoxide generation from endothelial nitric oxide synthase. A Ca^{2+}/calmodulindependent and tetrahydrobiopterin regulatory process. *Journal of Biological Chemistry* **273**, 804–808.
Yu, Y. H., Kuo, H. K. & Lai, Y. L. (2007) The association between serum folate levels and periodontal disease in older adults: data from the national health and nutrition examination survey 2001/02. *Journal of American Geriatrics Society* **55**, 108–113.

Address:
Ebru Olgun Erdemir
Department of Periodontology
Faculty of Dentistry
Kirikkale University
Kirikkale/TURKEY
E-mail: olgun_ebru@yahoo.com

Clinical Relevance

Scientific rationale for the study: Folic acid and vitamin B_{12} are involved in the regulation of homocysteine, a risk factor for several diseases including coronary heart disease. We have earlier observed that among periodontal disease patients smokers display significantly reduced levels of folic acid. We therefore investigated the possible influence of smoking on folic acid and vitamin B_{12} status during a 6-month period following non-surgical intervention.

Principal findings: Smoking exerted a negative influence on the clinical outcome following non-surgical intervention. Folic acid status deteriorated in smokers during the course of study, whereas it improved in non-smokers. At 6-months, the number of smokers with suboptimal folic acid levels remained elevated as compared with baseline.

Practical implications: Any periodontal treatment in smokers should include attempts to abolish smoking. Diets rich in folic acid and vitamin B_{12} may be beneficial in this group of patients. In future, RCTs are required to investigate the possible effects of dietary supplementation of folic acid and vitamin B_{12} on the periodontal tissues in smoking and non-smoking patients with chronic periodontal disease.