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Micronutritional approaches to periodontal therapy

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Abstract

Aim: Periodontitis results from the loss of a delicate balance between microbial virulence factors and a proportionate host response. Nutritional factors have been implicated in several chronic inflammatory diseases that are associated with periodontitis. This manuscript reviews the evidence for nutritional exposures in the etiology and therapeutic management of periodontitis, and makes recommendations for daily nutritional intake for vitamin C (ascorbic acid), vitamin D, calcium, and antioxidants.

Results and conclusion: Periodontitis is associated with low serum/plasma micronutrient levels, which may result from dietary and/or life-style factors as well as nutrigenetic characteristics. Early evidence suggests beneficial outcomes from nutritional interventions; supporting the contention that daily intake of certain nutrients should be at the higher end of recommended daily allowances. For prevention and treatment of periodontitis daily nutrition should include sufficient antioxidants, vitamin D, and calcium. Inadequate antioxidant levels may be managed by higher intake of vegetables, berries, and fruits (e.g. kiwi fruit), or by phytonutrient supplementation. Current evidence is insufficient to support recommendations of mono-antioxidant vitamin supplements and randomised controlled double-blind intervention studies are needed to provide evidence to underpin future recommendations. Inadequate supply of vitamin D and calcium may be addressed by implementing changes in diet/life style or by supplements.

Key words: antioxidants; micronutrients; nutrition; oxidative stress; periodontitis; vitamin C; vitamin D

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Periodontitis is a destructive inflammatory disease of the tissues that surround and support the teeth, and can in general be successfully treated by scaling, root surface debridement (RSD) and periodontal surgery. Nevertheless, some patients respond poorly to this form of therapy and have been referred to as suffering from ''refractory periodontitis'' (Adams 1992, Flemmig 1999). Regarding the onset/progression of periodontal breakdown a number of risk indicators/factors have been identified e.g. smoking (Grossi et al. 1995, Bergström et al. 2000), diabetes (Lalla et al. 2007a, b), genetics (Michalowicz et al. 1991, 2000, Torres de Heens et al. 2010), stress (Hugoson et al. 2002) and (subgingival) presence of periodontal pathogens like *Porphyromonas gingivalis*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* (Papapanou et al. 1997, Van der Velden et al. 2006). The onset and progression of disease depend upon a delicate equilibrium between the microbial challenge and the host response. In this respect, nutrition may be of great importance since it has been implicated in a number of inflammatory diseases and conditions, including cardiovascular diseases, type 2 diabetes, rheumatoid arthritis and inflammatory bowel disease, all of which have been associated with periodontitis.

There are six major classes of nutrients: carbohydrates, fats, minerals, protein, vitamins, and water. These nutrient classes can be categorized as either macronutrients (needed in relatively large amounts) or micronutrients. Micronutrients are dietary compounds, such as vitamins, minerals, trace elements, amino acids, poly-unsaturated fatty acids (PUFA) that are required only in small quantities (micrograms or milligrams per day) by living organisms and are essential for optimal health, proper growth, and metabolism. This paper will discuss the possible role of some micronutrients in the etiology and therapy of periodontal diseases. For this review the National Library of Medicine, Washington, DC (MEDLINE: PubMed) was used to select appropriate

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papers. MESH terms included: micronutrients, vitamin A (retinol), vitamin B complex [vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_5 (pantothenic acid), vitamin B_6 group (pyridoxine, pyridoxal, pyridoxamine), vitamin B_7 (biotin), vitamin B_8 (ergadenylic acid), vitamin B_9 (folic acid), vitamin B_{12} (cyanocobalamin), choline, inositol], vitamin C (ascorbic acid), vitamin D, vitamin E (tocopherols, tocotrienols), vitamin K, biotin, carotenoids (alpha carotene, beta carotene, crytoxanthin, lutein, lycopene, zeanxantin), flavonoids, glutathione (GSH), melatonin, polyphenolics, polyunsaturated fatty acids, omega 3 fatty acids, macrominerals (calcium, chloride, magnesium, phosphorus, potassium, sodium), and trace minerals (boron, cobalt, chloride, chromium, copper, fluoride, iodine, iron, manganese, molybdenum, selenium, zinc), periodontal diseases, periodontitis. Only studies written in English language were accepted, while pilot studies, case reports and reports in which micronutrients were not used as a nutritional intervention were not accepted for this review. In addition, reviews and studies on scurvy/vitamin C (ascorbic acid) deficiency were excluded for the assessment of the relationship between micronutrients and periodontal conditions. All reference lists of the selected studies were screened for additional papers. The paucity of nutritional intervention studies and the wide heterogeneity of study designs precluded a systematic approach with focussed questions. Therefore, this is a traditional evidence-based narrative review. However, for the assessment of the relationship between micronutrients and periodontitis all available studies that we could find, which were carried out in humans were included. In Table 1 an overview of these studies is presented.

Vitamin C

The importance of ascorbic acid, better known as vitamin C, for periodontal health has long been known. Sailors in the 18th century often suffered from scurvy, the vitamin C deficiency disease, associated with bleeding of the gums and loosening of the teeth. In 1747 James Lind conducted his classic experiments aboard the ship ''the Salisbury'', in which he cured scurvy with oranges and lemons (Sutton 2003). Investigations over several decades have sought to elucidate the mechanisms of action of vitamin C and provide evidence for its multiple functions.

Ascorbic acid is an essential nutrient, exhibiting rapid intestinal absorption and a very low renal-excretion threshold (Lee et al. 1988). It acts in single-electron reactions as an electron donor for different enzymes and is therefore a reducing agent that itself becomes oxidized to dehydroascorbic acid (DHAA). The latter can be used by cells to regenerate ascorbic acid, and directly or indirectly, it can change the redox state of many other molecules (Rodrigo et al. 2007). In addition, it participates in collagen hydroxylation, in the biosynthesis of norepinephrine from dopamine and in the modulation of tyrosine metabolism (Levine 1986).

Ascorbic acid can be synthesized from d-glucuronate by those vertebrates who retain this innate capacity. The last step in the pathway of vitamin C synthesis is the oxidation of L-gulonolactone to L-ascorbic acid by L-gulonolactone oxidase, an enzyme associated with the endoplasmic reticulum membrane and one that is deficient in man, guinea pigs, and other species due to mutations in the gene that encodes it (Linster & Van Schaftingen 2007). Therefore, ascorbic acid and its oxidized form DHAA are essential dietary sources of vitamin C in humans. Insufficient consumption of vegetables and fruits, the two major sources of vitamin C, can lead to depletion or deficiency states for the vitamin (Taylor et al. 2000, Wrieden et al. 2000). Both nutrients, ascorbic acid and DHAA, are absorbed from the lumen of the intestine and renal tubules by enterocytes and renal epithelial cells, respectively. Specific mechanisms of transport and metabolism concentrate vitamin C intracellularly to enhance its function as an enzyme cofactor and a scavenging antioxidant (Wilson 2005). Vitamin C is a powerful antioxidant radical scavenger within the aqueous phase, but upon oxidation forms an ascorbyl radical, which then breaks down to DHAA (Bergendi et al. 1999). DHAA can be converted back to ascorbate directly by reduced GSH or by the NAD-semi-dehydroascorbate reductase enzyme system, which also utilises GSH. These systems are intracellular and thus ascorbate within the extracellular fluids is rapidly depleted (oxidized) in conditions of oxidative stress (Frei et al. 1989) unless adequate GSH levels are present (Svardal et al. 1990). GSH (see later) is therefore a chain-breaking antioxidant, crucial to controlling cellular redox status and downstream inflammatory events and maintaining appropriate cell and tissue vitamin C levels, helps to preserve intracellular GSH (Chapple & Matthews 2007).

Ascorbic acid is a co-factor for lysyl and prolyl hydroxylase, two iron essential enzymes in the collagen biosynthesis pathway (Robertson 1961). Lysyl and prolyl hydroxylase catalyse the hydroxylation of lysine and proline residues on the collagen polypeptide, and these post-translational modifications allow the formation/stabilisation of the collagen triple helix, and its subsequent secretion into the extracellular space as procollagen. Procollagen is then transformed to tropocollagen by propeptide excision, and finally collagen fibres are formed by spontaneous spatial re-arrangement of tropocollagen molecules. Consequently, collagen hydroxylation is a critical step in collagen biosynthesis. Interestingly, lysyl hydroxylase is down regulated in oral epithelial cells when exposed to the challenge of periodontal pathogens, providing some evidence for collagen dysmetabolism as a feature of frustrated healing (Milward et al. 2007). The role of vitamin C is to promote the synthesis of a normal mature collagen network by preventing iron-dependent oxidation of lysyl and prolyl hydroxylase and protecting these enzymes against auto-inactivation (Puistola et al. 1980).

Periodontal ligament (PDL) cells are composed of fibroblastic and mineralized tissue-forming cells derived from fibrous and cellular connective tissues attaching teeth to bone. A large percentage of the cells differentiate into fibroblasts; and a substantial proportion of the cells exhibit an osteogenic response to appropriate stimulation (Ishikawa et al. 2004). PDL cells share some properties with osteoblasts that gingival fibroblasts lack or only weakly express, such as high alkaline phosphatase (ALP) activity, production of bone-associated proteins (including osteopontin, osteocalcin, bone sialoprotein, and bone morphogenetic protein-2/4), and mineralized nodule formation (Shiga et al. 2003). The mediators of osteogenic responses in these cells enhance the expression of ALP, type I collagen, osteocalcin, osteopontin, bone sialoprotein, and increase the formation of

Table 1. Publications that assessed the relationship between micronutrients and periodontitis in humans

Study	Background of participants	No. of subjects	Age (years) range or mean	Definition of periodontal disease	Study design	Nutrient measurements	Relationship between micronutrient and periodontal disease
Vitamin C							
Burrill (1942)	Patients attending the dental school (USA)	971	Not given	Pocket depth	Cross-sectional	Plasma vitamin C	No association
Barros & Witkop (1963)	Military personnel and civilians (Chile)	1877	<4–50+	PI*	Cross-sectional	Serum vitamin C	No association
Russell (1963)	Subjects from eight countries (Alaska, Ethiopia, Ecuador, S. Vietnam, Chile, Colombia, Thailand, Lebanon)	3065	5–50+	PI*	Cross-sectional	Plasma vitamin C	No association
Russell et al. (1965)	Military personnel and civilians (S. Vietnam)	2474	<4–70+	PI*	Cross-sectional	Serum vitamin C	No association
Enwonwu & Edozien (1970)	Low and high socio-economic subjects (Nigeria)	941	5–50+	PI*	Cross-sectional	Serum vitamin C	No association
Clark et al. (1990)	Pregnant woman (USA)	102	19–30	Alveolar bone loss	longitudinal	Dietary intake vitamin C	No association
Ismail et al. (1983)	NHANES I (USA)	8609	25–74	PI*	Cross-sectional	Dietary intake vitamin C	Inverse relation
Blignaut & Grobler (1992)	Fruit and grain farmer workers	313	15–76	CPITN†	Cross-sectional	Dietary fruit intake	Citrus farm workers have less frequently deep pockets compared to grain farm workers
Väänänen et al. (1993)	General population (Finland)	75 cases (low plasma vitamin C) and 75 controls (high plasma vitamin C)	20–64 20–64	Pocket depth	Case-control	Plasma vitamin C	Deeper pockets in cases
Nishida et al. (2000a, b)	NHANES III (USA)	12419	20–90+	Attachment loss	Cross-sectional	Dietary intake vitamin C	Inverse relation in current and former smokers
Chapple et al. (2007b)	NHANES III (USA)	11480	20–90	Pocket depth Attachment loss	Cross-sectional	Serum vitamin C	Inverse relation
Amarasena et al. (2005)	Community dwelling elderly (Japan)	413	mean 70	Pocket depth Attachment loss	Cross-sectional	Serum vitamin C	Inverse relation
Staudte et al. (2005)	Periodontitis patients and Control subjects (Germany)	58 patients 20 controls	22–75 (patients) 24–65 (controls)	Pocket depth	Case-control	Plasma vitamin C	Lower plasma vitamin C levels in patients
Panjamurthy et al. (2005)	Periodontitis patients and Control subjects (India)	25 patients 25 controls	Not given	Pocket depth	Case-control	Plasma vitamin C	Lower plasma vitamin C levels in patients
Amaliya et al. (2007)	Tea workers (Indonesia)	123	33–43	Attachment loss	Cross-sectional	Plasma vitamin C	Inverse relation
Vitamin D and calcium							
Nishida et al. (2000b)	NHANES III (USA)	12419 dietary Ca 11787 serum Ca	20–90+	Pocket depth Attachment loss	Cross-sectional	Dietary Ca intake and serum Ca	Inverse relation
Al-Zahrani (2006)	NHANES III (USA)	12764	⩾18	Pocket depth Attachment loss	Cross-sectional	Dairy products	Inverse relation

Krall (2001)	Community dwelling men (USA)	387 Ca < 1000 mg/day 165 Ca ⩾ 1000 mg/day	mean 62 mean 63	Alveolar bone loss	Cohort study	Dietary Ca intake Dietary vitamin D intake	No relationship at baseline. For Ca an inverse relation and for vitamin D no relation with disease progression
Dietrich et al. (2004)	NHANES III (USA)	11 202	20–90+	Attachment loss	Cross-sectional	Serum 25(OH)D	Inverse relation in subjects ⩾ 50 years
Liu et al. (2009)	Periodontitis patients and Control subjects (China)	52 (chronic periodontitis) 66 (aggressive periodontitis) 60 (controls)	18–64 (chronic periodontitis) 16–37 (aggressive periodontitis) 20–39 (controls)	Pocket depth Attachment loss	Case-control	Plasma 25(OH)D	Plasma 25(OH)D higher in aggressive periodontitis patients compared to controls
Magnesium (Mg)							
Meisel et al. (2005)	Residence registries (Germany)	4290	20–80	Pocket depth Attachment loss	Cross-sectional	Serum Mg and Ca	Inverse relation of Mg and Mg/Ca ratio
Antioxidants other than vitamin C							
Brock et al. (2004)	Periodontitis patients and Control subjects (UK)	20 (patients) 20 (controls)	23–60 (patients) 24–63 (controls)	Pocket depth	Case-control	Total antioxidant capacity in serum and plasma	Total antioxidant capacity in plasma lower in periodontitis patients compared to controls
Konopka et al. (2007)	Periodontitis patients and Control subjects (Poland)	56 (patients) 25 (controls)	18–55 (patients) 22–50 (controls)	Pocket depth Alveolar bone loss	Case-control	Total antioxidant status in serum	Total antioxidant status in plasma lower in periodontitis patients compared to controls
Panjamurthy et al. (2005)	Periodontitis patients and Control subjects (India)	25 patients 25 controls	Not given	Pocket depth	Case-control	Thiobarbituric acid reactive substances, Superoxide dismutase, catalase, glutathione peroxide, vitamin E, reduced glutathione	Lower plasma vitamin E and reduced glutathione levels in patients
Chapple et al. (2007b)	NHANES III (USA)	11 480	20–90	Pocket depth Attachment loss	Cross-sectional	Serum α-carotene, β-carotene, selenium, lutein, uric acid, β-cryptoxanthine, vitamins A, E and bilirubin. Total antioxidant capacity in serum was calculated	Inverse relation total antioxidant capacity and bilirubin.
Linden et al. (2009)	Men matching the social class structure of the population (Northern Ireland)	1258	60–70	Pocket depth Attachment loss	Cross-sectional	Retinol, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, β-cryptoxanthine, zeaxanthin, lutein and lycopene	Inverse relation α-carotene, β- carotene, β-cryptoxanthine,
Yu et al. (2007)	NHANES 2001/02 (USA)	844	⩾60	Pocket depth Attachment loss	Cross-sectional	Serum folic acid	Inverse relation serum folic acid
Esaki et al. (2009)	National Health and Nutrition Survey (Japan)	497	50–59	CPI† & bleeding on probing	Cross-sectional	Dietary folic acid intake	Inverse relation between dietary folic acid and

Table 1. (Contd.)

Study	Background of participants	No. of subjects	Age (years) range or mean	Definition of periodontal disease	Study design	Nutrient measurements	Relationship between micronutrient and periodontal disease
							bleeding on probing and no relation with CPI
Kushiyama et al. (2009)	Men of self-defence force (Japan)	940	49–59	Pocket depth Attachment loss	Cross-sectional	Dietery green tea intake	Inverse relation
Gómez-Moreno et al. (2007)	Periodontitis patients and Control subjects (Spain)	46 (patients) 26 (controls)	47.2 (patients) 47.2 (controls)	Pocket depth	Case-control	Plasma melatonin	Inverse relation
Polyunsaturated fatty acids							
Iwasaki et al. (2010)	Elderly population (Japan)	55	mean 74	Attachment loss	Cohort study	Dietary docosahexaenoid and eicosapentaenoic acid	Inverse relation between dietary docosahexaenoid intake and disease progression

*Periodontal Index (Russell 1956).
†Community Periodontal Index of Treatment Needs/Community Periodontal Index (WHO 2005).

mineralized nodules in PDL cells (Ishikawa et al. 2004). Because the PDL is comprised of cells with different physiologic functions, it is likely that the various cellular phenotypes show varied responses to ascorbic acid (Mimori et al. 2007). Similar to osteoblastic cells, bone-forming PDL cells lining the lamina dura may undergo further differentiation in the presence of ascorbic acid. In contrast, fibroblastic PDL cells may respond to ascorbic acid by increasing both collagen and collagenase-1 expression, maintaining a high state of matrix turnover necessary for an actively remodelling tissue like the PDL (Shiga et al. 2003, Hayami et al. 2007).

Neutrophilic polymorphonuclear leukocytes (PMNLs), mononuclear cells (MN), platelets, and endothelial cells accumulate high concentrations of ascorbic acid (Evans et al. 1982). The known transport mechanisms are facilitated diffusion of DHAA through glucose-sensitive and insensitive transporters, facilitated diffusion of ascorbic acid through specific channels, exocytosis of ascorbic acid in secretory vesicles, and secondary active transport of ascorbic acid through the sodium-dependent vitamin C transporters SVCT1 and SVCT2 proteins that are encoded by the genes Slc23a1 and Slc23a2, respectively (Wilson 2005). PMNLs and macrophages contain intracellular ascorbic acid concentrations that are 10–40 times higher than plasma (Oberritter et al. 1986). It has been suggested that high ascorbic acid levels achievable in leukocytes contribute to the ability of these cells to react to inflammatory stimuli (Boxer et al. 1979). The latter study demonstrated that the exposure of peripheral blood leukocytes to levels of ascorbic acid as high as 5 mM (880 mg) increased their chemotactic responsiveness as well as promoting the assembly of cellular microtubules that are involved in providing a structural framework for the cell. Ascorbic acid was not only shown to enhance chemotaxis of normal PMNLs but also to correct in vivo and in vitro the abnormal chemotaxis and lysosome degranulation, two microtubule-dependant functions, in PMNL from patients with Chediak–Higashi syndrome (CHS) – a rare autosomal recessive disorder characterized by impaired bacteriolysis (Goetzl et al. 1974, Boxer et al. 1979). It was suggested that the improved clinical course of patients with CHS following treatment with ascorbic acid (200 mg daily)

was related at least in part, to improvements in microtubule assembly and bactericidal activity of PMNLs (Boxer et al. 1979, Rister & Haneke 1980). These and other studies indicated that ascorbic acid may be useful in the treatment of conditions associated with impaired PMNL movement and chronic or recurrent bacterial or viral infections, rheumatoid arthritis, diabetes mellitus or allergic states (Anderson & Theron 1979).

Some authors have reported on the influence of ascorbic acid on improved phagocytic capacity of neutrophils (Leibovitz & Siegel 1978). Stankova et al. (1975) found similar phagocytic activities in normal and scorbutic guinea-pig neutrophils, including their ability to opsonize and kill *Staphylococcus aureus*. However, in an experiment on human PMNLs the same group observed a reduction in total and reduced ascorbic acid contents and a light increase in DHAA following phagocytosis, demonstrating that ascorbic acid is involved in, or affected by the phagocytic process. Two further studies on scorbutic guinea-pigs with 16 times lower ascorbic acid levels in leucocytes compared with normoscorbic animals, demonstrated that ascorbic acid deficiency did not appear to affect phagocytic activity itself, but diminished the in vitro effectiveness of circulating PMNLs in killing ingested, cell-associated, and extracellular *Actinomyces viscosus*, and led to changes in leukocyte morphology as well as to impaired or absent chemotactic responses (Goldschmidt et al. 1988, Goldschmidt 1991). The decreased bactericidal activity was reversed by adding vitamin C supplements to the diet of the scorbutic animals. Furthermore, in a non-scorbutic experimental periodontitis rat model it was shown that vitamin C supplements reduced the polymorphonuclear leukocyte infiltration in the gingiva (Ekuni et al. 2009).

The relationship between vitamin C deficiency and necrotizing ulcerative gingivitis has frequently been described (Melnick et al. 1988). However, in early epidemiological studies no relationship could be assessed between vitamin C and the degree of periodontal disease (Burrill 1942, Barros & Witkop 1963, Russell 1963, Russell et al. 1965, Enwonwu & Edozien 1970). Also, in a short-term study on the effects of pregnancy and vitamin C on the periodontal condition no association could be found between dietary intake of vitamin C and changes in alveolar crest morphology and pocket depth (Clark et al. 1990). In contrast, most recent studies have found a relationship between vitamin C and periodontitis. Vogel & Wechsler (1979) showed that the daily intake of vitamin C in a group of periodontitis patients was significantly less than in the control subjects. On the basis of the NHANES I study, Ismail et al. (1983) found a weak but significant negative correlation between dietary vitamin C intake and periodontal disease after controlling for the potentially confounding variables of age, gender, race, education, income, and oral hygiene status. Blignaut & Grobler (1992) compared the periodontal condition of workers in citrus fruit-producing farms to that of workers in grain-producing farms. The only significant difference in the diets of the groups was the large amounts of fresh fruit consumed by the fruit-farm workers who had free access to the fruit produced on the farms. Results showed that deeper pockets (CPITN code 3 and 4) occurred far less frequently in subjects who consumed citrus fruit. In a case control study matched for age, gender, and number of teeth, Väänänen et al. (1993) studied the periodontal condition of subjects with low [$\leqslant$4.4 mg/l (multiply mg/l by 5.678 to get μmol/l)] and high ($\geqslant$8.8 mg/l) plasma vitamin C levels. In the group with low-plasma vitamin C levels, 60% of the subjects had pockets $\geqslant$4 mm compared with 37% in the group with high plasma vitamin C levels. On the basis of the NHANES III survey, Nishida et al. (2000a) found that the dietary intake of vitamin C showed a weak, but statistically significant inverse relationship to periodontal disease in current and former smokers. Smokers with the lowest intake of vitamin C were likely to have the worst periodontal condition. Using the same NHANES III data set Chapple et al. (2007a) found a strong and consistent inverse association between serum vitamin C concentrations and the prevalence of periodontitis in adjusted models (multiple logistic regression analysis adjusted for age, gender, race/ethnicity, BMI, cigarette smoking, oral contraceptives and hormone replacement therapy use, diabetes, poverty income ratio, and education). Their results also showed stronger inverse associations between serum vitamin C concentrations and periodontitis among a sub-group of never-smokers than within the full sample. Amarasena et al. (2005) showed in an elderly population of community dwelling Japanese subjects an inverse relationship between serum vitamin C levels and attachment loss irrespective of smoking, diabetes, oral hygiene, gender, or number of teeth present. Staudte et al. (2005) found lower levels of plasma vitamin C in periodontitis patients compared with periodontally healthy controls i.e. 4.9 and 10.7 mg/l, respectively. Panjamurthy et al. (2005) also demonstrated lower plasma levels of vitamin C in periodontitis patients compared with healthy controls but these levels were 7.1 and 13.8 mg/l. In a more recent study, Amaliya et al. (2007) also found in an Indonesian population deprived of dental care a significant inverse relationship between plasma vitamin C levels and attachment loss. In addition, subjects with vitamin C deficiency (plasma vitamin C values $<$2.0 mg/l) comprising 14.7% of the study population, had more attachment loss compared with those with depletion or normal plasma vitamin C values. The relationship between periodontitis and vitamin C is also supported by a Finish study (Pussinen et al. 2003) which demonstrated an inverse relationship between plasma vitamin C values and serum antibody levels to *P. gingivalis* in a random subsample of Finnish and Russian men (linear regression analysis adjusted for age, number of teeth and dental fillings, serum carbohydrate-deficient transferrin concentrations, and number of cigarettes smoked per day).

For several years the recommended daily intake of vitamin C has been 60 mg (Havel et al. 1989). In 2000 the recommended guidelines for daily intake of vitamin C was increased by the Food and Nutrition Board, based upon more recent biochemical, molecular, epidemiological, and clinical data (FNB 2000). The Recommended Dietary Allowance (RDA) for men was increased from 60 to 90 mg daily and for women to 75 mg daily. However, some feel that these values are still too low. Levine et al. (2001a) concluded that the ideal vitamin C intake should be 200 mg daily from a variety of fresh fruits and vegetables.

Vitamin D and Calcium

At present it is common knowledge that an adequate supply of vitamin D and calcium are essential for optimal skele-

tal development and maintaining bone mass. However, vitamin D is not only important in relation to bone metabolism but also to a number of other diseases. Vitamin D deficiency has been associated with cancer, infectious diseases, chronic inflammatory and autoimmune diseases like inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and type I diabetes mellitus (Peterlik & Cross 2009).

Vitamin D_3 or the structurally related vitamin D_2, whether synthesized in the skin under the influence of ultraviolet light from the sun or absorbed from the diet, are both further metabolized within several hours in the liver by hydroxylation at C25 to form $25(OH)D_3$ mostly referred to as 25(OH)D. This form is largely stable and its half-life has been estimated to be approximately 60 days. Therefore serum levels of 25(OH)D are regarded as a reliable indicator of the vitamin D status of an individual. The active form of vitamin D is established when the kidneys hydrolyse 25(OH)D into $1,25(OH)_2D_3$, the biologically active hormone. When serum levels of 25(OH)D are low there is decreased calcium absorption and increased parathyroid hormone secretion. This hormone increases osteoclastic activity in bone to release stored calcium into the circulation and also increases the synthesis of $1,25(OH)_2D_3$, which stimulates the absorption of calcium from the intestine. An increase of extracellular Ca^{2+} firstly stimulates pre-osteoblasts to proliferate and to produce collagen (Yamaguchi et al. 1998). Then, activation of the vitamin D receptor (VDR) by $1,25(OH)_2D_3$ promotes osteoblast differentiation resulting in matrix maturation and mineralisation (Owen et al. 1991).

$1,25(OH)_2D_3$ not only plays an important role in systemic calcium and phosphate homeostasis, but is also important for the regulation of cellular proliferation, differentiation and function of a great number of cells throughout the body that express the VDR. In the early 1980s, it was first observed that malignant cells that had a VDR responded to $1,25(OH)_2D_3$ with marked inhibition of cell proliferation and induction of terminal differentiation (Tanaka et al. 1982). This opened the door for extensive research into the potential role of $1,25(OH)_2D_3$ in the treatment of cancer. Recently, the preventive role of $1,25(OH)_2D_3$ was confirmed in a 4-year, population-based, double-blind, randomized placebo-controlled trial which demonstrated that improving calcium and vitamin D nutritional status substantially reduced all-cancer risk in postmenopausal women (Lappe et al. 2007).

Reports suggest that vitamin D deficiency increases the risk for cardiovascular diseases. Conflicting reports were published on the relationship between 25(OH)D and blood pressure. Scragg et al. (2007) found that the vitamin D status was inversely associated with blood pressure whereas the study of Forman et al. (2005) did not support such a relationship. This discrepancy may be explained by the fact that it is not the 25(OH)D concentrations in blood that are important, but more the extent to which the kidneys are able to produce enough $1,25(OH)_2D_3$ to suppress rennin synthesis which is part of the system to control blood pressure (Peterlik & Cross 2009). Scragg et al. (1990) found in a community-based case–control study a significant relationship between low plasma levels of 25(OH)D and myocardial infarction. Subsequently, Wang et al. (2008) reported data from a 5 year follow-up study in which individuals with 25(OH)D levels <15 ng/ml (multiply ng/ml by 2.496 to get μmol/l 25(OH)D) had a more than 50% increased risk of incident cardiovascular events compared with those with 25(OH)D $\geqslant 15$ ng/ml. In a 7 year flow-up study Dobnig et al. (2008) showed that low levels of 25(OH)D i.e. <20 ng/ml were independently associated with all-cause and cardiovascular mortality.

During recent decades a wealth of evidence has become available suggesting that vitamin D is a potent regulator of innate immune responses against infectious diseases (Adams & Hewison 2008). Of great importance for the role of vitamin D in innate immunity is the fact that monocytes, macrophages, and dendritic cells all express the VDR, which when up regulated by activated Toll-like receptors-2/1, results in the intracellular production of $1,25(OH)_2D_3$ which in turn induces the release of LL-37 cathelicidin, a potent anti-microbial peptide (Liu et al. 2006). This mechanism is not restricted to immune cells alone. The promoter regions of the human cathelicidin antimicrobial peptide and defensin $\beta 2$ genes contain common vitamin D response elements that mediate $1,25(OH)_2D_3$-dependent gene transcription. $1,25(OH)_2D_3$ induces antimicrobial peptide gene expression in human keratinocytes, monocytes, and neutrophils (Wang et al. 2004). This may be a particularly important first line defence mechanism for epithelial cells. Furthermore, $1,25(OH)_2D_3$ inhibits differentiation of naïve $CD4^+$ T helper (Th-0) into Th-1 cells. Consequently, $1,25(OH)_2D_3$ reduces the number of $CD4^+$ cells that produce IL-2, TNF-α, and IFN-γ (Thien et al. 2005).

With regard to periodontal disease there is limited evidence that vitamin D and calcium are important determinants of periodontal health. Data from epidemiological studies suggest that periodontal alveolar bone loss is greater in subjects with osteoporosis (Jeffcoat 2005). Utilizing data from the third National Health and Nutrition Examination Survey (NHANES III) it has been shown that low dietary intake of calcium results in more severe periodontal disease (Nishida et al. 2000b). In addition, on the basis of the same data base an inverse relationship was found between dairy products, which are an important source of calcium and other nutrients, and the prevalence of periodontitis (Al-Zahrani 2006). In a 7-year prospective study of community dwelling men who were approximately 62 years at baseline, no relationship was found between dietary vitamin D and calcium and alveolar bone loss. However, after 7 years the number of teeth that progressed from low to high alveolar bone loss was 30% higher among men with low calcium (<1000 mg/day) than men with high calcium ($\geqslant 1000$ mg/day) intakes. There was no association between level of vitamin D intake and alveolar bone progression (Krall 2001). Using the NHANES III data set Dietrich et al. (2004) showed that 25(OH)D levels were significantly and inversely correlated with attachment loss in men and women aged $\geqslant 50$ years, independently of bone mineral density. In a subsequent analysis of the same data set they also found that low serum levels of 25(OH)D were associated with greater amounts of bleeding on probing. They concluded that vitamin D may reduce the susceptibility to gingival inflammation through its anti-inflammatory effect (Dietrich et al. 2005). Conflicting results were reported by Liu et al. (2009). They found that plasma 25(OH)D levels were not different between chronic periodontitis patients (35–41 years) and controls

(23–27 years) whereas in aggressive periodontitis patients (23–30 years) plasma 25(OH)D levels were higher than in controls. A potential role for vitamin D in periodontal health is supported by studies of polymorphisms in the VDR gene, which are reported in many studies to be associated with periodontitis (Laine et al. 2010). In a recent study (Miley et al. 2009) the use of calcium and vitamin D oral supplements by subjects attending periodontal disease maintenance programs was investigated. Participants who had been taking vitamin D ($\geqslant$400 IU/day) and calcium ($\geqslant$1000 mg/day) for more than 18 months were compared with subjects who were taking neither vitamin D nor calcium supplements. In the group of subjects receiving periodontal maintenance therapy with adjunctive vitamin D and calcium supplementation, there was a trend towards better periodontal health. Obviously there is a clear need for proper randomized controlled clinical trials to determine the effect of supplementation in the onset and treatment of periodontal diseases. In the light of the available evidence it may be suggested that serum 25(OH)D levels between 36 and 40 ng/ml are desirable (Bischoff-Ferrari et al. 2006). In this respect the National Osteoporosis Foundation (NOF 2008) recommend for adults younger than 50 years 1000 mg of calcium daily, and for adults $\geqslant$50 years 1200 mg of calcium daily. For vitamin D the recommendation for adults younger than 50 years, is 400–800 IU of vitamin D daily, and for adults $\geqslant$50 years 800–1000 IU of vitamin D daily.

Magnesium

Magnesium is an essential cation playing a crucial role in many physiological functions. Imbalances of magnesium are common and are associated with a great number of pathological situations responsible for human morbidity and mortality. Magnesium deficiency has been suggested to be involved in the aetiology of cardiovascular diseases, diabetes, pre-eclampsia, eclampsia, sickle cell disease and chronic alcoholism (Laires et al. 2004). Meisel et al. (2005) reported the results of the population-based cross-sectional health survey from northeastern Germany [Study of Health in Pomerania (SHIP), $N = 4290$] performed to identify risk indicators or risk determinants associated with periodontal disease. The concentrations of serum magnesium and calcium were determined and related to periodontal parameters. It was shown that a higher Mg/Ca ratio was associated with a significantly lower level of periodontitis. In a matched-pair study, 60 subjects from the same population using oral magnesium-containing drugs and 120 non-users were compared. In subjects aged 40 years and older, increased serum Mg/Ca was significantly associated with reduced probing depth, less attachment loss, and a higher number of remaining teeth. Subjects taking Mg supplements showed less attachment loss and a higher number of remaining teeth than did their matched counterparts. The authors suggested that nutritional magnesium supplementation may improve periodontal health and prevent or delay tooth loss.

Antioxidant Micronutrients

Recent data have shown that periodontitis is associated with a number of chronic inflammatory conditions such as cardiovascular disease (Dietrich et al. 2008), ischaemic stroke (Jiminez et al. 2009), rheumatoid arthritis (de Pablo et al. 2008), type 2 diabetes (Taylor 2001), and obesity (Pischon et al. 2007, Bullon et al. 2009). One key link between periodontitis and the chronic systemic inflammatory conditions that are associated with it, is that they are all strongly associated with the presence of oxidative stress. Oxidative stress was first described by Sies (1985, 1986) as ''a process in which the balance between oxidants and antioxidants is shifted toward the oxidant side. This shift can lead to antioxidant depletion and potentially to biological damage if the body has an insufficient reserve to compensate for consumed antioxidants''. Oxidative stress underpins the pathogenesis of periodontitis (Chapple & Matthews 2007), type 2 diabetes (Allen et al. 2009), cardiovascular disease (Siekmeier et al. 2007), and obesity/metabolic dysregulation (Bullon et al. 2009). It has been suggested that oxidative stress forms the key orchestration point for the pro-inflammatory cascades that underpin tissue damage in those inflammatory conditions that are associated with periodontitis (Chapple 2009). It is biologically plausible that co-morbidity with inflammatory disorders in which oxidative stress is a feature, may exceed physiological tolerance and trigger, for example, atherogenesis, or rheumatoid arthritis (de Pablo et al. 2009).

Oxidative stress may result from mitochondrial electron leakage at complex III of the hydrogen-electron transfer chain on the inner mitochondrial membrane during normal metabolism (Battino et al. 1999). This gives rise to the single electron reduction of molecular oxygen forming the superoxide radical. In this situation increased nutritional intake of refined sugars or saturated fats (which are macronutrients) can overload the Krebs cycle, producing excess superoxide radical formation and downstream reactive oxygen species (ROS), which overwhelm mitochondrial antioxidant defence systems (superoxide dismutase 2), generating oxidative stress. Another mechanism by which dietary refined carbohydrate and saturated fat intake generate oxidative stress is by receptor binding of advanced glycation end products (AGE) to their complimentary receptor (RAGE), or oxidized LDL to Toll-like receptor-4, on the neutrophil membrane. These latter events then signal via protein kinase-C to activate the NADPH-oxidase (the respiratory burst), thus generating further ROS in a functional manner (Chapple & Matthews 2007). Another endogenous source for oxygen radical formation is its functional generation by host defense cells (phagocytic lymphocytes) during an inflammatory response following stimulation by e.g. opsonized particles, bacterial DNA or peptides and activation of the hexose-monophosphate shunt which utilizes molecular oxygen and NADPH as electron donor (Waddington et al. 2000, Chapple & Matthews 2007). The superoxide (O2-) generated by this enzyme serves as the starting material for the production of a vast assortment of ROS including oxidized halogens, free radicals, and singlet oxygen. These oxidants are used by phagocytes to kill invading microorganisms, but they also cause a lot of collateral damage to nearby tissues, so their production has to be tightly regulated to make sure they are only generated when and where required (Babior 1999).

Other sources of ROS and antioxidant depletion include external stressors such as infection, some therapeutic drugs, heat, trauma, ultrasound, ultraviolet light, ozone, exhaust fumes, radiation,

excessive exercise, and smoking. The latter depletes serum vitamin C levels and more importantly, it reduces cell and tissue concentrations of the chain breaking and redox-regulating antioxidant reduced GSH (Rahman et al. 1996). Recently, it has been recognised that the above mechanisms result in a post-prandial oxidative stress, which has been termed ''meal induced inflammation'' (O'Keefe & Bell 2007, O'Keefe et al. 2008). Mechanisms are believed to involve post-prandial surges in glucose and triglycerides, consequent on the rapid absorption of glucose and lipids into the blood stream following intake of dietary refined carbohydrate and saturated fats, and elevations in CRP and pro-inflammatory cytokines result (Monnier et al. 2006). Oxidative stress also activates redox sensitive gene transcription factors which have a pro-inflammatory role, such as nuclear factor kappa B (NFkB) and activating protein-1 (AP-1). There are multiple points in the activation cascade for these transcription factors that are redox-regulated and depletion of intracellular antioxidants, such as GSH can trigger activation and pro-inflammatory cytokine production (Chapple 1996). Triggers for NFkB activation include oxidised-LDL, AGE-RAGE interactions, ROS generation and pro-inflammatory cytokines. Increasing intracellular GSH concentrations, or the GSH:GSSG ratio (a marker of intracellular redox status and also antioxidant levels; GSSG is the oxidized form of GSH and a non-radical) can down-regulate NFkB and downstream cytokine production (Rahman et al. 2005). In an experimental periodontitis model in rats it could be shown that vitamin C supplementation resulted in an elevated GSH:GSSG ratio in the gingival tissues of supplemented rats with periodontitis over non-supplemented rats with periodontitis. Furthermore, expression of genes encoding inflammatory peptides, including interleukin-1α and interleukin-1β, was more than twofold down-regulated by vitamin C intake (Tomofuji et al. 2009a). Interestingly, GSH concentrations have been shown to be significantly depleted in the GCF of periodontitis patients relative to periodontally healthy matched controls, and while resolution of periodontal inflammation by standard scaling and RSD restored the GSH:GSSG ratio to that of controls, total GSH levels remained lower (Grant et al. 2010). These data suggest that periodontitis patients may be predisposed to oxidative stress by possessing reduced GSH buffering capacity, something that may be protected by improving overall antioxidant micronutrient intake (e.g. vitamin C – see earlier).

Other causes of nutrition-induced dysmetabolism and oxidative stress were recently reviewed by Chapple (2009) and include, insulin-mediated liver lipoprotein synthesis with subsequent release of free fatty acids into the circulation; increased formation of adipocyte triglycerides, which in conjunction with insulin-induced decreases in lipolysis, create adiposity – adipose tissue then functions as an endocrine gland releasing cytokines such as tumour necrosis factor-alpha, interleukin-1 (IL-1), IL-6 and adipokines such as leptin and adiponectin (Rosen & Spiegleman 2006). The latter cytokines and adipokines are associated with the generation of further oxidative stress through activation of the NADPH-oxidase, NFkB, and AP-1 (Chapple 2009). Oxidative stress also reduces beta-cell function within the pancreas and creates a state of insulin-resistance, which further elevates plasma glucose levels, AGE concentrations and reduces cellular antioxidant concentrations (Schmidt et al. 1994, Gillery et al. 1998, Bruce et al. 2003, Houstis et al. 2006).

An antioxidant is defined as ''a substance which when present at low concentrations, compared with those of an oxidizable substrate will significantly delay or inhibit oxidation of that substrate'' (Halliwell & Gutteridge 1989). This term is now recognised as being too narrow to encompass the full range of biological activities possessed by many traditional antioxidants in vivo, where mechanisms involve a complex array of biological pathways, which do not necessarily involve ''free radical scavenging'' (Chapple & Matthews 2007). Moreover, antioxidant properties are difficult to measure or demonstrate in vivo because free radicals simply cannot be measured directly. In vitro systems called spin traps are used to measure radical species but there are currently no suitable spin traps/probes available for in vivo measurement of ROS production in the human, because of their unknown toxicity. The term ''antioxidant'' is therefore now limited to in vitro experimentation, and the term ''micronutrient'' is employed for in vivo studies, in recognition that a diverse range of bioactivities un-related to free radical scavenging may explain the health benefits of such species.

Numerous biological substances have traditional ''antioxidant capacities''. In relation to periodontal disease only a small number have been studied and include vitamin C, vitamin E (tocopherol), carotenoids (α and β-carotene, beta-cryptoxanthin, and zeoxanthin), polyphenols, bilirubin, GSH, uric acid, and melatonin. Non-antioxidant micronutrients such as the vitamin B group [niacin (B3), folate or folic acid (B9)], and omega-3 PUFAs have also been investigated.

Evidence for antioxidant micronutrient depletion in periodontitis has been shown in a number of studies. For example, the total antioxidant capacity (TAOC) in plasma is reduced in periodontitis patients (Brock et al. 2004, Konopka et al. 2007). In addition, Panjamurthy et al. (2005) demonstrated lower plasma levels of vitamin C, vitamin E and reduced GSH in periodontitis patients compared with healthy controls. Chapple et al. (2007a) studied in the NHANES III data set the relationship between periodontitis and a large number of antioxidants i.e. α-carotene, β-carotene, selenium, lutein, uric acid, β-cryptoxanthine, vitamins A, C, E, bilirubin, and TAOC. Serum TAOC was calculated as the weighted sum of the serum concentrations of uric acid, vitamin A, vitamin C, and vitamin E. Results showed an inverse relationship between plasma vitamin C, bilirubin and TAOC and periodontitis in the NHANES III data set, in adjusted models (multiple logistic regression adjusted for age, gender, race/ethnicity, BMI, cigarette smoking, oral contraceptives and hormone replacement therapy use, diabetes, poverty income ratio, and education). Higher serum antioxidant concentrations were associated with a reduced relative risk for severe periodontitis of 0.53 (CI 0.42–0.68) for vitamin C, 0.65 (CI 0.49–0.93) for bilirubin and 0.63 (CI 0.47–0.85) for TAOC. The inverse relationships were even stronger in a sub-analysis of never smokers. As discussed above the studies of Vogel & Wechsler (1979), Ismail et al. (1983), Blignaut & Grobler (1992), Nishida et al. (2000a) showed a significant inverse relationship between dietary vitamin C intake periodontitis and Väänänen et al. (1993), Amarasena et al. (2005), Staudte et al. (2005), Chapple et al. (2007a) and Amaliya et al. (2007) showed an inverse relationship between plasma ascorbic acid levels and periodontitis. More recently, Linden et al. (2009) investigated, in a representative

sample of 1258 men aged 60–70 years drawn from the population of Northern Ireland, the association between periodontal health and the serum levels of various antioxidants including retinol, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, and lycopene. The population was divided into a group with generalized severe periodontitis, moderate periodontitis and the remaining population. Compared with the remaining population the levels of α- and β-carotene, β-cryptoxanthin, and zeaxanthin were significantly lower both in the moderate and the generalized severe periodontitis group. There were no significant differences in the levels of lutein, lycopene, α- and γ-tocopherol or retinol in relation to periodontitis. In adjusted models (age, smoking, diabetes, socioeconomic status and BMI) there was an inverse relationship between α- and β-carotene and β-cryptoxanthin and moderate (low threshold) periodontitis. However, β-carotene and β-cryptoxanthin were the only antioxidants for which lower levels were associated with an increased risk of generalized severe (high threshold) periodontitis. The adjusted odds ratio for high-threshold periodontitis in the lowest fifth relative to the highest fifth of β-cryptoxanthin was 4.02 (CI 1.61–9.99).

Yu et al. (2007) studied the relationship between serum folic acid and periodontitis in the NHANES III data set. After controlling for demographics, educational level, body mass index, bleeding on probing, and probing sites, the odds ratio for periodontal disease was 0.74 (95% confidence interval = 0.59–0.93) for each standard deviation increase in natural-log-transformed folate levels. After additionally controlling for levels of vitamin B_{12} and homocysteine, chronic diseases (hypertension, diabetes mellitus, heart disease, and stroke), and health behaviours (smoking status and alcohol consumption), the negative association between folate level and periodontal disease remained statistically significant and essentially unchanged. They concluded that low levels of serum folic acid were independently associated with periodontal disease in older adults. The relationship between dietary folic acid intake and the periodontal health was studied by Esaki et al. (2010). Multiple regression analysis showed that dietary folic acid was significantly correlated with bleeding on probing but not with Community Periodontal Index scores (WHO 2005). Erdemir & Bergstrom (2006) examined 88 chronic periodontitis patients and found that the smokers among them have 26% lower serum folic acid concentration compared with non-smokers. They concluded that for periodontitis patients who cannot quit smoking and have insufficient intake and/or a deficient folic acid status, improved dietary intake or a folic acid supplement is advisable.

Catechin is a polyphenolic antioxidant present among others in green tea (*Camellia sinensis*). In the 1990s, Japanese researchers found that among the tea catechins tested, those containing galloyl radical [(-)-epicatechin gallate and (-)-epigallocatechin gallate] possess the ability to inhibit both eukaryotic and prokaryotic cell derived collagenase (Makimura et al. 1993) and inhibit the adherence of *P. gingivalis* onto the epithelial cells (Sakanaka et al. 1996). Kushiyama et al. (2009) showed that the intake of green tea was inversely correlated with mean pocket depth, mean clinical attachment level, and bleeding on probing in 940 Japanese men aged 49–59 years. In multivariate linear regression models, every one cup/day increment in green tea intake was associated with a 0.023-mm decrease in mean pocket depth ($p < 0.05$), a 0.028-mm decrease in the mean clinical attachment loss ($p < 0.05$), and a 0.63% decrease in bleeding on probing ($p < 0.05$), after adjusting for other confounding variables. The possible role of flavonoids in relation to periodontal disease may be supported by an animal study that investigated the effects of a cocoa-enriched diet on gingival oxidative stress in a rat-periodontitis model (Tomofuji et al. 2009b). Results showed that although experimental periodontitis was induced in the rats fed a cocoa-enriched diet, they did not show impairments in serum reactive oxygen metabolite levels and the GSH ratio was maintained within gingival tissues. However, those fed on a control diet demonstrated lower reduced:oxidized GSH rations and higher plasma levels of oxidative stress. In addition, alveolar bone loss and polymorphonuclear leukocyte infiltration after ligature placement were also inhibited by cocoa intake. However, although cocoa is a rich source of flavonoids with antioxidant properties such as picatechin, catechin, and procyanidins, recent findings suggest a regulatory effect of cocoa on the immune cells implicated in innate and acquired immunity (Ramiro-Puig & Castell 2009).

Melatonin was also studied in relation to periodontal disease. Melatonin is a hormone synthesized and secreted mainly in the pineal gland. Besides its function as synchronizer of the biological clock, melatonin also exerts a powerful antioxidant activity. In an age and gender matched case control study Gómez-Moreno et al. (2007) showed that plasma melatonin levels were increased in patients with a worse periodontal condition.

Studies on saliva/gingival crevicular fluid (GCF) antioxidant levels have also demonstrated compromise in periodontitis patients relative to controls (Brock et al. 2004, Panjamurthy et al. 2005). In addition, it was found that the levels of salivary melatonin decrease as the severity of disease increases (Cutando et al. 2006). Recently it was also shown that the amount of melatonin in GCF was reduced in periodontitis patients compared with controls (Srinath et al. 2010). The conflicting results regarding increased plasma and decreased GCF melatonin values needs further research. More importantly, GSH levels also appear significantly reduced in periodontitis (Chapple et al. 2002), implying a redox imbalance local to the periodontal tissues (albeit outside the tissues) which may have pro-inflammatory consequences. Interestingly, antioxidant enzyme levels appear elevated in periodontitis tissues (Akalin et al. 2005, Panjamurthy et al. 2005, Patel et al. 2009), which authors attribute to representing a defensive response to the oxidative stress induced by periodontal inflammation. Whether antioxidant depletion predisposes to or results from oxidative stress within the periodontium remains unproven. However, an intervention study that employed standard scaling and RSD to reduce periodontal inflammation, resulted in the restoration of GCF TAOC concentrations to those of healthy controls (Chapple et al. 2007b). This study provided evidence that the antioxidant depletion within the periodontal environment may result from the oxidative stress that arises within the inflammatory lesion.

Polyunsaturated Fatty Acids

The human body cannot synthesize omega-3 fatty acids de novo, but can

synthesize all the other necessary omega-3 fatty acids from the simpler omega-3 fatty acid α-linolenic acid. Therefore, α-linolenic acid is an essential nutrient which must be obtained from food, and the other omega-3 fatty acids which can be either synthesized from it within the body or obtained from food are sometimes also referred to as essential nutrients. Adequate dietary intake of omega-3 (n-3) polyunsaturated fatty acids (n-3 PUFAs) increases tissue concentrations of the types of fatty acids (e.g., eicosapentaenoic acid and docosahexaenoic acid) that downregulate inflammation (Ziboh 2000, Vedin et al. 2008). Studies suggest that n-3 PUFA metabolites may serve as ''stop signals'' for preventing neutrophil-mediated tissue damage (Van Dyke & Serhan 2003, Mori & Beilin 2004). Studies in animals have suggested a positive, modulating effect of n-3 PUFAs on gingival inflammation through biomarker analysis (Vardar et al. 2004, 2005, Kesavalu et al. 2007), and there are also reports of reduced bone loss in a *P. gingivalis* ligature-induced model of periodontitis (Kesavalu et al. 2006). Improved outcomes are attributed to the primary metabolites of omega-3 fish oils, eicosapentaenoic acid (EPA) and docoshexaenoic acid (DHA). In a 5-year longitudinal study of subjects 70 years of age at baseline, an inverse independent relationship was found between dietary DHA intake and periodontal disease events, after controlling for confounding factors. People with low DHA intake had an approximately 1.5 times higher incidence rate ratio of periodontal disease progression (Iwasaki et al. 2010). The effect of dietary supplementation was evaluated by El-Sharkawy et al. (2010) in double blind clinical study of parallel design. The control group was treated with scaling and root planing (SRP) and placebo while the test group received SRP followed by dietary supplementation of fish oil (900 mg EPA+DHA) and 81 mg aspirin daily. Results showed a significant reduction in pocket depth and attachment gain after 3 and 6 months in the test group compared with baseline and control group. In addition, supplementation with omega-3+aspirin resulted in a significant shift in the frequency of pockets <4 mm.

Evidence from Nutritional Benefit Intervention Studies

Although numerous studies evaluated the relationship between micronutrients and periodontal disease, intervention studies in humans are scarce. Leggott et al. (1986) studied the effect of a rotating 7-day diet, adequate in all nutrients except vitamin C, on periodontal health. No changes in plaque accumulation or probing depths were noted during any of the periods of depletion or supplementation. However, measures of gingival inflammation were directly related to the ascorbic acid status. The results suggested that ascorbic acid may influence early stages of gingivitis, particularly crevicular bleeding. In a subsequent study Leggott et al. (1991) investigated the relationship between varying vitamin C intakes and the periodontal status in subjects selected on the basis of the presence of pocket depth and attachment loss >4 mm. No significant changes in plaque accumulation, probing pocket depth, or attachment level were noted when different vitamin C groups were compared. By contrast, gingival bleeding increased significantly after the period of vitamin C depletion and returned to baseline values after the period of vitamin C repletion. Jacob et al. (1987) studied the effect of a rotation diet with vitamin C depletion, normal and supplementation in subjects with a periodontally healthy condition. Their results showed that the propensity of the gingiva to become inflamed or bleed on probing was reduced after normal (65 mg/day) vitamin C intakes as compared with deficient (5 mg/day) intakes and upon supplementary (605 mg/day) vitamin C intakes as compared with normal intakes. The results of these short-term studies suggest that vitamin C status may influence early stages of gingival inflammation and crevicular bleeding. The effect of megadoses (500 mg t.i.d.) of vitamin C was studied by Vogel et al. (1986) in an experimental gingivitis model. Results showed no differences between test and control groups. In a study by Woolfe et al. (1984), the effect of initial periodontal therapy with and without megadoses of vitamin C supplementation (250 mg q.i.d.) was investigated in 10 non-deficient patients. Two groups of five patients matched for age, periodontal status and oral hygiene level were created and received either vitamin C or placebo capsules. Results from this small study showed no difference between the two groups. It is however important to remember that high doses of vitamin C may generate toxicity due to the formation of vitamin C radicals, and therefore the findings of the above studies need to be interpreted carefully with this context in mind (Chapple & Matthews 2007). Staudte et al. (2005) evaluated the effect of supplementing the diet with increased grape fruit consumption. Systemically healthy subjects with chronic periodontitis were selected, who had not received antibiotics, dietary supplements or undergone periodontal treatment in the previous 6 months. In the test group the diet was supplemented with two grapefruits daily for a period of 2 weeks. Results showed that the intake of grapefruit leads to an increase in plasma vitamin C levels and improved sulcus bleeding scores.

Uhrbom & Jacobson (1984) reported a placebo controlled trial investigating the effect of calcium supplementation (1 g per day for 180 days) on the periodontal condition of periodontitis patients but were unable to show an effect. The use of calcium and vitamin D oral supplements was evaluated retrospectively by Miley et al. (2009) in subjects receiving periodontal maintenance therapy. The test group was selected on the basis of taking vitamin D ($\geqslant$400 IU/day) and calcium ($\geqslant$1000 mg/day) supplementation for an average of 10 years, and the control group did not take such supplementation. Compared with subjects who did not take vitamin D and calcium supplementation, supplement takers had shallower probing depths, fewer bleeding sites, lower gingival index values, fewer furcation involvements, less attachment loss, and less alveolar crest height loss. The repeated-measures analysis indicated that collectively these differences were of borderline significance ($p = 0.08$).

Campan et al. (1997) studied the effect of n-3 PUFA supplementation in the treatment of human experimental gingivitis. Thirty-seven healthy volunteers, who discontinued routine oral hygiene measures, were treated with either fish oil (6 g of n-3 PUFA) in 18 subjects, or placebo (olive oil) in 19 subjects for 8 days. The results showed a significant decrease in gingival index in the PUFA-treated group. The potential anti-inflammatory effects of PUFA supplementation was studied in adult periodontitis patients by administration of fish oil as a source of the n-3 PUFA, eicosapentaenoic acid, and borage oil as a source of the n-6 PUFA, gamma-linolenic acid (GLA). After 12 weeks, compared with control subjects an improvement in probing depth was seen in subjects treated with either fish oil or borage oil (Rosenstein et al. 2003).

Chapple et al. (2010) conducted a randomized controlled double blind intervention to elucidate the adjunctive effects of ingesting whole fruit, vegetable, and berry concentrates in a powdered capsular form during non-surgical periodontal therapy in 60 non-smokers with mild-moderate periodontitis. In an intention to treat analysis using two-tailed analysis of covariance with baseline measures as covariates, they demonstrated small but significant additional reductions in pocket depths ($p < 0.04$) and GCF volumes ($p < 0.02$) at 3-months post-therapy in the phytonutrient supplement verses the placebo groups.

Jenzsch et al. (2009) employed a guided nutritional intervention program in 20 female patients with mild-moderate periodontitis and metabolic syndrome as the sole intervention over a 12-month observation period. In this year the participants changed their dietary habits from an average German mixed diet towards wholesome nutrition. Wholesome nutrition had the following general features: (1) preference for foods of plant origins, (2) preference for food processed as little as possible, (3) plentiful consumption of unheated fresh food, (4) careful preparation of meals from fresh foods, and (5) the sparse use of fat. Such a diet mainly comprises vegetables, fruits, whole-grain products, potatoes, legumes and dairy products. The consumption of meat, fish and eggs was limited to one or two portions per week. Patients were examined at 2 weeks, 3, 6, and 12 months for clinical measures and microbiological and immunological outcomes. Unfortunately, plasma micronutrient status was not measured and there was no control group, but compliance with the nutritional intervention appeared favorable. The study demonstrated small but significant improvements in pocket depth and gingival index at 12 months relative to baseline measures. In this respect, the study reported by Baumgartner et al. (2009) is interesting. They studied the periodontal condition in a group of subjects who had over a 4-week period no access to traditional oral hygiene methods and who ate a diet available to stone age humans. Results showed that the consequence of having no access to modern oral hygiene methods was reflected by an increase in supragingival plaque scores. However, this increase was not accompanied by an anticipated increase in the severity of gingival inflammation. The insignificant increases in the subjects' mean gingival index from 0.38 to 0.43, with a decrease in bleeding on probing scores as well as a slight decrease in pocket depths was not expected by the authors. However, in the light of the information presented in this review these changes may not be surprising.

Muñoz et al. (2001) studied the effect of a nutritional supplement in a randomized, placebo controlled trial of 63 patients with early periodontitis. Subjects took the assigned tablet at breakfast and at dinner after brushing their teeth twice daily. The nutritional supplement consisted of seven ingredients i.e. folic acid, vitamin B_{12}, vitamin C, *Echinacea angustifolia*, *Vitis vinifera* seed, ubiquinone and *Piper nigrum* extract. After 60 days a significant reduction in gingival index, bleeding index, and pocket depth could shown for the experimental group. In a randomized, double-blind, placebo-controlled clinical trial (Neiva et al. 2005) 30 subjects with generalized moderate to severe chronic periodontitis were instructed to take one capsule a day of either vitamin-B complex (50 mg of the following: thiamine HCl, riboflavin, niacinamide, D-calcium pantothenate, and pyridoxine HCl; 50 mcg each of D-biotin and cyanocobalamin; and 400 mcg of folate) or placebo for 30 days following access flap surgery. Both groups experienced comparable levels of pocket depth reduction. However, the mean CAL gain was statistically significantly superior in vitamin-B complex supplemented subjects regarding both, shallow and deep sites.

Guidelines for Micronutrients in Relation to Periodontal Therapy

The exact need for micronutrients is still a matter for discussion. The dietary requirement for a micronutrient is defined as an intake level which meets a specified criterion for adequacy, thereby minimizing risk of nutrient deficit or excess. The well-known RDA from the Food and Nutrition Board (FNB) of the Institute of Medicine have been used for many years. However a new set of reference values were published by the FNB in 1997 which consisted of four types of daily intakes: (1) estimated average requirement (EAR), this is the average daily nutrient intake level that meets the needs of 50% of the ''healthy'' individuals in a particular age and gender group, (2) recommended nutrient intake (RNI) is the daily intake, set at the EAR plus 2 standard deviations, which meets the nutrient requirements of almost all apparently healthy individuals in an age- and gender-specific population group, (3) adequate intake (AI) is the intake that appears to sustain normal health and (4) upper limits (UL) of nutrient intake are defined as the maximum intake from food, water and supplements that is unlikely to pose a risk of adverse health effects from ''excess'' in almost all (97.5%) apparently healthy individuals in an age- and sex-specific population group.

Since in periodontal disease low serum/plasma levels of the various micronutrients have been found which may be partly under genetic control, daily intake of these nutrients may be recommended as being on the higher end of the scale. For vitamin C, Levine et al. (2001b) showed that at doses of 200 mg daily and higher, steady-state plasma values are obtained which were similar for both genders. Plasma is completely saturated at doses equal to or higher than 400 mg daily, producing a steady-state plasma concentration of approximately 80 μM. It is advisable to obtain the vitamin C from a variety of fresh fruits and vegetables. Good sources include peppers, strawberries, paprika, broccoli, Brussels sprouts, sweet potatoes, oranges, grapefruits and kiwi fruit. From these the kiwi fruit seems most attractive since green kiwi fruit contains 93 mg vitamin C/100 g fruit whereas e.g. oranges contain 53 mg/100 g fruit (USDA 2010). In addition, kiwifruit provides a dual protection against oxidative DNA damage, enhancing antioxidant levels and stimulating DNA repair (Collins et al. 2003). Furthermore, it has been reported that consuming two or three kiwi fruit per day for 28 days reduced platelet aggregation responses to collagen and adenosine diphosphate by 18% compared with controls and that consumption of kiwi fruit lowers blood triglycerides levels by 15% compared with controls (Duttaroy & Jørgensen 2004). In all, kiwi fruit seems to be one of the best natural sources of vitamin C to support health in general. One could argue that periodontal patients may benefit from consuming two kiwi fruits per day, since this together with the vitamin C present in the ''normal'' diet should be sufficient to obtain the recommended 200 mg vitamin C per day and may buffer against oxidative stress and preserve reduced GSH status.

Throughout evolution humans have been depended on the sun for their vitamin D requirements. However, high rates of vitamin D deficiency have been

reported in children and adults living in the United States, Europe, Middle East, India, Australia, New Zealand and Asia (Holick 2008). The zenith angle of the sun is critically important for the production of vitamin D in the skin. For example, early in the morning and late afternoon little if any vitamin D is produced no matter where one lives on the globe. During the winter, living above 35° latitude, almost no vitamin D is produced from October to March. Furthermore, due to changing living conditions, indoor working, transport in cars and the use of sun protectors less vitamin D is produced. In addition, very few foods, mostly oily fish, naturally contain vitamin D. 25(OH)D levels may be particularly low in the serum of elderly patients (Holick et al. 2005). This deficiency in vitamin D may have several causes, one of which is the decline with age in cutaneous levels of 7-dehydrocholesterol needed for the synthesis of vitamin D_3. The level of 7-dehydrocholesterol in a 70-year-old is approximately 25% of that of young subjects (MacLaughlin & Holick 1985). In periodontal patients an estimation by means of interview can be made concerning the likelihood that the patient suffers from vitamin D deficiency, or a blood sample may be taken in order to assess whether the plasma vitamin D levels are at the level of 40 ng/ml. Since vitamin D and calcium metabolism are interlinked, and low dietary intake of calcium results in more severe periodontal disease, it is advised also to evaluate daily calcium intake by patients. A diet low in milk and dairy products, or other calcium-containing foods will predispose to calcium deficiency. If necessary, by means of calcium supplements the required daily 1200 mg calcium (NOF 2008) may be obtained.

Regarding the suggested guidelines for micronutrients in relation to periodontal health it must be realized that these suggestions have not yet been validated by clinical trials. Nevertheless, as long as solid evidence is lacking these guidelines may be helpful in specific cases of refractory periodontitis in which all traditional risk indicators are not present.

Cautions and Nutrigenetic Issues

While epidemiological studies demonstrate a potential role for individual micronutrient supplements in managing periodontal diseases, such interventions have met with mix reports of success in the medical literature, when employed for other inflammatory or neoplastic conditions. α-Tocopherol may accelerate or prolong lipid peroxidation in vivo in low density lipoprotein particles (Siekmeier et al. 2007), unless it is reduced by vitamin C, ubiquinol-10 or GSH. Moreover, high concentrations of α-tocopherol may displace other lipid soluble antioxidants, thus perturbing the natural balance of the complex antioxidant systems within human cells and tissues (Miller et al. 2005). Meta-analyses of studies exploring the effects of vitamin supplementation with vitamin E largely demonstrate no benefit in studies of cardiovascular disease (Hooper et al. 2001) and there are some reports of increases in all cause mortality with long-term high dose supplementation (Miller et al. 2005, Bjelakovic et al. 2007). Similar data exists with the use of β-carotene supplementation in the management of lung cancer (The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group 1994, Omenn et al. 2006). The disconnect between epidemiological data and intervention studies may have many explanations, including confounding by supplement consumption and poorer lifestyles in supplement takers within epidemiological studies, as well as nutrigenetic issues, i.e. variations in the biological response of patients to nutritional interventions based upon their genetic makeup. For example, differential responses in TNF-α production by peripheral blood monocytes, resulting from interventions with omega-3 PUFAs have been demonstrated in certain patient groups who are heterozygous for a polymorphism in the lymphotoxin (TNF-β) gene (Grimble et al. 2002). Similar data exists for IL-1 responses of monocytes and IL-1 monocyte gene expression, in patients with a composite polymorphism in IL-1 (Kornman et al. 2004). Indeed, recently two single nucleotide polymorphisms in the β-carotene gene have been described in female patients, which may account in part, for reduced plasma retinal/retinol levels despite adequate beta-carotene intake (Leung et al. 2009). The *BCMO1* 379V or 267S+379S variants show a reduced efficiency in intestinal β-carotene conversion and are believed to contribute to a ''low converter trait'' for β-carotene. Based upon current evidence, the most appropriate advice to offer periodontitis patients currently would appear to be to increase consumption of antioxidants and other micronutrients through natural whole food consumption. Where this appears difficult, then the use of phytonutrient (plant-based nutrients e.g. polyphenolic flavenoids, carotenoids) supplements appears to offer some benefit (Chapple et al. 2010). Unfortunately, intervention studies are still scarce and include small numbers of subjects therefore it may be premature to discuss the clinical significance/relevance of micronutrient interventions.

In conclusion, while this review has taken an optimistic stance to the potential role for micronutrients in periodontal disease, we are mindful of the lessons from the medical literature. Nevertheless, for the prevention and treatment of periodontitis adequate daily intake of natural antioxidants, fish oils (omega-3 PUFAs), vitamin D and calcium are recommended. Deficiencies of vitamin D and calcium may be solved by either changing diet/life style or by specific supplements. Antioxidant micronutrient deficiencies can be met by a higher intake of vegetables, fruits, and berries. However, the majority of patients are not antioxidant vitamin deficient and mono-vitamin supplements may be associated with toxicity effects. Current evidence shows some benefit in reducing gingival inflammation from vitamin C supplementation, provided UL are not exceeded. The most appropriate sources of vitamin C are natural fruits such as kiwi fruit. The most appropriate sources of polyphenolic flavenoids and carotenoids are from natural fruit/vegetable/berry intake or the use of whole fruit, vegetable, and berry concentrates. An initial intervention study with a powdered capsular form of the latter phytonutrients showed promise as an adjunctive approach to standard periodontal therapy in improving pocket depth reductions. However, the potential public health benefits of such approaches in un-treated periodontitis patients remains to be investigated. The next decade will see an explosion in phytonutritional approaches to modulating inflammation through redox-regulated gene transcription factors (''nutrigenomics''), but early evidence from nutrigenetic studies indicates that nutritional interventions will provide differential effects according to the individual patients' genetic makeup and it is unlikely that a ''one size fits all'' approach will yield the best results.

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Clinical Relevance

Scientific rationale for the study: It has been shown that nutrition is a major lifestyle risk factor for a number of inflammatory diseases and conditions including cardiovascular diseases, type 2 diabetes, rheumatoid arthritis and inflammatory bowel disease, all of which have been associated with periodontitis. Therefore nutrition may also play an important role in periodontitis pathogenesis and management.

Principle findings: The evaluation of the reviewed literature showed that periodontal disease is associated with low serum/plasma levels of the various micronutrients, principally vitamin D, vitamin C, and other antioxidants. There is, however, a paucity of longitudinal intervention studies in this important area, to explore the impact of nutritional interventions on periodontal outcomes.

Practical implication: The available evidence would support the recommendation of an adequate daily intake of foods comprising natural sources of antioxidants, and supplements of vitamin D and calcium in deficient patients. Therefore, the medical history should include detailed information regarding the daily dietary intake of the various micronutrients as well as related life style characteristics. Inadequate intake of antioxidants may be met by a higher intake of vegetables, berries, and fruits (e.g. kiwi fruit). Inadequate supply of vitamin D and calcium may be addressed by either a change in diet/life style or by supplements.