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The potential impact of essential nutrients vitamins C and D upon periodontal disease pathogenesis and therapeutic outcomes

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Abstract
Diet has powerful effects upon inflammatory status, arguably as strong or stronger than microbial plaque. Despite a relationship between diet and periodontal inflammatory markers being established over 30 years ago, it is only recently that the mechanisms underpinning these effects have begun to be examined in detail. Following an analysis of the evidence base in 2011, this review focuses upon the most contemporaneous evidence relating specifically to the micronutrient vitamins C and D and their potential impact upon periodontal disease pathogenesis and/or therapeutic outcomes. The authors bring together both epidemiological and laboratory data, and aim to outline avenues for potential studies given the limited number of larger well conducted clinical interventional trials completed to date.

Introduction

The "signature" host response of periodontitis exhibits wide phenotypic heterogeneity as is the case with many complex chronic diseases, largely because it is driven by different exposures that individually impact to differing degrees of magnitude in different patients, and which also interact with each other in a variable manner (the biological phenotype). These exposure categories include genetic (gene polymorphisms), environmental (stress, bacteria), lifestyle/behavioural (exercise, nutrition) and pharmacological (different drugs) exposures. Individual constituents of these broad categories are component causes of periodontitis, since they contribute to a series of biological events that drive exaggerated inflammation [1] and/or inflammation that fails to resolve [2]. Nutrients are broadly categorized into 6 classes:

- Vitamins (A, C, D, E, F)
- Minerals/trace elements (Selenium, Zinc, Copper, Iron)
- Proteins
- Carbohydrates
- Fats (including essential fatty acids, ω3 & ω6)
- Water

Broadly, they may be categorised as or "micronutrients" (minerals, vitamins) required by the body in relatively small amounts, and "macronutrients" (proteins, fats, carbohydrates, poly-unsaturated fatty acids/PUFAs) required by the body in relatively large quantities from the diet [3]. It has long been recognised that diet adversely influences inflammatory periodontal status. A classical study in 1984 [4] observed that irrespective of levels of plaque, more gingival bleeding was experienced in young adults who undertook 21-days of experimental gingivitis whilst fed on multiple daily snacks of refined carbohydrates, than when the same volunteers crossed over within the same study to a low sugar diet. More recently, a Swiss study found that when 10 adults were placed in a "stone-age" environment for 4-weeks, with no oral hygiene and diets were "stone-age" in nature (*id est.* low in simple sugars and high in antioxidant micronutrients, fish oils and fibre), they remarkably demonstrated significant decreases in gingival bleeding and probing depths, despite significant increases in plaque accumulation [5]. Diet therefore has powerful effects upon inflammatory status, arguably as strong or stronger than microbial plaque, and until recently the mechanisms underpinning those effects were poorly understood.

The evidence base for nutrition as a factor influencing periodontal health and disease was recently reviewed by Van der Velden *et al* [3] and this chapter therefore focuses upon the most contemporaneous evidence relating specifically to vitamin C and vitamin D (and calcium) as essential nutrients that may impact upon periodontal disease pathogenesis and/or therapeutic outcomes.

Vitamin C

History, nutritional value and deficiency

"They all in general had putrid gums, the spots and lassitude, with weakness of the knees" - James Lind's observations of twelve sailors on board the Salisbury at the start of his experiment 20th May 1747, some eight weeks after leaving port. By the end of May, the two men assigned to the citrus fruit (vitamin C) arm of his study were almost fully recovered - although the Royal Navy did not adopt citrus rations and eliminate scurvy until 1795 [6]. Vitamin C is an essential micronutrient and an enzyme co-factor that acts as an electron donor that maintains metal ions in a reduced state for optimum enzyme activity. It is also a powerful water soluble antioxidant capable of regenerating vitamin E from its radical, which forms during oxygen radical attack on the lipid membranes of cells. The resulting vitamin C radical is ultimately restored to its non-radical state by the chain breaking antioxidant glutathione (GSH) (Figure 1).

Statistics from the Health and Social Care Information Centre that compiles data sent by more than 300 National Health Service (NHS) Trusts in England and approximately 200 independent sector organisations for activity commissioned by NHS England show a rise in the diagnoses of scurvy - Summer 2013-4 showed 15 cases where this was the primary cause for admission, and 94 cases where this was the primary or secondary diagnosis [7]. Despite these relatively low numbers, subclinical vitamin C (ascorbate) deficiency seems to be more commonplace. Assuming a normal ascorbate serum concentration range of 5-15mg/L, the risk of developing scurvy becomes significant at < 2mg/L. Fain (2003) defined < 5mg/L as ascorbate insufficiency and < 2mg/L as deficiency; in hospitalised individuals, 16% had levels < 2mg/L and 56% had less than 5mg/L [8].

The relevance of vitamin C in tissue biochemistry

The reduced form of vitamin C (ascorbic acid; ascorbate) can be synthesised from glucuronate by vertebrates that retain this capacity, however, the last enzymatic step in its synthesis cannot be achieved in

man due to a mutation in the gene that encodes this enzyme. Therefore, ascorbic acid and its oxidised form dehydroascorbic acid (DHAA) must be obtained through dietary intake. Furthermore, as this is a water soluble vitamin there is a limited storage pool and the threshold for renal excretion is low; the mechanistic case for ascorbate insufficiency and deficiency is therefore clear.

The relationship between chronic inflammatory diseases and redox imbalance has been established both in general terms and also in relation to periodontal disease. The antioxidant capacity of ascorbate as a powerful scavenger of reactive oxygen and nitrogen species is therefore central to its protective role. Post-translational modifications of polypeptide pro-collagen require iron-dependent enzymes, which are protected by vitamin C from reactive oxygen species (ROS) attack. These modifications help to promote the synthesis of a mature collagen network and allow secretion into the extracellular milieu within the periodontal tissues. Ascorbate also influences the biosynthesis of other connective tissue components including elastin, fibronectin, proteoglycans, bone matrix and elastin-associated fibrillin. Both ascorbate and DHAA are concentrated intracellularly, whereas ascorbate that has undergone radical scavenging can be recycled intracellularly via another potent antioxidant reduced glutathione (GSH) (Figure 1). GSH levels in extracellular fluids can be rapidly depleted in conditions of oxidative stress unless there is sufficient chain-breaking activity provided by further GSH synthesis. With these systems in mind, it is hardly surprising that high concentrations (10-40 times higher than plasma) of ascorbate are present within cells that are key to the periodontal inflammatory environment - namely endothelial cells, neutrophilic polymorphonuclear leukocytes (neutrophils) and macrophages. Furthermore, ascorbate has been shown not only to enhance chemotaxis of normal neutrophils, but also to improve microtubule assembly and their subsequent bactericidal activity with impaired lysosome degranulation, as is seen in the improved clinical course of patients with Chediak-Higashi syndrome, following ascorbate supplementation. Moreover, typical plasma ascorbate concentrations have been shown to scavenge hypochlorous acid (HOCl), one of many powerful oxidants generated by myeloperoxidase in activated neutrophils and monocytes [9]; it is in turn hypothesised that this protects against further tissue damage that alpha-1-antiprotease inactivation and low density lipoprotein (LDL) oxidation would contribute indirectly to as the result of uninhibited phagocyte-derived oxidants in the locally inflamed periodontal tissues.

A mechanistic link between vitamin C, excess glucose and periodontal tissue pathology?

The efficiency of vitamin C active transport in the small intestine appears to decrease with increasing doses and impaired intestinal absorption in the elderly has been suggested as one mechanism, although the majority of evidence indicates that there is no greater incidence of vitamin C deficiency with advancing age [10]. Plasma ascorbate concentration has a sigmoidal relationship with increased daily intake, the plateau of which is lower in women than males [11]. Furthermore, smokers typically exhibit a lower plasma ascorbate concentration compared with non-smokers or former smokers, even when adjusted for differences in vitamin C intake [12]; females also maintain higher plasma ascorbate levels than men at a given vitamin C intake. Extracellular oxidised vitamin C (dehydroascorbate) is partly transported intracellularly via facilitative glucose transporters; an excess of glucose may therefore competitively block DHA uptake and impair recycling / extracellular clearance of DHA as well as inhibit neutrophil functions as described above. In a concept that was developed nearly 30 years ago [13], recent research has begun to shed light on the importance that excess glucose has on ascorbate concentrations in red blood cells with subsequent microvascular angiopathy [14]. The significance that this may have on compromised host defences in the periodontal complex of poorly controlled diabetes patients remains to be determined.

Rich sources of vitamin C include kiwi fruit, yellow peppers and broccoli; the sensitivity of ascorbate to light, heat and air would indicate that fresh products need to be consumed in a raw state or only lightly cooked. Pharmacokinetic models have shown that maximum bioavailability is usually attained at *lower* doses when supplements are used and declines with elevation in supplement dose (70-90% for usual dietary intakes of 30-180mg/day, <50% for doses 1250mg) [15,16]. One must also consider the attendant risk of tooth wear that regular use of non-buffered chewable vitamin C preparations may pose [17]. Furthermore, supplementation with high doses of vitamin C in isolation also acts as a clear risk factor for renal stone formation [18]. The relative importance or significance of a given antioxidant may therefore rely not only on its reaction and concentration in a given compartment, but also on its ability to interact with antioxidant regeneration systems of varying types, such as intracellular dehydroascorbate recycling via GSH-dependent mechanisms, and the synergistic relationship between vitamin C and vitamin E whereby water soluble ascorbate can interrupt free radical chain propagation in the lipid phase.

Vitamin C and periodontal diseases

The recommended dietary allowance (RDA) of 90mg/day for adult men and 75mg/day for adult women is based on vitamin C intake to maintain near-maximum neutrophil concentrations with minimal urinary excretion of ascorbate, with an increase of 35mg/day in smokers given the increased oxidative stress that

smoking poses. Scorbutic gingivitis (Figure 2) is reported at levels <10mg/day [19]. Nevertheless, data on which these recommendations were made is limited and therefore on the basis of more recent evidence, some authorities have recommended that the ideal daily intake of vitamin C should be up to 200mg from a variety of fresh fruit and vegetables [20-22].

The vast majority of studies from the early 1980s onwards report an inverse association between serum/plasma vitamin C concentrations and periodontal status, although the measures and populations within which each study has been conducted vary widely [3]. Furthermore, most research to date is of cross-sectional design. Although smoking clearly reduces mean serum/plasma vitamin C concentration, NHANES III data analysis has shown this inverse relationship in current, former and never smokers, although the inverse association appears to be stronger in never smokers [23].

A recent study comparing systemically healthy subjects (n=150) and type 2 diabetes patients with (n=150) and without (n=150) periodontitis showed the same trend, with decreased serum vitamin C concentrations in volunteers with diabetes regardless of periodontal status, although mean concentrations in those subjects who also had periodontitis as a co-morbidity were even lower [24].

So does hampered neutrophil function due to reduced plasma vitamin C concentration explain this relationship? A recent study with a relatively small group of periodontitis (n=21) and control (n=21) subjects did show a negative correlation between neutrophil vitamin C concentrations in patients' and probing pocket depth, although there were no significant differences in peripheral neutrophil and peripheral blood mononuclear cell vitamin C concentrations between these two volunteer groups [25]. Whilst the relationship between low plasma vitamin C concentration and periodontitis could not therefore be explained by a hampered peripheral leucocyte function due to a lack of intracellular vitamin C, it may be that neutrophils with lower vitamin C concentrations may exit periodontal tissues via gingival crevicular fluid rather than returning to systemic circulation. An alternative explanation is that leukocyte vitamin C concentrations are protected by GSH oxidation, as recent data has demonstrated GSH deficiency and a lowered ratio of GSH to its oxidized counterpart in neutrophils from periodontitis patients relative to controls [26]. Furthermore, those patients with vitamin C insufficiency (i.e. not sufficiently deficient to develop signs of scurvy) presented with greater bone loss compared with patients whose plasma vitamin C was within normal limits. The data corroborates a previous study where a significant inverse association was found between plasma vitamin C levels and the severity of attachment loss in a relatively large population deprived of regular dental care [27], as well as in an elderly Japanese population [28].

A recent longitudinal retrospective cohort study with a two-year follow-up aimed to explore the relationship between intake of dietary antioxidants and periodontal disease in community-dwelling older Japanese [29]. The results suggest that antioxidant micronutrient levels (including vitamin C) which can be obtained through diet rather than supplements have a positive protective impact on the periodontal tissues. Interestingly, the mean levels of vitamin C intake were approximately 200mg/day, matching the suggested intake of Levine [30] but exceeding the 70mg/day found in the British National Diet and Nutrition Survey of over 65s [31], although estimates were made using a food frequency questionnaire which has a tendency to overestimate levels of intake. Nevertheless, these data concur with the former Japanese studies reported in 2007 and 2009 and imply that dietary modification to increase the consumption of fruits and other vegetables (containing not only vitamin C) may be beneficial to oral health and have a therapeutic and preventative role in periodontal disease [32,33]. This cohort was observed over a short time period, did not report any additional supplement use, had a limited number of teeth and did not report any related diseases, therefore the authors report that overestimation due to residual confounding of the observed association remained a risk in their analysis.

The impact of vitamin C on therapeutic outcomes

There are very few intervention studies examining whether greater intake of fruits and vegetables or specific micronutrients with antioxidant/anti-inflammatory activity are associated with improved clinical and dietary measures after routine non-surgical periodontal therapy. Javid *et al.* examined the impact of personalised dietary intervention designed to increase consumption of fruits, vegetables and whole grains on these measures [34]. At 3 and 6 months' post-intervention (including routine non-surgical therapy), those subjects given tailored advice (n=18) showed a significantly higher intake of fruits and vegetables in addition to a significantly increased plasma total antioxidant capacity compared with unadvised controls (n=19). As part of this, vitamin C intake was approximately 50mg/day higher following advice delivery, a difference that was maintained throughout the study period. The findings provide support for delivering personalised dietary interventions to patients with chronic periodontitis to improve their fruit and vegetable intake and showed that baseline intake was consistently lower than UK [35] and World Health Organisation recommendations [36]. The authors suggested that the lack of any significant changes in periodontal indices may have resulted from the magnitude of the dietary change being insufficient to alter these indices or that the period allocated after

the dietary intervention or the power of study were not sufficient to determine any significant differences in these measures between the two groups.

A North American cohort study of 63 non-smokers and 23 smokers showed that non-smokers with greater intakes of fruits and vegetables (analysed by a validated food questionnaire) had a lower percentage of sites with probing depths >3mm after debridement compared with patients with lower intakes, having adjusted for BMI and bleeding on probing. Vitamin C was the nutrient most strongly correlated with periodontal healing, although higher dietary intakes of the antioxidants vitamin C, β-carotene and α-tocopherol were all associated with greater probing depth reductions. *However, associations were consistently attenuated when contributions from dietary supplements were included in the authors' analyses*, suggesting again that the synergistic effect of whole foods rather than purified compounds in supplements offers a more robust approach, in addition to the potential benefit of more than 5000 plant-based phytochemicals that have not been examined here [37]. This may also explain the findings of a small intervention study where 2 groups (n=15 per group) of chronic periodontitis patients were treated with a standard non-surgical protocol, however, one group was supplemented with 2g/day vitamin C for 4-weeks post-therapy [38]. Despite both groups' total plasma antioxidant capacity significantly increasing following therapy, vitamin C supplementation showed no additional clinical nor plasma antioxidant benefit. Whilst this may also be explained by low subject numbers and clinical therapeutic outcomes, one may speculate that supplementation with single vitamin has limitations given that antioxidants *in* vivo act in concert. Willershausen *et al*. examined a small cohort of dental students before and after their final examinations, having divided the cohort into a non-supplemented group and a test group provided with a daily micronutrient combination for 3 months. Measurements for vitamins C and E increased after 3-months supplementation; despite poorer plaque control in both test and control groups thought to be related to exams, a smaller increase (albeit non-significant) in sulcular bleeding was reported in those subjects taking the micronutrient combination compared with the control group [39].

A similar 2-arm 8-week study with non-smoking volunteers screened for mild to severe periodontitis included a group who were supplemented with a complex multivitamin and phytonutrient formulation group (n=40) and a placebo formulation (n=45). The nutritional supplement modestly decreased periodontal inflammation; for probing depths of ≥4 mm, the nutritional supplement was associated with a significant decrease in the gingival index score and although not statistically significant, the test supplement group demonstrated a decrease in bleeding scores [40].

The role of dietary intervention to increase intake of foods rich in antioxidants (rather than by synthetic supplementation) in patients with periodontal disease

Larger, well controlled clinical studies are required to build upon the currently limited evidence regarding improved tissue resistance to periodontitis as a preventive or therapeutic strategy, or before it develops, during treatment and with the aim of preventing disease recurrence.

Studies investigating correlations between vegetarianism and oral health are rare. The first such cross sectional study with 200 age, smoking and gender-matched participants split equally between vegetarians (with at least a 2-year dietary history) and non-vegetarians, reported significantly lower probing pocket depths and % bleeding on probing in vegetarian participants [41]. The reported association may relate to a healthier lifestyle in general, and therefore factors such as obesity/BMI may play a role, as well as frequency of dental visits and better dental home care. However, the reported findings may also have a nutritional basis with greater quantities of antioxidant micronutrient consumption. Jenzsch *et al.* [42] have previously reported that a planned dietary intervention without changes to oral hygiene regimen or professional dental input can influence probing pocket depths and inflammatory markers and therefore these cross sectional findings may genuinely represent positive effects of a vegetarian diet upon periodontal tissues.

Vitamin D and calcium

Supplementation is not universally agreed upon

There has been a significant volume of scientific literature published on vitamin D compared with other vitamins although some authorities are advising caution in "the recent rush to elevate serum 25(OH)D concentrations universally" [43]. The classical expression of vitamin D deficiency is poor skeletal development and bone and joint deterioration, however, as well as promoting calcium absorption in the gut and maintaining adequate serum calcium and phosphate concentrations for bone growth and remodelling,

vitamin D plays significant roles in the modulation of cell growth, neuromuscular, immune and inflammatory function [44].

Vitamin D is a fat-soluble vitamin that is naturally present in very few foods. Endogenous production can also be triggered in the skin under the influence of ultraviolet light. Both dietary and endogenous forms are biologically inert, and undergo two hydroxylation reactions for activation: the first in the liver (vitamin D to 25-hydroxyvitamin D: 25(OH)D, calcidiol), the second primarily in the kidney to form physiologically active 1,25-dihydroxyvitamin D ($1,25(OH)_2D$, calcitriol). Serum 25(OH)D concentrations are regarded as a reliable indicator of vitamin status and function as a biomarker of exposure (given a circulating half-life of 15 days) [45] although it is not clear to what extent this serves as a biomarker of health status/outcomes [46]. In contrast, calcitriol has a short half-life and is therefore an unreliable indicator of vitamin D status. Furthermore, considerable variability has existed in serum calcidiol measurements until 2009 when a standard reference material that permits cross-laboratory and cross-assay standardisation was made available; indeed the relationship between increased vitamin D intake and serum calcidiol levels is reported to be non-linear.

The major source of vitamin D is exposure to natural sunlight; furthermore, vitamin D produced in the skin may last at least twice as long in the blood compared with ingested vitamin D. Whilst topical sunscreen application, increased skin pigmentation and obesity can dramatically reduce vitamin D synthesis, alteration in the zenith angle of the sun due to a change in latitude and season or even time of day also have a profound influence on the skin's vitamin D synthesis [47]. Seasonal variation, location and body fat therefore significantly affect the diagnosis of vitamin D sufficiency and should be carefully considered when conducting investigations [48].

Vitamin D exerts its biological effects by binding to the vitamin D receptor (VDR); a potential role for vitamin D in periodontal health is supported by polymorphisms in the VDR gene which have been reported in many studies to be associated with periodontitis [49,50]. The vitamin D–VDR complex stimulates RANKL (receptor activator of nuclear factor Kappa-B ligand) expression in cells such as osteoblasts and down regulates OPG (osteoprotegerin), thus favouring differentiation and activation of osteoclasts and increased bone resorption. Low vitamin D levels may stimulate an increase in circulating levels of parathyroid hormone which indirectly stimulate bone resorption in order to liberate vitamin D. Although vitamin D therefore appears to stimulate bone resorption, the catabolic effects can be transient and after longer periods of exposure may facilitate osteoblast proliferation, the relevance being the length of time for which future intervention trials are conducted for [for review, see 51].

The debate over optimal vitamin D intake

There is ongoing debate regarding what constitutes adequate vitamin D intake. In 2010, the United States Institute of Medicine released new recommendations for dietary intake of vitamin D: 400 IU/d (0–12 months of age); 600 IU/d (1–70 years of age); and 800 IU/d for older adults (> 70 years of age) (IU=25ng). Several experts feel that although these Institute of Medicine recommendations may prevent clinical vitamin D insufficiency (typically reported as < 30 ng/mL) and deficiency (< 20 ng/mL), they may be suboptimal with regard to autocrine mechanisms of vitamin D action [51]. Indeed, even at the higher levels recommended by the International Osteoporosis Foundation [52], some authorities suggest that even these would only normalise serum calcidiol concentrations in not more than half the US population (reviewed in [53]).

It is therefore not surprising that optimal serum vitamin D concentrations for bone health are the subject of continuing debate, although the Endocrine Society has issued clinical practice guidelines of >30ng/mL to maximise the effect of vitamin D on calcium, bone and muscle metabolism [47]. Nationally representative data estimated that 9.9% of the US population is vitamin D deficient (25[OH]D < 20 ng/mL) and 32% is insufficient (25[OH]D 21-29ng/mL), using cut points to maintain healthy bones defined by the Institute of Medicine (IOM).

Review of the contemporaneous scientific literature recently led the United States Agency for Healthcare Research and Quality to conclude that it is still not possible to specify a relationship between vitamin D and health outcomes other than bone health [54]. Furthermore, a meta-review of systematic reviews and meta-analyses suggests that there is no highly convincing evidence for a clear role for vitamin D supplementation in a wide range of health outcomes (skeletal, malignant, cardiovascular, autoimmune, infectious metabolic and other diseases), although "associations with a selection of outcomes are probable" [55]. To illustrate this, a recent publication found that patients given high doses of vitamin D or those on lower doses that increased vitamin D blood levels within the optimal range had a 20-30% increased rate of fractures and falls compared with those on low doses or who failed to reach "optimal blood levels" [56]. The strategy of supplementation to achieve plasma concentrations of 30ng/mL has not been established by randomised controlled trials to

reduce the risks of fractures or falls, but on the basis of observations showing healthy subjects have higher plasma vitamin concentrations.

The relationship between vitamin D and periodontal disease prevalence

Epidemiological (cross sectional) data regarding the prevalence of periodontitis and serum vitamin D levels initially appears somewhat conflicting, although trends do seem to exist. Authors who examined the US NHANES dataset have previously reported serum calcidiol levels to be significantly and inversely associated with attachment loss in men and women ≥ 50 years independently of bone mineral density [57]; subsequent analysis of the same dataset also found that low calcidiol levels were associated with greater amounts of bleeding on probing. The most recent analysis of a Korean dataset demonstrated that periodontal status (examined by CPITN measures) was inversely correlated in current smokers alone, the authors suggesting that this may be due to the clinical measures employed to assess attachment loss or that smoking influences the effect of vitamin D on periodontal tissues [58]. Alshouibi *et* al. also reported that total vitamin D intake was inversely associated with the odds of having severe periodontitis where total intake was ≥ 800 IU/day in an older all-male North American patient sample (mean age ~63 years) [59].

One study reported a *positive* association between plasma calcidiol and periodontal inflammation in young Chinese patients with aggressive periodontitis (n=66; median age 27) whereas those patients with chronic periodontitis (n=52) did not present with significantly different plasma calcidiol concentrations relative to healthy control subjects [60]. However, there was no statistically significant correlation between plasma calcidiol levels and attachment loss or probing depth, and all subjects had sub-optimal serum vitamin D levels and were therefore vitamin D insufficient or deficient.

The association between vitamin D and the progression of periodontitis

Prospective studies examining associations between disease progression and calcidiol concentrations that reflect intake from all sources (diet, supplements and sun exposure) are limited. Zhan et al (2014) concluded that increasing concentrations of calcidiol were associated with a reduced risk for tooth loss but not progression of clinical attachment loss over the follow up period (mean 5.9 years) [61]. This was equivalent to a 13% decreased risk of tooth loss with each 10 ng/mL increase in 25(OH)D concentration. There may be a small favourable effect on reduced CAL loss, however, measurement error and adjustment for vitamin D supplement use may have attenuated findings [62]. Two other prospective studies also observed no association between calcidiol and progression of periodontitis, although one did observe a protective effect of 'optimal' vitamin D concentration and tooth loss [63,64]. However, at baseline, Millen *et al* found a 33% lower odds of prevalent periodontal disease (defined using the AAP definition), among women with adequate compared with inadequate vitamin D status, and an inverse association between serum vitamin D concentration and percentage of sites that bled on probing [65].

A further prospective study in a cohort of older American men followed this trend with more severe periodontitis significantly associated with lower levels of calcidiol, but not with disease progression. Furthermore, bleeding on probing was greater in those subjects with the lowest serum vitamin D concentrations [66].

A cross-sectional and subsequent prospective cohort study in post-menopausal females also showed that calcium and vitamin D supplementation had a modest positive effect (less BOP) on periodontal health over 12 months in subjects enrolled on a periodontal maintenance programme, Nevertheless, consistent dental care improved periodontal clinical parameters regardless of such supplements. The authors pointed out that between group differences of serum vitamin D concentrations were limited, and therefore not all subjects who used supplements may have had sufficient supplementation to ensure obtaining serum concentrations within normal limits, thus potentially limiting clinical improvement [67].

Only one study to date has investigated the association between the vitamin D metabolite calcitriol and severity of chronic periodontitis, possibly because this vitamin D marker is more labile than calcidiol. All volunteers were type 1 diabetes patients (mean HbA1c 8.5% over 1 and 3 years). The serum level of calcitriol was associated with periodontal disease severity in all subjects and non-smokers; subjects with greater calcitriol concentrations were significantly more likely to belong to the group of subjects with no or mild periodontitis than the group with moderate or severe disease. Moreover, eight weeks following non-surgical therapy, a significant increase in calcitriol concentrations was reported, despite virtually no effect on calcidiol concentrations [68]. The authors concluded that this metabolite may vary in concentration between systemic and local pools, although both smoking and diabetes may influence these data.

Vitamin D, vitamin D receptor polymorphisms and the innate immune response

Vitamin D can also affect bone metabolism via anti-inflammatory mechanisms. For example, calcitriol interferes with nuclear factor-kappa B activation and significantly downregulates *P gingivalis*-induced expression of IL-8, an important pro-inflammatory cytokine, in cultured human periodontal ligament cells although the various pathways through which this may occur are yet to be fully elucidated [69].

Assuming that reduced vitamin D can lead to increased clinical signs of periodontal inflammation, this hypothesis also fits with vitamin D receptor gene polymorphisms' association with periodontitis. While this has been traditionally ascribed to the role of vitamin D in bone loss, vitamin D-mediated gene regulation (via VDR) of the innate immune response, i.e. the *first line* of defence against colonisation by periodontal pathogens may also play at least as important a role. The enhancing of innate antimicrobial defence by host peptides such as LL-37 by active vitamin D (calcitriol) in gingival epithelial cells certainly points to the potential importance of vitamin D in the primary prevention of the inflammatory periodontal diseases [69].

In summary, these data suggest that adequate vitamin D levels may exert a protective influence in periodontal health, both in a primary prevention and a maintenance phase of care. This appears likely to relate to reducing acute measures of periodontal inflammation rather than measures that reflect historical periodontal disease status. Interestingly, Dodington et al. (2015) did not find an association between vitamin D intake (as measured by a food frequency / supplement questionnaire) and greater reduction in mean pocket depths following non-surgical therapy in chronic generalised periodontitis patients, despite finding the opposite for other nutrients (with proven antioxidant or anti-inflammatory activity such as vitamin C, α-tocopherol and β-carotene), which showed a positive association with healing in non-smokers [37].

Conclusion

Five years following the review by Van der Velden and colleagues [3], the conclusions made regarding vitamin D and periodontitis remain valid and there remains "a clear need for proper randomised controlled trials to determine the effect of supplementation in the onset and treatment of periodontal diseases".

This review has updated the evidence base on the role of vitamins C and D as risk factors for periodontal diseases. Despite continued research activities into the roles of these two important micronutrients, there remains a lack of definitive data from large randomized controlled trials to enable clear statements to be made on their role in disease pathogenesis, or in adjunctive therapeutic interventions. As far as the authors are aware, the only randomized, controlled interventional setting to examine an oral health optimized diet on gingival inflammation since 2009 [5] has recently been published [71]. Despite a small sample size (determined by power analysis of data from [5]) and constant plaque values in both test and control groups, all inflammatory clinical parameters significantly decreased in the experimental subject group (placed on a four-week diet low in carbohydrates, rich in omega-3 fatty acids and rich in vitamins C, D, antioxidants and fibre) compared with the control group.

This contemporaneous evidence continues to support the hypothesis that supplementation via whole food nutritional approaches may provide some benefit in patients who are depleted of micronutrients, but the evidence base requires further data from larger well-conducted trials.