

# A review of the evidence for pathogenic mechanisms that may link periodontitis and diabetes

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## Abstract

**Aims:** To review the evidence for the molecular and cellular processes that may potentially link periodontal disease and diabetes. The pathogenic roles of cytokines and metabolic molecules (e.g. glucose, lipids) are explored and the role of periodontal bacteria is also addressed. Paradigms for bidirectional relationships between periodontitis and diabetes are discussed and opportunities for elaborating these models are considered.

**Methods:** Database searches were performed using MeSH terms, keywords, and title words. Studies were evaluated and summarized in a narrative review.

**Results:** Periodontal microbiota appears unaltered by diabetes and there is little evidence that it may influence glycaemic control. Small-scale clinical studies and experiments in animal models suggest that IL-1 $\beta$ , TNF- $\alpha$ , IL-6, OPG and RANKL may mediate periodontitis in diabetes. The AGE-RAGE axis is likely an important pathway of tissue destruction and impaired repair in diabetes-associated periodontitis. A role for locally activated pro-inflammatory factors in the periodontium, which subsequently impact on diabetes, remains speculative.

**Conclusion:** There is substantial information on potential mechanistic pathways which support a close association between diabetes and periodontitis, but there is a real need for longitudinal clinical studies using larger patient groups, integrated with studies of animal models and cells/tissues in vitro.

Key words: adipokines; AGE; cytokines; diabetes; hyperglycaemia; inflammation; periodontal disease; plaque bacteria; RAGE

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## Introduction

The increasing recognition over the last decade of the clinical and pathogenic relationship of periodontitis

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with overall health and disease (including diabetes) has revealed new perspectives regarding our understanding of periodontal pathogenesis with many potential clinical applications (Nassar et al. 2007, Lalla & Papanou 2011, Preshaw et al. 2012).

A dysregulated immune system is central to the pathogenesis of diabetes and associated complications. Type 2 diabetes (T2DM) and related conditions such as obesity are associated with a number of physiological, nutritional and metabolic changes including hyperglycaemia, production of

advanced glycation end-products (AGEs), hyperlipidaemia and increased adiposity; these changes have a number of consequences including immune-dysregulation manifested by a pronounced long-lasting inflammatory state and weakened self-limitation and resolution of immune responses (Kolb & Mandrup-Poulsen 2010). Systemic changes in cytokine levels are central to pathogenesis in T2DM (Pickup & Crook 1998) and these immune changes (often described as chronic, low-grade inflammation) can underpin insulin

resistance and islet cell apoptosis leading to insulin deficiency and therefore progression of the diabetic state (Donath & Shoelson 2011, Fernandez-Real & Pickup 2012). Critically, localized effects of immune dysregulation may underpin complications associated with T2DM; these include vascular disease, retinopathy, nephropathy and impaired healing (Graves & Kayal 2008, King 2008). A number of clinical trials provide evidence for the utility of cytokine analysis in pre-clinical diagnosis of T2DM (Carstensen et al. 2010) and for cytokine blockade in therapy for T2DM (Larsen et al. 2009). Cytokines are also critical in the development of type 1 diabetes (T1DM) and modulation of cytokines is a potential therapeutic modality (Mandrup-Poulsen et al. 2010, Baumann et al. 2012).

The overall aim of this Supplement article was to provide a review of the molecular and cellular processes which might underpin the relationship between periodontal disease and diabetes. Evidence from clinical studies and experiments in model systems (in vivo and in vitro) are summarized and evaluated. Existing paradigms for bidirectional relationships between periodontitis and diabetes are discussed and opportunities for elaborating these models briefly alluded to. Given the nature of the subject area, it remains clear that this literature search cannot be considered a systematic review.

A variety of search strategies were adopted which included electronic database searches of Medline and Pubmed from 1946 up to, and including, June 2012, using MeSH terms, keywords and title words during the search. The terms used for these searches were as follows: [diabetes mellitus type 1 OR diabetes mellitus type 2 OR diabetes OR diabetic] AND [bacteria OR microflora OR microbiota OR microbiology OR pathogen OR microorganism OR host-pathogen interactions OR *P. gingivalis* OR *A. actinomycetemcomitans*] AND [periodontitis OR chronic periodontitis OR aggressive periodontitis OR periodont\* OR gingivitis OR gingival]; [diabetes mellitus type 1 OR diabetes mellitus type 2 OR diabetes OR diabetic] AND [periodontitis OR chronic periodontitis OR aggressive periodontitis OR periodont\* OR gingivitis OR gingival] AND [cytokine OR interleukin OR

prostaglandin OR matrix metalloproteinase OR adipokine OR leptin OR adiponectin]; [diabetes OR diabetes mellitus OR diabetic OR hyperglycemia OR dysglycemia] AND [periodontitis OR periodont\* OR gingivitis OR gingival] AND [mechanisms OR pathogenesis OR infection OR pathogen OR *P. gingivalis*]. Reference lists of identified articles were also reviewed. Only English language articles were considered and case reports were not included in the review. The key features of identified relevant research studies were evaluated and the conclusions summarized in a narrative review.

### The impact of diabetes on periodontal disease pathogenesis

#### Microbial factors

Whether diabetes has an impact on the periodontal microbiota has been debated for many years. This question was first posed by researchers in the 1980s, who proposed that elevated glucose levels in gingival crevicular fluid (GCF) in people with diabetes might favour the growth of certain bacterial species in the subgingival environment, leading to increased susceptibility to periodontitis and accelerated disease progression. Studies that have investigated the impact of diabetes on the periodontal microbiota are summarized in Table 1. Comparisons between the studies are difficult due to the varying clinical and laboratory protocols that were utilized. Also, there are a number of other studies of the periodontal microbiota in diabetes not included in Table 1, which are inconclusive as they did not adequately report or control for the clinical periodontal status of the patients involved in the research (Sanchez-Cordero et al. 1979, Thorstenson et al. 1995, Novaes et al. 1997, Collin et al. 1998, Campus et al. 2005, Ciantar et al. 2005, Hinta et al. 2007, Colhoun et al. 2008, Dasanayake et al. 2008, Kamaraj et al. 2011) or because they did not present statistical analysis in support of stated conclusions (Zambon et al. 1988). Therefore, in these studies, it is not always clear if identified differences in the periodontal microbiota between diabetic and non-diabetic patients result truly from an impact of the diabetic state on the

periodontal bacteria, or simply result from more severe periodontitis (i.e. deeper pockets) in people with diabetes. For example, in many studies, patients with diabetes and periodontitis are compared to control groups of periodontally healthy, non-diabetic patients. Ideally, studies of the subgingival microbiota in patients with periodontitis and diabetes should involve control groups comprising non-diabetic individuals with matched periodontal status. Another limitation of these studies is the restricted analysis of biofilm species; global (and unbiased) analysis of the plaque microbiota using the techniques of microbiomics and metagenomics could reveal more detailed information about the influence of diabetes on the oral microbiome (Zarco et al. 2012).

From the studies listed in Table 1, it is apparent that the presence of diabetes (T1DM or T2DM) has no significant effect on the composition of the periodontal microbiota. Furthermore, the level of glycaemic control in people with diabetes also does not significantly influence the composition of the subgingival biofilm.

#### Cytokines and adipokines

The vast majority of studies of cytokines, adipokines and other mediators [growth factors, prostanoids and matrix metalloproteinases (MMPs)] in periodontitis and diabetes have been small-scale clinical studies using GCF, saliva or gingival tissue samples (Table 2). Most reports focus on chronic periodontitis in diabetes, but a limited number include gingivitis (Andriankaja et al. 2009) and aggressive periodontitis (Davies et al. 2011). The majority are cross-sectional studies and are designed to assess whether diabetes quantitatively or qualitatively influences the cytokine profile of patients with periodontal disease. The interpretations in Table 2 are restricted to information relevant to the relationship of diabetes with periodontal disease. A number of studies not included in Table 2 are inconclusive in this regard because they were designed to compare periodontal treatment regimens in diabetic subjects and did not include non-diabetic control groups with matched periodontal status and therefore any

Table 1. Principal findings from clinical studies that investigated the potential influence of diabetes on the periodontal microbiota

| Author/year                | Study population   | Study design   | Analytical methods   | Main findings   | Interpretation   |
|----------------------------|--|--|--|---|--|
| Sastrowijoto et al. 1989   | 22 adults with type 1 diabetes, 12 with HbA1c $\leq 7.7\%$ and 10 with HbA1c $\geq 9.9\%$            | Cross-sectional comparison of selected bacterial species in subgingival plaque samples   | Culture techniques for selected bacterial species  | Clinical periodontal status did not differ significantly between the groups. Similarly, there were no significant differences in the frequency of identification of the bacterial species between the groups  | NO INFLUENCE of level of glycaemic control on the periodontal microbiota |
| Sastrowijoto et al. 1990   | 6 patients with poorly controlled insulin-dependent diabetes who then commenced insulin therapy      | 8-month longitudinal study with subgingival plaque samples collected at months 0, 4 and 8  | Culture techniques for selected bacterial species  | Glycaemic control significantly improved over the course of the study as a result of the insulin therapy, but no significant changes in the periodontal microbiota were identified either in deep ( $\geq 4$ mm) or shallow ( $\leq 3$ mm) sites (other than an increase in streptococci species in deep sites) | NO INFLUENCE of level of glycaemic control on the periodontal microbiota |
| Tervonen et al. 1994       | 60 adults with type 1 diabetes and 47 adults with type 2 diabetes                                    | Cross-sectional comparison of occurrence of 5 periodontal pathogens, with logistic regression to evaluate impact of diabetes state on carriage of the selected species                                 | Monoclonal antibodies utilized in immunoassays selective for <i>A. actinomycetemcomitans</i> , <i>F. nucleatum</i> , <i>E. corrodens</i> , <i>P. gingivalis</i> and <i>P. intermedia</i> | Factors such as the type of diabetes (type 1 or type 2), time since diagnosis of diabetes and level of glycaemic control had no statistically significant effect on the prevalence of the periodontal bacteria  | NO INFLUENCE of diabetes on the periodontal microbiota                   |
| Sbordone et al. 1995, 1998 | 16 children (9–17 years old) with insulin-dependent diabetes and 16 non-diabetic cohabiting siblings | Cross-sectional baseline (Sbordone et al. 1995) and longitudinal continuation study (Sbordone et al. 1998), with clinical data and subgingival plaque samples collected at baseline, year 2 and year 3 | Culture of subgingival plaque samples for <i>P. gingivalis</i> , <i>P. intermedia</i> , <i>A. actinomycetemcomitans</i> and <i>Capnocytophaga</i> species                                | No significant differences in the composition of the subgingival microbiota (or clinical periodontal parameters) between cases and controls were identified at any time-point   | NO INFLUENCE of diabetes on the periodontal microbiota                   |
| Pinducciu et al. 1996      | 131 adults with type 1 diabetes, and 20 (non-matched) non-diabetic controls                          | Cross-sectional comparison of total bacterial counts and <i>Capnocytophaga</i> species   | Culture of supragingival plaque samples to determine total bacterial and <i>Capnocytophaga</i> counts  | No significant quantitative differences in microaerophilic and anaerobic microbial flora or <i>Capnocytophaga</i> between the diabetic and non-diabetic groups. Gingival index was higher in the diabetic group, but mobility, oral hygiene and CPTN index did not differ significantly between the groups      | NO INFLUENCE of diabetes on the periodontal microbiota                   |
| Yuan et al. 2001           | 105 adults with non-insulin-dependent diabetes and 141 age- and sex-matched non-diabetic individuals | Cross-sectional comparison of detection rates of <i>A. actinomycetemcomitans</i> , <i>P. gingivalis</i> , <i>E. corrodens</i> , <i>T. denticola</i> and <i>C. albicans</i>                             | PCR analysis for the presence of the selected microorganisms   | No significant differences in the prevalence rates of the 5 microorganisms between the diabetic and non-diabetic individuals. There were no significant differences in mean plaque index, gingival index, probing depths or attachment levels (all recorded at Ramfjord teeth) between the two groups           | NO INFLUENCE of diabetes on the periodontal microbiota                   |

Table 1. (continued)

| Author/year          | Study population   | Study design  | Analytical methods   | Main findings   | Interpretation   |
|----------------------|--|---|--|---|--|
| Salvi et al. 2005    | 9 patients with type 1 diabetes and 9 age- and gender-matched non-diabetic controls  | 3-week experimental gingivitis study (days 0-21) followed by 2 weeks of resumption of normal oral hygiene (days 21-35). Plaque index and gingival index were recorded at days 0, 7, 14, 21 and 35, and plaque samples were collected at days 0, 21 and 35 | Subgingival plaque samples were assessed by DNA-DNA hybridization for a panel of 40 subgingival microbial species                          | Mean total DNA bacterial counts did not differ significantly between the two groups (type 1 diabetes vs. control) at days 0, 21 and 35. Mean DNA probe counts of the red and orange complex species increased significantly from day 0 to day 21, and then decreased significantly from day 21 to day 35 in both groups, but there were no significant differences in the mean DNA proportions between the two groups. The only significant difference in bacterial levels between the groups was for <i>E. nodatum</i> , which was significantly elevated in the diabetes patients. When analysis was stratified by probing depths at sampled sites, no significant differences between the groups were recorded. There were no differences in serum IgG titres between the groups | NO INFLUENCE of diabetes on the periodontal microbiota       |
| Lalla et al. 2006    | 50 adults with type 1 diabetes, and 50 age- and gender-matched non-diabetic controls with a similar level of periodontitis | Cross-sectional comparison of subgingival plaque samples  | Checkerboard hybridization for 12 periodontal bacteria, and checkerboard immunoblotting for serum IgG titres against the selected bacteria | Periodontal status improved following treatment, but no changes in HbA1c occurred. Reductions in the frequency of bacterial recovery occurred over the 3 months, but there were no significant differences between the two groups   | NO INFLUENCE of diabetes on the periodontal microbiota       |
| da Cruz et al. 2008  | 20 adults with periodontitis, of whom 10 also had type 2 diabetes  | Longitudinal follow-up study (3 months) following non-surgical periodontal therapy  | PCR analysis of subgingival plaque samples for <i>A. actinomycetemcomitans</i> , <i>P. gingivalis</i> and <i>T. forsythia</i>              | Similar pathogens were found in periodontitis sites in subjects with and without type 2 diabetes, though <i>P. gingivalis</i> , <i>A. actinomycetemcomitans</i> and <i>Campylobacter</i> species were found more frequently ( $p < 0.05$ ) in subjects with type 2 diabetes compared to those without. Also, serum IgG responses were broadly similar between the two groups, apart from a significant elevation in antibody to <i>C. rectus</i> in the diabetic subjects   | NO INFLUENCE of diabetes on the periodontal microbiota       |
| Ebersole et al. 2008 | 39 Hispanic Americans with type 2 diabetes, and 24 non-diabetic controls   | Cross-sectional comparison of subgingival plaque samples and serum antibody levels to selected oral microorganisms  | DNA checkerboard hybridization of plaque samples, and ELISA analysis of serum IgG levels   | Similar pathogens were found in periodontitis sites in subjects with and without type 2 diabetes, though <i>P. gingivalis</i> , <i>A. actinomycetemcomitans</i> and <i>Campylobacter</i> species were found more frequently ( $p < 0.05$ ) in subjects with type 2 diabetes compared to those without. Also, serum IgG responses were broadly similar between the two groups, apart from a significant elevation in antibody to <i>C. rectus</i> in the diabetic subjects   | POSSIBLE INFLUENCE of diabetes on the periodontal microbiota |

CPTN, Community Periodontal Index of Treatment Need; ELISA, enzyme linked immunosorbent assay; HbA1c, glycated haemoglobin A1c; IgG, immunoglobulin G; PCR, polymerase chain reaction.

Table 2. Principal findings from clinical studies that investigated the influence of diabetes on cytokines in periodontal disease

| Author/year        | Study population  | Study design   | Analytical methods   | Main findings   | Interpretation   |
|--------------------|---|--|--|---|--|
| Salvi et al. 1997c | 10 adults with T1DM and gingivitis or mild chronic periodontitis, 29 adults with T1DM and moderate or severe chronic periodontitis, 43 systemically healthy adults with varying degrees of chronic periodontitis and 21 healthy adult controls. T1DM subjects had moderate to poor glycaemic control. Note: duration of diabetes prior to study not indicated | Cross-sectional comparison of IL-1 $\beta$ and PGE <sub>2</sub> in GCF                                   | ELISA (IL-1 $\beta$ ) and RIA (PGE <sub>2</sub> )  | IL-1 $\beta$ and PGE <sub>2</sub> elevated in T1DM as compared to non-diabetes controls irrespective of level of probing depth. IL-1 $\beta$ and PGE <sub>2</sub> elevated in T1DM patients with moderate or severe chronic periodontitis as compared to patients with T1DM and gingivitis or mild chronic periodontitis  | POSSIBLE ASSOCIATION between T1DM and IL-1 $\beta$ and PGE <sub>2</sub> in GCF |
| Cutler et al. 1999 | 6 adults with poorly controlled T2DM and chronic periodontitis, 5 periodontally healthy adults with poorly controlled T2DM, 6 periodontally healthy adults with well-controlled T2DM, 7 systemically healthy adults with chronic periodontitis and 6 healthy adult controls   | Cross-sectional comparison of IL-1 $\beta$ in GCF and IL-1 $\beta$ , IL-6 and PDGF-AB in gingival tissue | ELISA  | No differences in IL-1 $\beta$ (in GCF or tissue) or IL-6 between the different groups. Clinical parameters of chronic periodontitis (irrespective of diabetes status) correlated with GCF IL-1 $\beta$ and all mediators (except PDGF-AB). Increase in PDGF-AB was significantly less in patients with poorly controlled diabetes as compared to those with well-controlled diabetes | NO ASSOCIATION between T2DM and gingival IL-1 $\beta$ , IL-6 and PDGF-AB       |
| Kurtis et al. 1999 | 24 adults with T2DM and chronic periodontitis, 24 systemically healthy adults with chronic periodontitis and 24 healthy control adults. Note: No indication of level of diabetes control and duration of diabetes in T2DM patients  | Cross-sectional comparison of IL-6 in GCF  | ELISA  | IL-6 elevated in patients with T2DM as compared to chronic periodontitis and control groups. IL-6 elevated in chronic periodontitis as compared to health   | POSSIBLE ASSOCIATION between T2DM and IL-6 in GCF                              |
| Collin et al. 2000 | 45 adults with T2DM and 77 systemically healthy adults. Note: although clinical measures of chronic periodontitis were similar in both groups, 20 T2DM patients and 23 controls were edentulous   | Cross-sectional comparison of MMP-1, MMP-8, MMP-9 and MMP-13 in saliva                                   | IFMA (MMP-8), WB (all MMPs), zymography and <sup>125</sup> I-gelatin assay (gelatinase activity) | MMP-8 and gelatinase activity higher in dentate groups. No differences in MMP-8 levels or gelatinase activity between T2DM group and controls. Major MMPs in saliva were MMP-8 and MMP-9. MMP-8 correlated with HbA1c and parameters of chronic periodontitis in the T2DM group   | POSSIBLE ASSOCIATION between glycaemic control in T2DM and salivary MMP-8      |

Table 2. (continued)

| Author/year              | Study population  | Study design   | Analytical methods                     | Main findings   | Interpretation  |
|--------------------------|---|--|--|---|---|
| Unlu et al. 2003         | 10 adults with well-controlled T2DM and chronic periodontitis, 10 systemically healthy adults with chronic periodontitis and 10 healthy adult controls  | Cross-sectional comparison of VEGF in gingival tissue  | IHC                                    | VEGF was not expressed in healthy gingival tissues from systemically healthy patients and in only 2 of the diseased sites sampled. In patients with diabetes, VEGF expression was present in 4 healthy sites and 6 diseased sites   | POSSIBLE ASSOCIATION between T2DM and gingival VEGF expression  |
| Engelbretson et al. 2004 | 45 adults with T2DM and chronic periodontitis   | Cross-sectional comparison of IL-1 $\beta$ in GCF  | ELISA                                  | IL-1 $\beta$ in GCF significantly correlated with parameters of glycaemic control (HbA1c and random glucose) independently of clinical measures of periodontitis  | POSSIBLE ASSOCIATION between glycaemic control and IL-1 $\beta$ in GCF  |
| Engelbretson et al. 2006 | 45 adults with T2DM and chronic periodontitis and 32 systemically healthy adults with chronic periodontitis   | Cross-sectional comparison of IL-8 and $\beta$ -glucuronidase in GCF                           | ELISA (IL-8) and enzyme activity assay | IL-8 and $\beta$ -glucuronidase both significantly lower in chronic periodontitis patients with T2DM compared to systemically healthy patients with chronic periodontitis. IL-8 and $\beta$ -glucuronidase levels did not correlate with HbA1c in the group with T2DM and chronic periodontitis | POSSIBLE ASSOCIATION between T2DM and IL-8 and $\beta$ -glucuronidase in GCF                                    |
| Lalla et al. 2006        | 13 adults with T1DM and 13 age- and gender-matched non-diabetic controls with similar levels of periodontal disease<br>Note: Overall, HbA1c measurements indicated good metabolic control. Mean diabetes duration was 20 years (range 6-41) | Cross-sectional comparison of multiple cytokines and mediators including adiponectin in serum. | Multiplex                              | T1DM group had significantly higher adiponectin, sE-selectin and VCAM-1 levels and lower PAI-1 levels as compared to controls   | POSSIBLE INFLUENCE of T1DM on serum adiponectin, sE-selectin, VCAM-1 and PAI-1 levels in periodontitis patients |
| Takeda et al. 2006       | 69 adults with T2DM and chronic periodontitis, 28 adults with T2DM only. Note: all T2DM patients were poorly controlled but information regarding duration of diabetes was not presented  | Cross-sectional comparison of serum CRP and TNF- $\alpha$                                      | Not known (outsourced)                 | No differences in serum CRP or TNF- $\alpha$ levels between groups  | NO ASSOCIATION between serum CRP and TNF- $\alpha$ and T2DM in patients with chronic periodontitis              |

Table 2. (continued)

| Author/year                 | Study population   | Study design   | Analytical methods   | Main findings  | Interpretation  |
|-----------------------------|--|--|--|--|---|
| Duarte et al. 2007a         | 20 adults with T2DM and chronic periodontitis, 20 systemically healthy adults with chronic periodontitis and 10 healthy adult controls. Note: diabetes status based on blood glucose levels  | Cross-sectional comparison of IL-1 $\beta$ , IL-6, IL-8 and IFN $\gamma$ in gingival tissue.   | ELISA  | IL-1 $\beta$ , IL-6, IL-8 and IFN $\gamma$ higher in chronic periodontitis compared to healthy controls, irrespective of diabetes status. IL-1 $\beta$ and IL-6 only higher in patients with both chronic periodontitis and T2DM compared to systemically healthy patients with similar periodontitis  | POSSIBLE ASSOCIATION between T2DM and IL-1 $\beta$ and IL-6 expression in gingival tissue |
| Duarte et al. 2007b         | 20 adults with well-controlled T1DM and chronic periodontitis, 20 systemically healthy adults with chronic periodontitis and 10 healthy adult controls. Note: diabetes status based on blood glucose levels                            | Cross-sectional comparison of TNF- $\alpha$ , IL-1 $\beta$ , IL-1Ra, IL-6, IL-8, IL-10, RANKL, OPG and IFN $\gamma$ in gingival tissue | qRT-PCR.   | Elevation in tissue cytokine expression in both groups with chronic periodontitis as compared to controls with the exception of OPG and IL-10 which were lower. IL-1 $\beta$ , IL-8, IL-10, TNF- $\alpha$ , RANKL and OPG were significantly lower in chronic periodontitis patients with T2DM as compared to systemically healthy patients with chronic periodontitis | POSSIBLE ASSOCIATION between T1DM and OPG and IL-10 mRNA expression in gingival tissue.   |
| Engelbreton et al. 2007     | 46 adults with well-controlled T2DM and chronic periodontitis; almost identical to the cohort reported in Engelbreton et al. 2004  | Cross-sectional comparison of IL-1 $\beta$ in GCF, TNF- $\alpha$ in serum, endotoxin in plasma   | ELISA (IL-1 $\beta$ , TNF- $\alpha$ ), LAL assay (endotoxin) | Plasma endotoxin correlated with serum TNF- $\alpha$ . TNF- $\alpha$ correlated with some but not all clinical measures of chronic periodontitis but not with serum glucose or HbA1c   | NO ASSOCIATION between glycaemic control and serum TNF- $\alpha$                          |
| Navarro-Sanchez et al. 2007 | 10 adults with well-controlled T2DM and chronic periodontitis and 10 systemically healthy adults with chronic periodontitis. Note: differences in some but not all clinical measures of chronic periodontitis between study groups     | Longitudinal follow-up study (6 months) comparing IL-6 and TNF- $\alpha$ in GCF after non-surgical treatment of chronic periodontitis  | ELISA  | Both mediators similarly reduced 3 months after treatment of chronic periodontitis. No differences in the levels of either mediator between the groups at any time, HbA1c also reduced after treatment   | NO ASSOCIATION between T2DM and gingival IL-1 $\beta$ or TNF- $\alpha$                    |
| Cole et al. 2008            | 7 adults with T2DM and chronic periodontitis (of whom 5 had well-controlled diabetes), 11 systemically healthy adults with chronic periodontitis and 9 healthy adult controls. Note: information on duration of diabetes not presented | Cross-sectional comparison of MMP-1, MMP-8, IL-6, TNF- $\alpha$ , IL-1 $\beta$ mRNA in gingival tissue.                                | qRT-PCR  | IL-6 expression elevated in disease groups, with highest expression in the T2DM and chronic periodontitis group. No significant differences in the expression of the other mediators between the 3 study groups  | POSSIBLE ASSOCIATION between T2DM on gingival IL-6 mRNA expression                        |

Table 2. (continued)

| Author/year             | Study population  | Study design  | Analytical methods                           | Main findings   | Interpretation  |
|-------------------------|---|---|--|---|---|
| Kardesler et al. 2008   | 17 adults with T2DM and chronic periodontitis, 17 systemically healthy adults with chronic periodontitis, and 17 healthy adult controls. Note: Mean HbA1c data presented but level of glycaemic control in groups not indicated | Cross-sectional comparison of IL-1 $\beta$ , PGE <sub>2</sub> , t-PA and PAI-2 in GCF                                   | ELISA  | IL-1 $\beta$ was significantly lower in the T2DM patients with chronic periodontitis as compared to systemically healthy chronic periodontitis patients. No difference between these groups with respect to the other mediators   | POSSIBLE ASSOCIATION between T2DM and gingival IL-1 $\beta$                                       |
| Andriankaja et al. 2009 | 340 adults with healthy gingiva (30 of whom had T2DM) and 385 adults with gingivitis (50 of whom had T2DM). Note: diagnosis of diabetes based on criteria other than HbA1c  | Cross-sectional comparison of IL-1 $\beta$ and PGE <sub>2</sub> in GCF and IL-6 in serum                                | ELISA  | GCF IL-1 $\beta$ and PGE <sub>2</sub> elevated in subjects with gingivitis compared to those with healthy gingiva irrespective of diabetes status. Elevation in IL-1 $\beta$ in subjects with diabetes higher than that noted for non-diabetes subjects. Serum IL-6 elevated in subjects with gingivitis compared to those with healthy gingiva in diabetes subjects only | POSSIBLE ASSOCIATION between T2DM and gingival IL-1 $\beta$ and between serum IL-6 and gingivitis |
| Dag et al. 2009         | 15 adults with well-controlled T2DM and chronic periodontitis, 15 adults with poorly controlled T2DM and chronic periodontitis and 15 systemically healthy adults with chronic periodontitis                                    | Longitudinal follow-up study (3 months) comparing serum TNF- $\alpha$ levels following non-surgical periodontal therapy | Chemiluminescent assay using Immulite system | TNF- $\alpha$ levels decreased in all groups after treatment but no differences in TNF- $\alpha$ levels at baseline or after treatment between individual groups of patients  | NO ASSOCIATION between serum TNF- $\alpha$ and T2DM in chronic periodontitis patients             |
| Lappin et al. 2009      | 26 adults with T1DM and chronic periodontitis, 26 adults with T1DM, 17 systemically healthy adults with chronic periodontitis and 19 healthy adult controls   | Cross-sectional comparison of OPG, RANKL, osteocalcin, ICTP in serum.   | ELISA  | Osteocalcin lower in patients with diabetes compared to patients without diabetes. No differences in RANKL or ICTP. OPG elevated in patients with diabetes as compared to patients without diabetes and was highest in T1DM/chronic periodontitis group. Correlations between HbA1c and OPG (positive) and osteocalcin (negative)   | POSSIBLE ASSOCIATION between glycaemic control and osteocalcin and OPG in serum of T1DM patients  |

Table 2. (continued)

| Author/year            | Study population  | Study design  | Analytical methods                      | Main findings   | Interpretation   |
|------------------------|---|---|---|---|--|
| Chen et al. 2010       | Adults with T2DM and chronic periodontitis divided up into 46 adults with 'low' probing depths, 48 adults with 'middle' probing depths and 46 adults with 'high' probing depths. Patients all had well-controlled T2DM      | Cross-sectional comparison of serum CRP and TNF- $\alpha$   | Not known (outsourced)                  | Serum TNF- $\alpha$ was not significantly different between the groups. CRP was significantly correlated with probing depth   | POSSIBLE ASSOCIATION between serum CRP and clinical measures of chronic periodontitis  |
| Costa et al. 2010      | 22 adults with T2DM and chronic periodontitis, 20 adults with T2DM, 24 systemically healthy adults with chronic periodontitis and 22 healthy adult controls. T2DM patients had poorly controlled diabetes                   | Cross-sectional comparison of IL-6, MMP-8, OPG in saliva  | ELISA                                   | IL-6 was elevated in both groups with chronic periodontitis and correlated with HbA1c in the T2DM and chronic periodontitis group. MMP-8 was elevated in all disease groups but OPG was only elevated in patients with T2DM but without chronic periodontitis                         | POSSIBLE ASSOCIATION between glycaemic control and IL-6  |
| Kardesler et al. 2010a | 12 adults with T2DM and gingivitis, 12 adults with T2DM and chronic periodontitis, 12 systemically healthy adults with gingivitis, 13 systemically healthy adults with chronic periodontitis, and 24 healthy adult controls | Cross-sectional comparison of MMP-8, MMP-13 and TIMP-1 in GCF.  | IFMA (MMP-8), ELISA (MMP-13 and TIMP-1) | MMP-8 was significantly lower in the healthy controls as compared to all other groups but otherwise there were no differences in mediators levels between the groups. No correlations between mediators and HbA1c presented. Note: level of glycaemic control in groups not indicated | NO ASSOCIATION between T2DM and gingival MMP-8, MMP-13 and TIMP-1  |
| Kardesler et al. 2010b | 12 adults with chronic periodontitis and poorly controlled T2DM, 13 adults with chronic periodontitis and well-controlled T2DM and 15 systemically healthy adults with chronic periodontitis                                | Longitudinal follow-up study (3 months) to compare serum levels of leptin, adiponectin, IL-6, sICAM-1, TNF- $\alpha$ and CRP following non-surgical therapy | ELISA                                   | Levels of all mediators were similar in all groups at baseline and after 1 and 3 months following treatment   | NO ASSOCIATION of T2DM and serum leptin, adiponectin, IL-6, sICAM-1, TNF- $\alpha$ and CRP in patients with chronic periodontitis and no effect of treatment on these mediators. |
| Ross et al. 2010       | 10 adults with T2DM and chronic periodontitis (of whom 9 had well-controlled diabetes), 17 systemically healthy adults with chronic periodontitis and 8 healthy adult controls  | Cross-sectional comparison of IL-6, IL-8, TNF- $\alpha$ , MCP-1, CCRI-5, CXCR1-5 mRNA and protein in gingival tissue  | qRT-PCR, WB                             | IL-6 expression elevated in disease groups with highest expression in the T2DM and chronic periodontitis group  | POSSIBLE ASSOCIATION between T2DM and gingival IL-6  |

Table 2. (continued)

| Author/year         | Study population   | Study design  | Analytical methods                             | Main findings   | Interpretation  |
|---------------------|--|---|--|---|---|
| Salvi et al. 2010   | 9 adults with well-controlled T1DM and 9 healthy adult controls  | Experimental gingivitis model with cessation of oral hygiene for 3 weeks and monitoring for a further 2 weeks to compare changes in IL-1 $\beta$ , IL-8, MMP-8 and MMP-9 in GCF | ELISA  | IL-1 $\beta$ higher at baseline in T1DM patients and was increased significantly at 7 days. IL-1 $\beta$ increased significantly after 14 days in controls. MMP-8 and MMP-9 elevated in both groups in parallel but higher in T1DM group. No change in IL-8 throughout  | POSSIBLE ASSOCIATION between T2DM and gingival IL-1 $\beta$ levels and changes of IL-1 $\beta$ (and MMP-8 and MMP-9) during development of gingivitis |
| Santos et al. 2010b | 20 adults with poorly controlled T2DM and chronic periodontitis and 18 adults with well-controlled T2DM and chronic periodontitis  | Longitudinal follow-up study (6 months) comparing IL-4, IL-17, IL-23, IFN $\gamma$ and TNF- $\alpha$ in GCF following non-surgical periodontal therapy                          | ELISA  | IL-4 and IL-17 higher in poorly controlled T2DM. IFN $\gamma$ elevated in well-controlled T2DM. No changes in mediators at either 3 or 6 months after treatment. IL-4, IL-17 and TNF- $\alpha$ levels all correlated with HbA1c. HbA1c did not change as the result of treatment  | POSSIBLE ASSOCIATION between glycaemic control and gingival IL-4 and IL-17 in chronic periodontitis   |
| Santos et al. 2010a | 20 adults with poorly controlled T2DM and chronic periodontitis and 18 adults with well-controlled T2DM and chronic periodontitis; same patient cohort and clinical study as reported in Santos et al. 2010a         | Cross-sectional comparison of RANKL and OPG in GCF  | ELISA  | RANKL and RANKL/OPG ratios significantly elevated in patients with poorly controlled diabetes and at 3 and 6 months after non-surgical treatment for chronic periodontitis. RANKL/OPG ratios decreased after treatment only in the well-controlled diabetes group. HbA1c did not change as the result of treatment but did correlate significantly with RANKL and OPG | POSSIBLE ASSOCIATION between glycaemic control and gingival RANKL and OPG in chronic periodontitis.   |
| Sun et al. 2011     | 75 adults with T2DM and chronic periodontitis undergoing no periodontal treatment and 82 adults with T2DM and chronic periodontitis undergoing periodontal treatment. Note: T2DM patients had poor glycaemic control | Longitudinal follow-up study (3 months) to compare serum levels of adiponectin, CRP, TNF- $\alpha$ and IL-6 following therapy (both surgical and non-surgical)                  | ELISA and automated biochemical analyser (CRP) | Levels of all mediators were similar in both groups at baseline. Adiponectin levels significantly increased after treatment. Similarly, the other mediators declined after treatment.   | POSSIBLE INFLUENCE of periodontal treatment on adiponectin, CRP, TNF- $\alpha$ and IL-6 in serum  |

Table 2. (continued)

| Author/year                | Study population  | Study design  | Analytical methods | Main findings  | Interpretation  |
|----------------------------|---|---|--------------------|--|---|
| Duarte et al. 2011         | 10 adults with poorly controlled T2DM and chronic periodontitis, 10 systemically healthy adult smokers with chronic periodontitis, 10 systemically healthy adult non-smokers with chronic periodontitis and 10 healthy adult controls | Cross-sectional comparison of IL-17 +, IL-15 + and Foxp3 + cells in gingival tissue   | IHC                | Elevated Foxp3 + cells in all chronic periodontitis groups as compared to healthy controls. The poorly controlled T2DM and chronic periodontitis group had the highest IL-15 +, IL-17 + and Foxp3 + cells associated with elevated fibrosis  | POSSIBLE ASSOCIATION between T2DM and development of T helper 17 and T regulatory cells in the gingiva        |
| Kardesler et al. 2011      | 20 adults with well-controlled T2DM and chronic periodontitis and 22 systemically healthy adults with chronic periodontitis. Note: baseline BOP measurements were significantly higher in the T2DM group                              | Longitudinal follow-up study (3 months) comparing GCF IL-6, tPA and PAI-2 levels following non-surgical periodontal therapy | ELISA              | IL-6 was significantly higher in the T2DM group with chronic periodontitis at baseline as compared to the group with chronic periodontitis alone. IL-6 and tPA declined after treatment in the T2DM/chronic periodontitis group but not in the chronic periodontitis group   | POSSIBLE ASSOCIATION between T2DM and gingival IL-6 and changes in IL-6 and t-PA after non-surgical treatment |
| Kim et al. 2011            | 12 adults with poorly controlled T2DM and chronic periodontitis, 12 systemically healthy adults with chronic periodontitis and 12 healthy adult controls. Note: no HbA1c data presented   | Cross-sectional comparison of CRP, MMP-14, TIMP-2 in gingival tissue  | WB                 | All mediators elevated in both groups with chronic periodontitis. CRP and MMP-14 highest in patients with both T2DM and chronic periodontitis  | POSSIBLE ASSOCIATION between T2DM and CRP, MMP-14 and TIMP-2 in gingival tissue                               |
| Vieira Riberto et al. 2011 | 20 adults with poorly controlled T2DM and chronic periodontitis, 17 adults with well-controlled T2DM and chronic periodontitis and 20 systemically healthy adults with chronic periodontitis  | Cross-sectional comparison of OPG, RANKL, IFN $\gamma$ , IL-4, IL-17 and IL-23 in GCF                                       | ELISA              | OPG, RANKL, IFN $\gamma$ , IL-17 and IL-23 were elevated in T2DM patients with chronic periodontitis as compared to systemically healthy patients with chronic periodontitis but IL-4 was significantly reduced. Patients with poorly controlled diabetes had significantly elevated IL-17 and RANKL/OPG ratios but significantly reduced IFN $\gamma$ compared to well-controlled diabetes. Otherwise, there were no differences in the levels of mediators in between the two groups with diabetes | POSSIBLE ASSOCIATION between T2DM and gingival OPG, RANKL, IFN $\gamma$ , IL-17, IL-23 and IL-4               |

Table 2. (continued)

| Author/year            | Study population   | Study design  | Analytical methods | Main findings  | Interpretation  |
|------------------------|--|---|--------------------|--|---|
| Hiroshima et al. 2012b | 18 adults with T2DM and chronic periodontitis, 24 systemically healthy adults with chronic periodontitis and 22 healthy adult controls. Note: information regarding duration of diabetes, gender distribution, diabetic control and clinical measures of periodontitis in study groups not presented | Cross-sectional comparison of resistin in GCF           | ELISA              | Resistin was significantly elevated in both patient groups as compared to controls but not significantly elevated in the T2DM patients. Resistin levels were correlated with gingival index measurements but not with HbA1c levels   | POSSIBLE ASSOCIATION between elevated GCF resistin and chronic periodontitis  |
| Pradeep et al. 2012    | 10 adults with well-controlled T2DM and chronic periodontitis, 10 adults with chronic periodontitis and 10 healthy adult controls. Note: Gender distribution in individual study groups not presented  | Cross-sectional comparison of visfatin in GCF and serum | ELISA              | Visfatin was elevated in both serum and GCF of T2DM/chronic periodontitis and chronic periodontitis groups compared to control but was highest in the T2DM/chronic periodontitis group. Both serum and GCF visfatin correlated to BMI and clinical measures of chronic periodontitis | POSSIBLE ASSOCIATION between elevated GCF and serum visfatin and chronic periodontitis. POSSIBLE ASSOCIATION between elevated GCF and serum visfatin and T2DM |

BMI, body mass index; BOP, bleeding on probing; CAL, clinical attachment loss; CRP, C-reactive protein; CCR1-5, chemokine receptor 1-5; CXCL1, CXC chemokine ligand 1; CXCR1-5, CXC chemokine receptor 1-5; ELISA, enzyme-linked immunosorbent assay; Foxp3, forkhead box P3; GCF, gingival crevicular fluid; HbA1c, glycated haemoglobin; ICTP, type I collagen carboxy terminal telopeptide; GRO $\alpha$ , growth-related oncogene  $\alpha$ ; IFN $\gamma$ , interferon  $\gamma$ ; IHC, immunohistochemistry; IFMA, immunofluorometric assay; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; OPG, osteoprotegerin; PAI-2, plasminogen activator inhibitor-2; PDGF-AB, platelet-derived growth factor AB; PGE $_2$ , prostaglandin E $_2$ ; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; RANKL, Receptor activator of nuclear factor-kappa B ligand; RIA, radioimmunoassay; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; sICAM, soluble intercellular adhesion molecule; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TIMP-1, TIMP metalloproteinase inhibitor 1; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ ; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor; WB, Western blot.

possible influence of diabetes on the mediators could not be identified (Iwamoto et al. 2001, 2003, Talbert et al. 2006, O'Connell et al. 2008, Aspriello et al. 2011, Passoja et al. 2011), lacked information about the clinical periodontal status of the subject groups (Araya et al. 2003, Gomes et al. 2006, Shin et al. 2010) or compared groups with different clinical indices of periodontitis (Bulut et al. 2001, Safkan-Seppala et al. 2006, Correa et al. 2008, Matsumoto et al. 2009, Venza et al. 2010, Surdacka et al. 2011). It is possible to assume that altered local, rather than systemic, mediators may have a more important role in periodontitis; however, evidence of quantitative and qualitative differences in these mediators in patients with diabetes is very inconsistent (Table 2). The literature on cytokines in T1DM patients with periodontitis is very limited and the elevation of IL-1 $\beta$  and PGE $_2$  in GCF from T1DM patients as compared to systemically healthy controls with similar levels of periodontitis is the only consistent finding (Salvi et al. 1997a, 2010). Some studies report altered cytokine levels in the GCF, saliva and/or gingival tissue of T2DM patients with chronic periodontitis as compared to systemically healthy chronic periodontitis patients (Kurtis et al. 1999, Unlu et al. 2003, Engbretson et al. 2006, Duarte et al. 2007a, 2011, Cole et al. 2008, Andriankaja et al. 2009, Ross et al. 2010, Santos et al. 2010b, Vieira Ribeiro et al. 2011), but many other studies found no such influence (Cutler et al. 1999, Collin et al. 2000, Navarro-Sanchez et al. 2007, Costa et al. 2010). Also, many of the individual changes reported have not been replicated by independent investigations. Perhaps, the most consistent findings have been associations between elevated levels of IL-1 $\beta$  (Duarte et al. 2007a, Kardesler et al. 2008, Andriankaja et al. 2009) and IL-6 (Kurtis et al. 1999, Duarte et al. 2007a, Cole et al. 2008, Andriankaja et al. 2009, Ross et al. 2010, Kardesler et al. 2011) and T2DM in patients with chronic periodontitis. Importantly, a number of other mediator changes are noted in models of periodontitis in diabetic animals: these include elevated pro-inflammatory cytokines such as IFN $\gamma$  and IL-1 $\beta$  as well as elevated chemokines such as macrophage inhibitory protein-2

(MIP-2) and monocyte chemotactic protein-1 (MCP-1) and blocking experiments with anti-TNF- $\alpha$  antibodies demonstrate these changes may be secondary to the effects of TNF- $\alpha$  (Naguib et al. 2004, Pacios et al. 2012). Interestingly, increased levels of IL-1 $\beta$  and platelet-derived growth factor-B (PDGFB) in periodontitis were ameliorated by diabetes in a rat model (Doxey et al. 1998). Also, MCP-1 expression was enhanced in the gingival tissues of diabetic rats (Sakallioglu et al. 2008). A number of studies of animal models have highlighted the importance of TNF- $\alpha$  in possibly prolonging the immune response to plaque bacteria in both T1DM and T2DM and thereby promoting periodontitis in these conditions (Lalla et al. 2000a,b, Graves et al. 2004, Naguib et al. 2004, Liu et al. 2006a,b, Watanabe et al. 2008, Pacios et al. 2012). The role of TNF- $\alpha$  (and IL-6) in the enhanced immune responses to periodontal bacteria in diabetic mouse models has also been highlighted (Graves et al. 2005, Nishihara et al. 2009, Takano et al. 2010). However, evidence for any association between levels of TNF- $\alpha$  in oral fluids or gingival tissues and T2DM in chronic periodontitis is inconsistent (Duarte et al. 2007b, Navarro-Sanchez et al. 2007, Ross et al. 2010, Santos et al. 2010b).

The majority of studies have focused on single or a limited number of mediators and most have investigated pro-inflammatory cytokines (Table 2). There is a possible role for T2DM in modulating the oral levels of receptor activator of nuclear factor-kappa B ligand (RANKL)/osteoprotegerin (OPG) in chronic periodontitis (Santos et al. 2010a, Vieira Ribeiro et al. 2011). With the exception of IL-8 (CXCL8), few studies have investigated the role of chemokines. Similarly, there are few substantial reports of studies investigating growth factors, soluble adhesion molecules and MMPs, all which have direct or indirect immunoregulatory potential. Although some studies have studied multiple mediators simultaneously in the same samples (Surdacka et al. 2011, Vieira Ribeiro et al. 2011), there are only isolated reports of multiplex (Lalla et al. 2006) or proteomic (Kido et al. 2012) analysis of the oral immune response in patients with diabetes and co-existing periodontal disease.

Hyperglycaemia can lead to dysregulation of immune responses through direct effects on immune cell function as described in the sections below. Thus, the effect of diabetic control on cytokines in chronic periodontitis patients is of great interest. Indeed, GCF IL-1 $\beta$  (Collin et al. 2000, Engebretson et al. 2004) and salivary IL-6 were found to be correlated with HbA1c (Costa et al. 2010), and GCF OPG and RANKL associated with poor glycaemic control in T2DM in patients with chronic periodontitis; these data are consistent with previously described associations of these mediators with T2DM in periodontal disease.

Conclusions from cross-sectional studies which suggest associations between mediators and periodontitis in diabetes would be strengthened if such findings could be replicated in longitudinal studies; this has been approached in studies of the effect of periodontal treatment on mediators, and although these studies demonstrate improvement in clinical measures of periodontitis in chronic periodontitis patients irrespective of diabetes status, there is no consensus with respect to post-treatment changes in mediators. Also, in an experimental gingivitis model, IL-1 $\beta$  was elevated in a cohort of patients with T1DM as compared to a healthy control group after 7–21 days whilst IL-1 $\beta$  levels increased later in the control group (14–21 days) (Salvi et al. 2010). Also, MMP-9 levels were elevated in experimental gingivitis at 7–14 days in T1DM patients as compared to healthy controls (Salvi et al. 2010).

A limited number of studies have investigated the role of adipokines in periodontal disease and diabetes. One study found no effect of T2DM on serum leptin and adiponectin in chronic periodontitis (Kardesler et al. 2010b). In another study, serum adiponectin was elevated in T1DM patients with chronic periodontitis as compared to systemically healthy patients with periodontitis (Lalla et al. 2006). There has only been a single study of resistin in diabetes patients with periodontitis but no effect of T2DM was revealed (Hiroshima et al. 2012a). Visfatin (pre-B-cell colony enhancing factor) has also been assessed in a single study which suggested a possible association

between visfatin and T2DM in chronic periodontitis patients (Pradeep et al. 2012).

In summary, there is good evidence for elevated IL-1 $\beta$ , IL-6 and RANKL/OPG ratios in patients with diabetes and periodontitis as compared to patients with periodontitis alone as well as a quantitative relationship between these cytokines and glycaemic control. The majority of studies relate to T2DM but changes in IL-1 $\beta$  may be important in T1DM also. Substantial evidence from animal studies supports a role for TNF- $\alpha$  in periodontitis in animal models of diabetes (both T1DM and T2DM) but evidence from clinical studies of TNF- $\alpha$  is inconclusive.

#### Immune cell function

There is evidence that monocytes from patients with T1DM have a hyper-inflammatory phenotype and the finding that these cells respond to LPS from periodontal bacteria to produce significantly higher levels of IL-1 $\beta$ , TNF- $\alpha$  and PGE<sub>2</sub> than cells from patients without diabetes has led to the suggestion that this might be one pathway relevant to periodontal pathogenesis in diabetes (Salvi et al. 1997a,b). However, these findings were not corroborated in studies of whole blood cell cultures from T1DM patients with aggressive periodontitis (Araya et al. 2003).

The role of neutrophils in the pathogenesis of periodontitis in diabetic patients has been analysed directly using numerous assays of neutrophil function. Although the dogma is that neutrophil function in periodontitis and diabetes is defective, the evidence obtained has been variable likely due to the variety of assays employed and the technical challenges in quantifying neutrophil function *ex vivo* (Engebretson et al. 2006, Chapple & Matthews 2007). Also, these studies have without exception investigated the activity of peripheral and not gingival neutrophils. Importantly, levels of the neutrophil-derived enzyme  $\beta$ -glucuronidase and the neutrophil chemotactic factor IL-8 are depressed in chronic periodontitis patients with T2DM as compared to systemically healthy chronic periodontitis patients which might suggest compromised gingival neutrophil-mediated immune responses in diabetes (Engebretson et al. 2006).

However, other studies of IL-8 expression in gingival tissue and in GCF did not replicate these findings (Duarte et al. 2007a, Ross et al. 2010, Salvi et al. 2010). Studies in diabetic rats have provided evidence of compromised gingival neutrophil function in vivo and in vitro (Ramamurthy et al. 1979, Golub et al. 1982) and studies in diabetic mice also suggest changes in gingival neutrophil function (Gyurko et al. 2006, Sima et al. 2010).

Recent evidence suggests that T-cells, which accumulate in insulin sensitive tissues, are important in metabolic disturbances associated with obesity and diabetes, possibly through their ability to regulate macrophage function (Feuerer et al. 2009, Ilan et al. 2010). T-cell subsets are diverse and plastic and their function is determined by the local cytokine milieu. However, although there is a substantial role for altered balance of Th1/Th2 cells in the pathogenesis and progression of periodontitis, there is no direct information about the role of specific T-cell subsets in periodontitis in patients with diabetes. Significantly, Th17 and Th1 cells have a role in regulation of bone resorption and are a source of cytokines such as RANKL and IL-17, which are elevated in periodontitis patients with diabetes (Santos et al. 2010a, b, Vieira Ribeiro et al. 2011); the role of these T-cell subsets in periodontal pathogenesis in diabetic patients is therefore worthy of investigation. In this regard, there is preliminary evidence for an association between glycaemic control and IL-4 (Th1 cytokine) and IL-17 levels in GCF from periodontitis patients with diabetes (Santos et al. 2010b, Vieira Ribeiro et al. 2011) and an immunohistochemical study recently provided evidence for elevated Th17 and T<sub>reg</sub> cells in patients with poorly controlled T2DM and periodontitis as compared to patients with periodontitis alone (Duarte et al. 2011).

In summary, evidence for a role of altered monocyte and T-cell function in diabetic patients with periodontitis is limited. Clinical and animal model studies provide some evidence for aberrant neutrophil function in diabetes and periodontitis, but the complexity of neutrophil functions and the diversity of experimental approaches make identification of

precise functional changes and their relationship to pathogenesis difficult.

#### Hyperglycaemia and cellular stress

The enhanced susceptibility to periodontal disease in diabetes appears to be primarily driven by an altered host response to the bacterial challenge. A key precipitating factor is the hyperglycaemia that characterizes diabetes, which can affect the periodontium in a manner similar to its well-described effects on several other organs. Indeed, hyperglycaemia has been shown to contribute to the development and progression of other diabetic complications by exerting stress on multiple cell types critical to the pathogenesis of these complications (Brownlee et al. 1988).

In the setting of diabetes-associated periodontitis, it was proposed early on that impairment of neutrophil adherence, chemotaxis and phagocytosis may facilitate bacterial persistence and increase periodontal destruction (Manouchehr-Pour et al. 1981a,b). A more recent study showed a positive correlation between severity of periodontitis and level of glycaemic control, and demonstrated neutrophil priming in moderately and poorly controlled diabetic patients, caused by increased levels and activity of protein kinase C (Karima et al. 2005). Again, as suggested from the evidence reviewed in the section above, it appears that distinct aspects of neutrophil function may be differentially affected in diabetes.

In addition, hyperglycaemia may lead to oxidative stress via a number of pathways with subsequent effects on inflammatory responses, but their role in periodontal pathogenesis remains largely obscure (Graves & Kayal 2008). For example, it is known that reactive oxygen species stimulate pro-inflammatory cytokine production through activation of intracellular signalling pathways such as MAP kinase, NF- $\kappa$ B and the NALP3 inflammasome (Graves & Kayal 2008, Martinon 2010). Reactive oxygen species also have more wide ranging effects including effects on bone formation and recently revealed pathways involving the interaction of reactive oxygen species, Wnt signalling and activation of FoxO transcription factors in the regulation of osteoblast activity suggest another novel pathway which

may link periodontitis and diabetes (Almeida et al. 2011, Galli et al. 2011). Hyperleptinaemia, a condition also associated with diabetes and obesity, can also promote oxidative stress which may exacerbate the effect of hyperglycaemia in promoting a pro-inflammatory state (Bullon et al. 2009).

Finally, a priming effect on monocytes, key cells in the hyper-inflammatory cytokine response described above, has been documented in a number of studies (Yalda et al. 1994, Salvi et al. 1997a,c). Effects on other relevant cell types have also been reported, such as decreased collagen production and increased collagenolytic activity by gingival and periodontal ligament fibroblasts (Ramamurthy & Golub 1983, Sasaki et al. 1992, Yu et al. 2012) and a hyper-inflammatory response by oral epithelial cells (Amir et al. 2011).

In summary, the hyperglycaemic state in diabetes has a number of effects on cellular function relevant to the pathogenesis of periodontitis, but the relative contribution of these individual observations remains to be fully determined.

#### Hyperglycaemia, advanced glycation end-products (AGEs) and their receptor RAGE

Hyperglycaemia has both acute and chronic effects. An important chronic effect involves the irreversible non-enzymatic glycation of proteins and lipids leading to the formation of AGEs. AGEs can lead to cellular stress by exerting pro-inflammatory/oxidant effects directly, or through interaction with cell-surface receptors. Expression of AGEs, along with markers of oxidant stress, was demonstrated for the first time in gingival tissues of diabetic patients with periodontitis by Schmidt and colleagues (Schmidt et al. 1996). Subsequently, AGE proteins were found in saliva of diabetic patients and were linked to dental plaque levels (Yoon et al. 2004), and AGE levels in serum were shown to be significantly associated with the extent of periodontitis in type 2 individuals (Takeda et al. 2006).

The first attempt to explore the most proximal changes induced in the periodontium by diabetes, focused on the role of the receptor for advanced glycation end-products (RAGE) (Lalla

et al. 1998b, 2000a). RAGE is a multi-ligand signalling receptor and member of the immunoglobulin superfamily of cell-surface molecules (Schmidt et al. 1992). RAGE expression is increased in diabetes and its activation through ligand interaction has an established role in the development and progression of other diabetic complications (Yan et al. 2009). In a model of oral infection and diabetes in mice, *P. gingivalis*-induced alveolar bone loss was increased in diabetic animals compared to non-diabetic controls and was accompanied by enhanced expression of RAGE, inflammatory AGEs and tissue destructive MMPs in the gingival tissues (Lalla et al. 1998a). Further, treatment with soluble RAGE (sRAGE), the extracellular ligand-binding domain of RAGE which antagonizes interaction of ligands with the whole receptor, decreased levels of TNF- $\alpha$ , IL-6 and MMPs in gingival tissues and suppressed alveolar bone loss in a dose-dependent manner in diabetic animals (Lalla et al. 2000b). Importantly, the beneficial effects of RAGE blockade were paralleled by suppressed expression of the receptor and its ligands in gingival tissues and were independent of the level of glycaemia. Increased RAGE expression was subsequently reported in other experimental models of diabetes-associated periodontitis (Chang et al. 2012a,b, Claudino et al. 2012) and in gingival tissues of diabetic individuals with periodontitis, and its expression was correlated to that of NF- $\kappa$ B (Katz et al. 2005, Abbass et al. 2012, Yu et al. 2012). These findings demonstrated that AGE-RAGE interaction may lead to the exaggerated inflammatory response and periodontal tissue destruction seen in diabetes.

Importantly, and as shown in studies of excisional wounds in diabetic mice, RAGE may contribute to impaired repair following destruction, as inhibition of RAGE signalling enhanced the rate of wound closure and repair and down-regulated MMP activity (Goova et al., 2001). The contribution of RAGE and its interaction with the AGE ligand carboxymethyllysine (CML)-albumin have been reported in delayed bone healing (in the absence of infection) using osteoblast cultures and craniotomy defects in mice (Santana et al. 2003). Using the

same experimental approach, the apoptotic effect of CML-collagen on osteoblasts was shown to be mediated through RAGE. Increased p38, JNK, caspase 3 and caspase 8 activity were all involved in the process (Alikhani et al. 2007).

AGEs can also interact with toll-like receptors. Increased expression of TLR2, TLR4 and TLR9 in gingival tissues of periodontitis patients with diabetes was observed by immunofluorescence when compared to periodontitis subjects without diabetes (Rojo-Botello et al. 2012). TLR4 has been shown to have similar pro-inflammatory responses to RAGE in diabetes (Velooso et al. 2011), and the role of TLRs in diabetes-associated periodontitis warrants further investigation.

Taken together, hyperglycaemia in diabetes drives the irreversible formation of AGEs that can have direct pro-inflammatory and pro-oxidant effects on cells. Importantly, when AGEs bind to their signalling receptor RAGE, cellular phenotype and function are critically impacted, and enhanced inflammation, oxidative stress [both of which can further drive AGE formation (Anderson et al. 1999, Giacco & Brownlee 2010)] and impaired tissue repair ensues. Periodontal infection further potentiates this vicious cycle in the susceptible diabetic host leading to accelerated and more severe periodontal destruction.

#### **Hyperglycaemia and alveolar bone homeostasis**

A number of studies focusing on osteoclastogenesis-related factors have reported elevated levels of RANKL in diabetes-associated periodontal tissues (Mahamed et al. 2005, Duarte et al. 2007b, Lappin et al. 2009). Studies in gingival crevicular fluid demonstrated that RANKL and the RANKL to OPG ratio are higher in poorly controlled diabetic patients with periodontitis compared to well-controlled or non-diabetic subjects with similar periodontal status (Santos et al. 2010a, Vieira Ribeiro et al. 2011). These studies have proposed that hyperglycaemia may modulate the RANKL/OPG ratio in periodontal tissues and this would, at least in part, explain enhanced alveolar bone destruction in diabetes. Interestingly, the AGE-RAGE axis has also been

suggested to contribute to osteoclastogenesis via increased RANKL expression and OPG down-regulation in various cell types (Ding et al. 2006, Yoshida et al. 2009).

Studies of *P. gingivalis* inoculation using the calvarial defect or the ligature-induced alveolar bone loss models in rodents suggested that diabetes may impair bone formation following resorption through increased apoptosis of bone-lining cells (He et al. 2004, Liu et al. 2006b). In the calvarial model, diabetic mice also displayed increased fibroblast apoptosis following *P. gingivalis* injury (Liu et al. 2004). The higher rate of fibroblast apoptosis could be explained by enhanced levels of activated caspase-3 and healing was significantly improved by a caspase inhibitor (Al-Mashat et al. 2006) or by anti-TNF- $\alpha$  treatment (Liu et al. 2006a). These results were confirmed in diabetic mice using intraoral wounds where, in addition, increased translocation of the pro-apoptotic transcription factor FoxO1 was found (Desta et al. 2010, Siqueira et al. 2010). More recently, TNF- $\alpha$  inhibition in diabetic rats with ligature-induced periodontitis was shown to restore the bone coupling process, reduce apoptosis and increase the proliferation of bone-lining cells and therefore the capacity of the animals to form new bone (Pacios et al. 2012). In this study, impaired expression of growth factors that control proliferation, differentiation or apoptosis of osteoblasts and their precursors was seen. Also, enhancement of gingival IL-17, IL-23 and RANKL/OPG ratios in periodontitis in murine T1DM has been reported (Silva et al. 2012a,b); these cytokines influence osteoclastogenesis and the duration of inflammatory responses.

In summary, evidence from clinical studies of mediators of bone resorption (e.g. RANKL/OPG) as well as relevant animal models strongly suggest that altered alveolar bone homeostasis is an important pathway in the pathogenesis of periodontitis in diabetes and there is evidence that this pathway may be important in both T1DM and T2DM.

#### **The impact of periodontal disease on diabetes**

Most of the published literature that has investigated the mechanistic links

between periodontal disease and diabetes has focused on the impact of diabetes on periodontal pathogenesis; there is relatively little mechanistic evidence available regarding the potential impact of periodontitis on the disease processes of diabetes. Most studies have been clinical investigations which do appear to clearly show an impact of periodontal inflammation on the diabetic state and/or diabetes complications. For example, periodontitis has been associated with poorer glycaemic control in people with diabetes (Taylor et al. 1996), increased risk of diabetic complications (Saremi et al. 2005, Shultis et al. 2007) and increases in HbA1c in people without diabetes (potentially suggesting that periodontitis may increase incident diabetes) (Demmer et al. 2010). There is also evidence from recent systematic reviews that treatment of periodontitis is associated with improved glycaemic control and reductions in HbA1c of around 0.4% (Simpson et al. 2010). The presumption is that resolution of periodontal inflammation following therapy results in reduced levels of inflammatory mediators locally, and therefore reduced levels of these mediators in the circulation. Key mediators in this process could be IL-6 and TNF- $\alpha$ , which are inducers of acute phase proteins such as CRP, and both have been shown to impair intracellular insulin signalling (Hotamisligil 2000, Rotter et al. 2003). Reductions in the levels of these mediators as a result of periodontal treatment could theoretically, therefore, lead to improved diabetes control. However, these hypotheses remain to be formally tested. The few mechanistic studies that were identified that investigated the impact of periodontitis on diabetes (both microbial and inflammatory factors) are considered in the following sections.

#### Microbial factors

Whereas the question as to whether the diabetic state influences the periodontal microbiota (as reviewed earlier in this Supplement article) has received much attention, the converse question, that is, whether the periodontal microbiota has any direct impact on diabetes or glycaemic control, has barely been addressed.

One study of 30 Japanese adults with chronic periodontitis and type 2

diabetes considered this issue (Makiura et al. 2008). All patients received non-surgical periodontal therapy, and subgingival plaque samples were collected at baseline and up to 12 months following therapy. It was observed that post-treatment, *P. gingivalis* was detected more frequently in those who had increased HbA1c values compared to those with decreased values relative to baseline, and more specifically, *P. gingivalis* with type II fimbriae was detected only in patients with an increase in HbA1c. The authors postulated that glycaemic control in patients with periodontitis and diabetes is potentially influenced by the persistence of *P. gingivalis*, particularly clones with type II fimbriae, following treatment (Makiura et al. 2008). It has also been reported in in vitro studies that cytokine induction (specifically IL-1 $\beta$ , IL-8, IL-12 and TNF- $\alpha$ ) by *P. gingivalis* with type II fimbriae is greater than that induced by *P. gingivalis* with type I fimbriae (Sugano et al. 2004), and in animal studies that *P. gingivalis* inoculation can lead to elevated serum levels of TNF- $\alpha$  and IL-6 (Nishihara et al. 2009). Clearly, more research is required, integrating both microbial assessment and analysis of serum cytokine levels. At the present time, however, there is no strong evidence to suggest that the periodontal microbiota has any direct impact on the diabetic state or on glycaemic control.

#### Inflammatory factors

Chronic dysregulation of the peripheral cytokine pool is a feature of pre-diabetic conditions as well as both T1DM and T2DM and is now considered a central pathogenic factor in diabetes (Kolb & Mandrup-Poulsen 2010). Therefore, it is certainly possible that periodontal inflammation may impact upon the diabetic state. It is known that circulating mediators (CRP, TNF- $\alpha$  and IL-6) may be elevated in periodontal diseases and correlated with clinical periodontal parameters (Bretz et al. 2005, Engbretson et al. 2007, Paraskevas et al. 2008). Nevertheless, in cross-sectional studies, a lack of elevation of serum TNF- $\alpha$  and CRP in T2DM patients with periodontitis as compared to controls is a consistent finding (Takeida et al. 2006, Dag et al. 2009, Chen et al. 2010, Kardesler et al. 2010b).

Longitudinal studies may be more revealing, for example, increases in HbA1c levels over a 5 year period in patients with periodontitis were highest in individuals with high levels of CRP suggesting an interaction between periodontitis and systemic inflammation (Demmer et al. 2010).

Oxidative stress is inextricably linked with chronic inflammation (Chapple et al. 1996, Graves & Kayal 2008). Biochemical markers of systemic oxidative stress are elevated in both diabetes and periodontitis (Bullon et al. 2009) and are positively correlated with CRP in periodontitis patients as compared to healthy controls (D'Aiuto et al. 2010). It has been proposed that hyperactive neutrophils, possibly activated in the periodontium, may be an important source of reactive oxygen species which lead to an activation of pro-inflammatory pathways and promote insulin resistance in patients with periodontitis and diabetes (Allen et al. 2011).

Increased oxidative stress may also result in elevated lipid peroxidation which in turn may have a pro-inflammatory effect; a recent study has demonstrated higher levels of GCF markers of lipid peroxidation in diabetes patients which correlated with clinical parameters of periodontitis and levels of inflammatory mediators (Bastos et al. 2012).

In summary, there is little evidence for a role of changes in the circulating levels of inflammatory mediators (CRP, TNF- $\alpha$  and IL-6) in patients with co-existing periodontitis and diabetes but more research is needed to investigate this further. Oxidative stress in diabetes may activate pro-inflammatory pathways in the periodontium which could influence diabetes, but there is, as yet, little direct evidence for this.

#### Summary and future directions

To date, most models proposed to explain the clinical and pathogenic relationships between diabetes and periodontal disease have supported the concept of a '2-way' interaction between these disorders (Grossi & Genco 1998, Mealey & Oates 2006, Santacroce et al. 2010, Lalla & Papapanou 2011, Preshaw et al. 2012). These models have provided a role for pro-inflammatory mediators (cytokines, AGEs,

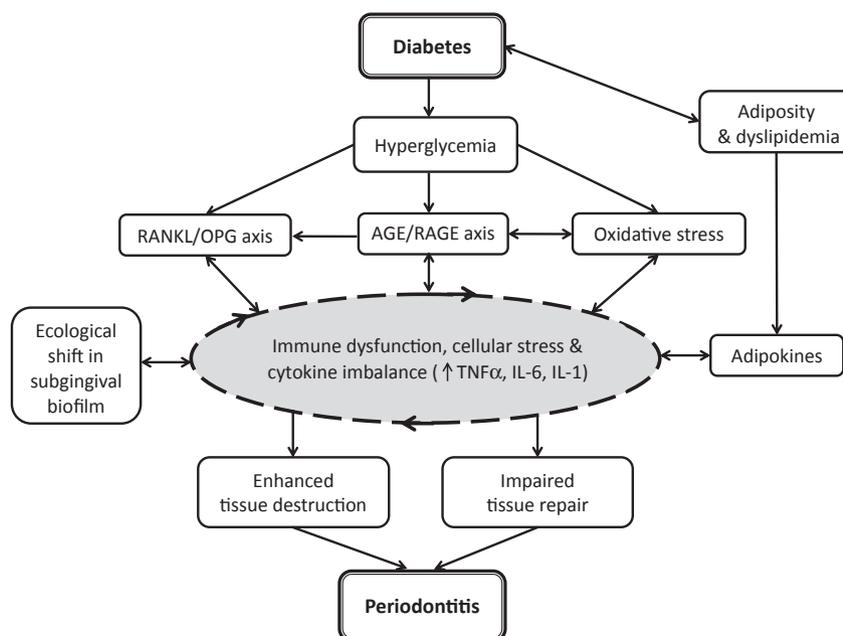
oxidative stress, etc.) in determining periodontal disease susceptibility and progression in subjects with diabetes by leading directly to inflammation in central and peripheral tissues and indirectly to compromised immune cell function and tissue homeostasis and hence increased susceptibility to periodontal destruction.

However, most investigations to date have been small-scale clinical studies which have focused on a limited number of mediators and many are inconclusive because of limitations in study design. Nevertheless, there are promising data on certain mediators such as IL-1 $\beta$ , IL-6, TNF- $\alpha$  and emerging data on RANKL and OPG; these are likely to have a central role in the pathogenesis of periodontitis in diabetic patients

(Fig. 1). A major challenge will be to gain a holistic understanding of the role of the numerous molecules with action relevant to disease pathogenesis. Complex interactions between individual mediators and emergent pathways, for example, synergy in cytokine signalling, will not be apparent from simple disease association studies of a limited number of molecules (Preshaw & Taylor 2011). However, we have not yet exploited the power of multiplex analysis of mediators or indeed global molecular analysis (e.g. proteomics) to address these issues.

Diabetes is the pathological consequence of a number of physiological changes and the resulting metabolic dysregulation, hyperglycaemia and chronic inflammation potentially

impact on tissue integrity and repair (Fig. 1). Although there is now considerable data on the relationship of these processes to periodontitis (Bullon et al. 2009), we are not yet able to bring this together in a unified view of cross-susceptibility between periodontitis, diabetes and diabetes-associated disorders such as obesity. Schematics such as that presented in Fig. 1 provide a helpful perspective, but need further supportive evidence. For example our understanding of the relationship, if any, of dyslipidaemia and adipokines to periodontal disease is insubstantial. There is a need for clinical-based studies of molecular and cellular mediators which underpin susceptibility to periodontitis, possibly via prospective studies of periodontal disease in these susceptibility groups



*Fig. 1.* Network of potential mechanisms involved in the pathogenesis of periodontitis in diabetes. The hyperglycaemic state that characterizes diabetes has several deleterious effects. It drives the formation of irreversible advanced glycation end-products (AGEs) and the expression of their chief signalling receptor RAGE. This interaction, in turn, leads to immune cell dysfunction, alters phenotype and function of other key cells in the periodontium, and contributes to cytokine imbalance with increased generation of certain pro-inflammatory cytokines. Hyperglycaemia also contributes to enhanced levels of reactive oxygen species (ROS) and a state of oxidative stress, both directly and indirectly through the AGE/RAGE axis, promoting quantitative and qualitative shifts in cytokine profiles. Finally, hyperglycaemia modulates the RANKL/OPG ratio, again directly and indirectly via the AGE/RAGE axis, tipping the balance towards enhanced inflammation and destruction. All the above, complemented by the effects of ecological shifts in the subgingival biofilm and the circulating adipokines generated due to diabetes-associated adiposity and dyslipidaemia, drive this vicious cycle of cellular dysfunction and inflammation. The end result is a loss of equilibrium where enhanced periodontal tissue destruction and impaired repair ensue, leading to accelerated and severe periodontitis. Importantly, as shown, several of the associations between the different elements in the figure are bidirectional, for example, the pro-inflammatory state further feeds the generation of AGEs, ROS, and adipokines, increases the RANKL/OPG ratio and helps pathogenic subgingival bacteria thrive. It is also important to note that a) the amount and quality of evidence supporting the various pathways in this figure varies, and b) although the goal is to depict the major mechanisms and networks described in the literature, other pathways and links among the various elements shown do exist, but cannot easily be demonstrated in a single schematic. Finally, the processes outlined are potentially modified by several other factors, such as genetics, age, smoking, stress, all of which may contribute significantly to inter-individual variations in disease experience.

(Genco et al. 2005, Pischon et al. 2007).

Animal models have the advantage of being able to combine analysis at the molecular, cellular and anatomical level and allow for longitudinal observations. To fully appreciate the significance of such model work, it will be important to integrate experimental findings with clinical studies.

Inter-individual differences in disease experience are another important dimension to a full understanding of these associations; a number of important correlations between clinical and biological parameters have emerged from cross-sectional clinical studies, but there have been few biological studies to endorse the relevance of these findings.

A shared genetic susceptibility has been considered one possible contributing factor to the alternative model of cross-susceptibility between periodontitis and diabetes although there is no substantial evidence to support this notion either (Soskolne & Klinger 2001, Preshaw et al. 2007). This is important because the considerable inter-individual variation in disease experience observed clinically is likely influenced by numerous genetic, epigenetic and environmental factors (Preshaw et al. 2007, 2012).

In summary, more research is needed to reinforce extant information on the importance of the limited number of factors studied to date and provide data on additional, potentially relevant mediators. The rubric for such research should shift from cross-sectional clinical studies to longitudinal studies of disease progression and treatment effects, integrated with in vitro analyses of cellular responses and holistic studies using animal models. The conclusions of this review suggest that more detailed studies of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, RANKL and OPG are all warranted, integrated with studies of pro-inflammatory pathways activated by AGE/RAGE and possibly oxidative stress particularly in the context of bone and tissue turnover and repair. Delineating the role of the cellular elements of inflammation, for example, monocytes/macrophage, neutrophils and T-cells is more challenging but equally important. The appropriate design of

future studies is critical for a more definitive understanding of the complex processes involved.

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**Clinical Relevance**

*Scientific rationale for the study:* The purpose was to review the evidence for the pathogenic processes which underpin the clinical relationship between periodontitis and diabetes.

*Principal findings:* Cytokines such as IL-1 $\beta$ , IL-6, TNF- $\alpha$ , OPG and

RANKL and the AGE-RAGE axis are likely to be important in tissue destruction and compromised repair in these disorders but our knowledge is incomplete.

*Practical implications:* The development of appropriate treatment strategies for periodontitis in patients

with diabetes relies on knowledge of the pathogenesis, but there is a need for substantial longitudinal clinical studies integrated with studies of animal models and cells/tissues *in vitro*.