

# 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

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

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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, Hinto 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 CPITN 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	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	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
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>	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
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

CPITN, 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), 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
Engelbreton 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
Engelbreton 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 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, CCR1-5, 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. 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 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	periodontitis group but not in the chronic periodontitis group. 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 Ribeiro 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, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, CXCL18, CXCL19, CXCL20, CXCL21, CXCL22, CXCL23, CXCL24, CXCL25, CXCL26, CXCL27, CXCL28, CXCL29, CXCL30, CXCL31, CXCL32, CXCL33, CXCL34, CXCL35, CXCL36, CXCL37, CXCL38, CXCL39, CXCL40, CXCL41, CXCL42, CXCL43, CXCL44, CXCL45, CXCL46, CXCL47, CXCL48, CXCL49, CXCL50, CXCL51, CXCL52, CXCL53, CXCL54, CXCL55, CXCL56, CXCL57, CXCL58, CXCL59, CXCL60, CXCL61, CXCL62, CXCL63, CXCL64, CXCL65, CXCL66, CXCL67, CXCL68, CXCL69, CXCL70, CXCL71, CXCL72, CXCL73, CXCL74, CXCL75, CXCL76, CXCL77, CXCL78, CXCL79, CXCL80, CXCL81, CXCL82, CXCL83, CXCL84, CXCL85, CXCL86, CXCL87, CXCL88, CXCL89, CXCL90, CXCL91, CXCL92, CXCL93, CXCL94, CXCL95, CXCL96, CXCL97, CXCL98, CXCL99, CXCL100, CXCL101, CXCL102, CXCL103, CXCL104, CXCL105, CXCL106, CXCL107, CXCL108, 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CXCL997, CXCL998, CXCL999, CXCL1000.

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, Saffkan-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<sub>2</sub> 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 (Sakallioğlu 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 (Feurerer 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, Martignon 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,

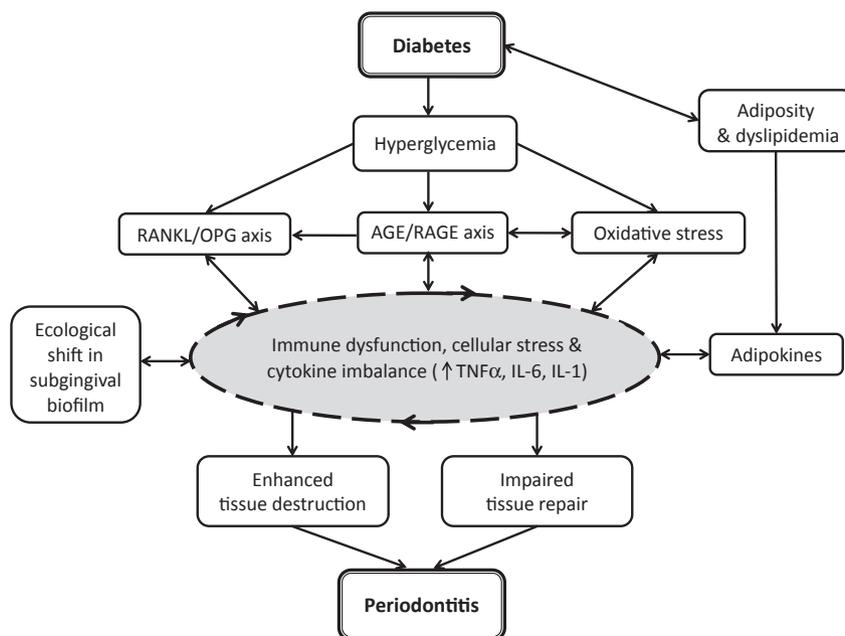
oxidative stress, etc.) in determining periodontal disease susceptibility and progression in subjects with diabetes by leading directly to inflammation in central and peripheral tissues and indirectly to compromised immune cell function and tissue homeostasis and hence increased susceptibility to periodontal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

## References

- Abbass, M. M., Korany, N. S., Salama, A. H., Dmytryk, J. J. & Safiejko-Mroccka, B. (2012) The relationship between receptor for advanced glycation end products expression and the severity of periodontal disease in the gingiva of diabetic and non diabetic periodontitis patients. *Archives of Oral Biol* **57**, 1342–1354.
- Alikhani, M., Alikhani, Z., Boyd, C., MacLellan, C. M., Raptis, M., Liu, R., Pischon, N., Trackman, P. C., Gerstenfeld, L. & Graves, D. T. (2007) Advanced glycation end products stimulate osteoblast apoptosis via the MAP kinase and cytosolic apoptotic pathways. *Bone* **40**, 345–353.
- Allen, E. M., Matthews, J. B., DJ, O. H., Griffiths, H. R. & Chapple, I. L. (2011) Oxidative and inflammatory status in Type 2 diabetes patients with periodontitis. *Journal of Clinical Periodontology* **38**, 894–901.
- Al-Mashat, H. A., Kandru, S., Liu, R., Behl, Y., Desta, T. & Graves, D. T. (2006) Diabetes enhances mRNA levels of proapoptotic genes and caspase activity, which contribute to impaired healing. *Diabetes* **55**, 487–495.
- Almeida, M., Han, L., Ambrogini, E., Weinstein, R. S. & Manolagas, S. C. (2011) Glucocorticoids and tumor necrosis factor alpha increase oxidative stress and suppress Wnt protein signaling in osteoblasts. *Journal of Biological Chemistry* **286**, 44326–44335.
- Amir, J., Waite, M., Tobler, J., Catalfamo, D. L., Koutouzis, T., Katz, J. & Wallet, S. M. (2011) The role of hyperglycemia in mechanisms of exacerbated inflammatory responses within the oral cavity. *Cellular Immunology* **272**, 45–52.
- Anderson, M. M., Requena, J. R., Crowley, J. R., Thorpe, S. R. & Heinecke, J. W. (1999) The myeloperoxidase system of human phagocytes generates N-epsilon-(carboxymethyl)lysine on proteins: a mechanism for producing advanced glycation end products at sites of inflammation. *Journal of Clinical Investigation* **104**, 103–113.
- Andriankaja, O. M., Barros, S. P., Moss, K., Panagakos, F. S., DeVizio, W., Beck, J. & Offenbacher, S. (2009) Levels of serum interleukin (IL)-6 and gingival crevicular fluid of IL-1beta and prostaglandin E(2) among non-smoking subjects with gingivitis and type 2 diabetes. *Journal of Periodontology* **80**, 307–316.
- Araya, A. V., Pavez, V., Perez, C., Gonzalez, F., Columbo, A., Aguirre, A., Schiattino, I. & Aguilon, J. C. (2003) Ex vivo lipopolysaccharide (LPS)-induced TNF-alpha, IL-1beta, IL-6 and PGE2 secretion in whole blood from Type 1 diabetes mellitus patients with or without aggressive periodontitis. *European Cytokine Network* **14**, 128–133.
- Aspriello, S. D., Zizzi, A., Tirabassi, G., Buldreghini, E., Biscotti, T., Faloia, E., Stramazzotti, D., Boscaro, M. & Piemontese, M. (2011) Diabetes mellitus-associated periodontitis: differences between type 1 and type 2 diabetes mellitus. *Journal of Periodontal Research* **46**, 164–169.
- Bastos, A. S., Graves, D. T., Loureiro, A. P., Rossa Junior, C., Abdalla, D. S., Faulin Tdo, E., Olsen Camara, N., Andriankaja, O. M. & Orrico, S. R. (2012) Lipid peroxidation is associated with the severity of periodontal disease and local inflammatory markers in patients with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* **97**, E1353–1362.
- Baumann, B., Salem, H. H. & Boehm, B. O. (2012) Anti-inflammatory therapy in type 1 diabetes. *Current Diabetes Reports* **12**, 499–509.
- Bretz, W. A., Weyant, R. J., Corby, P. M., Ren, D., Weissfeld, L., Kritchevsky, S. B., Harris, T., Kurella, M., Satterfield, S., Visser, M. & Newman, A. B. (2005) Systemic inflammatory markers, periodontal diseases, and periodontal infections in an elderly population. *Journal of the American Geriatric Society* **53**, 1532–1537.
- Brownlee, M., Cerami, A. & Vlassara, H. (1988) Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *New England Journal of Medicine* **318**, 1315–1321.
- Bullon, P., Morillo, J. M., Ramirez-Tortosa, M. C., Quiles, J. L., Newman, H. N. & Battino, M. (2009) Metabolic syndrome and periodontitis: is oxidative stress a common link? *Journal of Dental Research* **88**, 503–518.
- Bulut, U., Develioglu, H., Taner, I. L. & Berker, E. (2001) Interleukin-1 beta levels in gingival crevicular fluid in type 2 diabetes mellitus and adult periodontitis. *Journal of Oral Science* **43**, 171–177.
- Campus, G., Salem, A., Uzzau, S., Baldoni, E. & Tonolo, G. (2005) Diabetes and periodontal disease: a case-control study. *Journal of Periodontology* **76**, 418–425.
- Carstensen, M., Herder, C., Kivimaki, M., Jokela, M., Roden, M., Shipley, M. J., Witte, D. R., Brunner, E. J. & Tabak, A. G. (2010) Accelerated increase in serum interleukin-1 receptor antagonist starts 6 years before diagnosis of type 2 diabetes: whitehall II prospective cohort study. *Diabetes* **59**, 1222–1227.
- Chang, P. C., Chien, L. Y., Yeo, J. F., Wang, Y. P., Chung, M. C., Chong, L. Y., Kuo, Y. P., Chen, C. H., Chiang, H. C., Ng, B. N., Lee, Q. Q., Phay, Y. K., Ng, J. R. & Erk, K. Y. (2012a) Progression of periodontal destruction and the roles of advanced glycation end-products in experimental diabetes. *Journal of Periodontology* **83**, 644–652.
- Chang, P. C., Chung, M. C., Wang, Y. P., Chien, L. Y., Lim, J. C., Liang, K., Chong, L. Y., Kuo, Y. P., Chen, C. H. & Chiang, H. C. (2012b) Patterns of diabetic periodontal wound repair: a study using micro-computed tomography and immunohistochemistry. *Journal of Periodontology* **83**, 644–652.
- Chapple, I. L. & Matthews, J. B. (2007) The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontology* **2000** **43**, 160–232.
- Chapple, I. L., Socransky, S. S., Dibart, S., Glenwright, H. D. & Matthews, J. B. (1996) Chemiluminescent assay of alkaline phosphatase in human gingival crevicular fluid: investigations with an experimental gingivitis model and studies on the source of the enzyme within crevicular fluid. *Journal of Clinical Periodontology* **23**, 587–594.
- Chen, L., Wei, B., Li, J., Liu, F., Xuan, D., Xie, B. & Zhang, J. (2010) Association of periodontal parameters with metabolic level and systemic inflammatory markers in patients with type 2 diabetes. *Journal of Periodontology* **81**, 364–371.
- Ciantar, M., Gilthorpe, M. S., Hurel, S. J., Newman, H. N., Wilson, M. & Spratt, D. A. (2005) Capnocytophaga spp. in periodontitis patients

- manifesting diabetes mellitus. *Journal of Periodontology* **76**, 194–203.
- Claudino, M., Gennaro, G., Cestari, T. M., Spadella, C. T., Garlet, G. P. & Assis, G. F. (2012) Spontaneous periodontitis development in diabetic rats involves an unrestricted expression of inflammatory cytokines and tissue destructive factors in the absence of major changes in commensal oral microbiota. *Experimental Diabetes Research* **2012**, 356841.
- Cole, C. M., Sundararaj, K. P., Leite, R. S., Nareika, A., Slate, E. H., Sanders, J. J., Lopes-Virella, M. F. & Huang, Y. (2008) A trend of increase in periodontal interleukin-6 expression across patients with neither diabetes nor periodontal disease, patients with periodontal disease alone, and patients with both diseases. *Journal of Periodontal Research* **43**, 717–722.
- Colhoun, H. M., Slaney, J. M., Rubens, M. B., Fuller, J. H., Sheiham, A. & Curtis, M. A. (2008) Antibodies to periodontal pathogens and coronary artery calcification in type 1 diabetic and nondiabetic subjects. *Journal of Periodontal Research* **43**, 103–110.
- Collin, H. L., Sorsa, T., Meurman, J. H., Niskanen, L., Salo, T., Ronka, H., Kontinen, Y. T., Koivisto, A. M. & Uusitupa, M. (2000) Salivary matrix metalloproteinase (MMP-8) levels and gelatinase (MMP-9) activities in patients with type 2 diabetes mellitus. *Journal of Periodontal Research* **35**, 259–265.
- Collin, H. L., Uusitupa, M., Niskanen, L., Kontturi-Narhi, V., Markkanen, H., Koivisto, A. M. & Meurman, J. H. (1998) Periodontal findings in elderly patients with non-insulin dependent diabetes mellitus. *Journal of Periodontology* **69**, 962–966.
- Correa, F. O., Goncalves, D., Figueredo, C. M., Gustafsson, A. & Orrico, S. R. (2008) The short-term effectiveness of non-surgical treatment in reducing levels of interleukin-1beta and proteases in gingival crevicular fluid from patients with type 2 diabetes mellitus and chronic periodontitis. *Journal of Periodontology* **79**, 2143–2150.
- Costa, P. P., Trevisan, G. L., Macedo, G. O., Palitoto, D. B., Souza, S. L., Grisi, M. F., Novaes, A. B. Jr & Taba, M. Jr (2010) Salivary interleukin-6, matrix metalloproteinase-8, and osteopontin in patients with periodontitis and diabetes. *Journal of Periodontology* **81**, 384–391.
- da Cruz, G. A., de Toledo, S., Sallum, E. A., Sallum, A. W., Ambrosano, G. M. B., de Cassia Orlandi Sardi, J., da Cruz, S. E. B. & Goncalves, R. B. (2008) Clinical and laboratory evaluations of non-surgical periodontal treatment in subjects with diabetes mellitus. *Journal of Periodontology* **79**, 1150–1157.
- Cutler, C. W., Machen, R. L., Jotwani, R. & Iacopino, A. M. (1999) Heightened gingival inflammation and attachment loss in type 2 diabetics with hyperlipidemia. *Journal of Periodontology* **70**, 1313–1321.
- Dag, A., Firat, E. T., Arikkan, S., Kadiroglu, A. K. & Kaplan, A. (2009) The effect of periodontal therapy on serum TNF-alpha and HbA1c levels in type 2 diabetic patients. *Australian Dental Journal* **54**, 17–22.
- D'Aiuto, F., Nibali, L., Parkar, M., Patel, K., Suvan, J. & Donos, N. (2010) Oxidative stress, systemic inflammation, and severe periodontitis. *Journal of Dental Research* **89**, 1241–1246.
- Dasanayake, A. P., Chhun, N., Tanner, A. C. R., Craig, R. G., Lee, M. J., Moore, A. F. & Norman, R. G. (2008) Periodontal pathogens and gestational diabetes mellitus. *Journal of Dental Research* **87**, 328–333.
- Davies, R. C., Jaedicke, K. M., Barksby, H. E., Jitprasertwong, P., Al-Shahwani, R. M., Taylor, J. J. & Preshaw, P. M. (2011) Do patients with aggressive periodontitis have evidence of diabetes? A pilot study. *Journal of Periodontal Research* **46**, 663–672.
- Demmer, R. T., Desvarieux, M., Holtfreter, B., Jacobs, D. R. Jr, Wallaschofski, H., Nauck, M., Volzke, H. & Kocher, T. (2010) Periodontal status and A1C change: longitudinal results from the study of health in Pomerania (SHIP). *Diabetes Care* **33**, 1037–1043.
- Desta, T., Li, J., Chino, T. & Graves, D. T. (2010) Altered fibroblast proliferation and apoptosis in diabetic gingival wounds. *Journal of Dental Research* **89**, 609–614.
- Ding, K. H., Wang, Z. Z., Hamrick, M. W., Deng, Z. B., Zhou, L., Kang, B., Yan, S. L., She, J. X., Stern, D. M., Isaacs, C. M. & Mi, Q. S. (2006) Disordered osteoclast formation in RAGE-deficient mouse establishes an essential role for RAGE in diabetes related bone loss. *Biochemical and Biophysical Research Communications* **340**, 1091–1097.
- Donath, M. Y. & Shoelson, S. E. (2011) Type 2 diabetes as an inflammatory disease. *Nature Reviews Immunology* **11**, 98–107.
- Doxey, D. L., Cutler, C. W. & Iacopino, A. M. (1998) Diabetes prevents periodontitis-induced increases in gingival platelet derived growth factor-B and interleukin 1-beta in a rat model. *Journal of Periodontology* **69**, 113–119.
- Duarte, P. M., de Oliveira, M. C., Tambeli, C. H., Parada, C. A., Casati, M. Z. & Nociti, F. H. Jr (2007a) Overexpression of interleukin-1beta and interleukin-6 may play an important role in periodontal breakdown in type 2 diabetic patients. *Journal of Periodontal Research* **42**, 377–381.
- Duarte, P. M., Neto, J. B., Casati, M. Z., Sallum, E. A. & Nociti, F. H. Jr (2007b) Diabetes modulates gene expression in the gingival tissues of patients with chronic periodontitis. *Oral Diseases* **13**, 594–599.
- Duarte, P. M., Santos, V. R., Dos Santos, F. A., de Lima Pereira, S. A., Rodrigues, D. B. & Nampimoga, M. H. (2011) Role of smoking and type 2 diabetes in the immunobalance of advanced chronic periodontitis. *Journal of Periodontology* **82**, 429–438.
- Ebersole, J. L., Holt, S. C., Hansard, R. & Novak, M. J. (2008) Microbiologic and immunologic characteristics of periodontal disease in Hispanic americans with type 2 diabetes. *Journal of Periodontology* **79**, 637–646.
- Engelbreton, S., Chertog, R., Nichols, A., Hey-Hadavi, J., Celenti, R. & Grbic, J. (2007) Plasma levels of tumour necrosis factor-alpha in patients with chronic periodontitis and type 2 diabetes. *Journal of Clinical Periodontology* **34**, 18–24.
- Engelbreton, S. P., Hey-Hadavi, J., Ehrhardt, F. J., Hsu, D., Celenti, R. S., Grbic, J. T. & Lamster, I. B. (2004) Gingival crevicular fluid levels of interleukin-1beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. *Journal of Periodontology* **75**, 1203–1208.
- Engelbreton, S. P., Vossughi, F., Hey-Hadavi, J., Emingil, G. & Grbic, J. T. (2006) The influence of diabetes on gingival crevicular fluid beta-glucuronidase and interleukin-8. *Journal of Clinical Periodontology* **11**, 784–790.
- Fernandez-Real, J. M. & Pickup, J. C. (2012) Innate immunity, insulin resistance and type 2 diabetes. *Diabetologia*, **55**, 273–278.
- Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A. B., Benoist, C., Shoelson, S. & Mathis, D. (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nature Medicine* **15**, 930–939.
- Galli, C., Passeri, G. & Macaluso, G. M. (2011) FoxOs, Wnts and oxidative stress-induced bone loss: new players in the periodontitis arena? *Journal of Periodontal Research* **46**, 397–406.
- Genco, R. J., Grossi, S. G., Ho, A., Nishimura, F. & Murayama, Y. (2005) A proposed model linking inflammation to obesity, diabetes, and periodontal infections. *Journal of Periodontology* **76**, 2075–2084.
- Giacco, F. & Brownlee, M. (2010) Oxidative stress and diabetic complications. *Circulation Research* **107**, 1058–1070.
- Golub, L. M., Nicoll, G. A., Iacono, V. J. & Ramamurthy, N. S. (1982) In vivo crevicular leukocyte response to a chemotactic challenge: inhibition by experimental diabetes. *Infection and Immunity* **37**, 1013–1020.
- Goova, M. T., Li, J., Kislinger, T., Qu, W., Lu, Y., Bucciarelli, L. G., Nowygrod, S., Wolf, B. M., Caliste, X., Yan, S. F., Stern, D. M. & Schmidt, A. M. (2001) Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. *American Journal of Pathology* **159**, 513–525.
- Gomes, M. A., Rodrigues, F. H., Afonso-Cardoso, S. R., Buso, A. M., Silva, A. G., Favoreto, S. Jr & Souza, M. A. (2006) Levels of immunoglobulin A1 and messenger RNA for interferon gamma and tumor necrosis factor alpha in total saliva from patients with diabetes mellitus type 2 with chronic periodontal disease. *Journal of Periodontal Research* **41**, 177–183.
- Graves, D. T., Al-Mashat, H. & Liu, R. (2004) Evidence that diabetes mellitus aggravates periodontal diseases and modifies the response to an oral pathogen in animal models. *Compendium of Continuing Education in Dentistry* **25**, 38–45.
- Graves, D. T. & Kayal, R. A. (2008) Diabetic complications and dysregulated innate immunity. *Frontiers in Bioscience* **13**, 1227–1239.
- Graves, D. T., Naguib, G., Lu, H., Leone, C., Hsue, H. & Krall, E. (2005) Inflammation is more persistent in type 1 diabetic mice. *Journal of Dental Research* **84**, 324–328.
- Grossi, S. G. & Genco, R. J. (1998) Periodontal disease and diabetes mellitus: a two-way relationship. *Annals of Periodontology* **3**, 51–61.
- Gyurko, R., Siqueira, C. C., Caldon, N., Gao, L., Kantarci, A. & Van Dyke, T. E. (2006) Chronic hyperglycemia predisposes to exaggerated inflammatory response and leukocyte dysfunction in Akita mice. *Journal of Immunology* **177**, 7250–7256.
- He, H., Liu, R., Desta, T., Leone, C., Gerstenfeld, L. C. & Graves, D. T. (2004) Diabetes causes decreased osteoclastogenesis, reduced bone formation, and enhanced apoptosis of osteoblastic cells in bacteria stimulated bone loss. *Endocrinology* **145**, 447–452.
- Hintao, J., Teanpaisan, R., Chongsuvivatwong, V., Ratarasan, C. & Dahlen, G. (2007) The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus. *Oral Microbiology and Immunology* **22**, 175–181.
- Hiroshima, Y., Bando, M., Inagaki, Y., Mihara, C., Kataoka, M., Murata, H., Shinohara, Y., Nagata, T. & Kido, J. (2012a) Resistin in gingival crevicular fluid and induction of resistin

- release by *Porphyromonas gingivalis* lipopolysaccharide in human neutrophils. *Journal of Periodontal Research* **47**, 554–562.
- Hiroshima, Y., Bando, M., Inagaki, Y., Mihara, C., Kataoka, M., Murata, H., Shinohara, Y., Nagata, T. & Kido, J. (2012b) Resistin in gingival crevicular fluid and induction of resistin release by *Porphyromonas gingivalis* lipopolysaccharide in human neutrophils. *Journal of Periodontal Research* **47**, 554–562.
- Hotamisligil, G. S. (2000) Molecular mechanisms of insulin resistance and the role of the adipocyte. *International Journal of Obesity and Related Metabolic Disorders* **24**(Suppl 4), S23–S27.
- Ilan, Y., Maron, R., Tukpah, A. M., Maioli, T. U., Murugaiyan, G., Yang, K., Wu, H. Y. & Weiner, H. L. (2010) Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. *Proceedings of the National Academy of Sciences U S A* **107**, 9765–9770.
- Iwamoto, Y., Nishimura, F., Nakagawa, M., Sugimoto, H., Shikata, K., Makino, H., Fukuda, T., Tsuji, T., Iwamoto, M. & Murayama, Y. (2001) The effect of antimicrobial periodontal treatment on circulating tumor necrosis factor- $\alpha$  and glycated hemoglobin level in patients with type 2 diabetes. *Journal of Periodontology* **72**, 774–778.
- Iwamoto, Y., Nishimura, F., Soga, Y., Takeuchi, K., Kurihara, M., Takashiba, S. & Murayama, Y. (2003) Antimicrobial periodontal treatment decreases serum C-reactive protein, tumor necrosis factor- $\alpha$ , but not adiponectin levels in patients with chronic periodontitis. *Journal of Periodontology* **74**, 1231–1236.
- Kamaraj, D. R., Bhushan, K. S., Laxman, V. K. & Mathew, J. (2011) Detection of odoriferous subgingival and tongue microbiota in diabetic and nondiabetic patients with oral malodor using polymerase chain reaction. *Indian Journal of Dental Research* **22**, 260–265.
- Kardesler, L., Biyikoglu, B., Cetinkalp, S., Pitkala, M., Sorsa, T. & Buduneli, N. (2010a) Crevicular fluid matrix metalloproteinase-8, -13, and TIMP-1 levels in type 2 diabetics. *Oral Diseases* **16**, 476–481.
- Kardesler, L., Buduneli, N., Biyikoglu, B., Cetinkalp, S. & Kutukculer, N. (2008) Gingival crevicular fluid PGE<sub>2</sub>, IL-1 $\beta$ , t-PA, PAI-2 levels in type 2 diabetes and relationship with periodontal disease. *Clinical Biochemistry* **41**, 863–868.
- Kardesler, L., Buduneli, N., Cetinkalp, S. & Kinane, D. F. (2010b) Adipokines and inflammatory mediators after initial periodontal treatment in patients with type 2 diabetes and chronic periodontitis. *Journal of Periodontology* **81**, 24–33.
- Kardesler, L., Buduneli, N., Cetinkalp, S., Lappin, D. & Kinane, D. F. (2011) Gingival crevicular fluid IL-6, tPA, PAI-2, albumin levels following initial periodontal treatment in chronic periodontitis patients with or without type 2 diabetes. *Inflammation Research* **60**, 143–151.
- Karima, M., Kantarci, A., Ohira, T., Hasturk, H., Jones, V. L., Nam, B. H., Malabanan, A., Trackman, P. C., Badwey, J. A. & Van Dyke, T. E. (2005) Enhanced superoxide release and elevated protein kinase C activity in neutrophils from diabetic patients: association with periodontitis. *Journal of Leukocyte Biology* **78**, 862–870.
- Katz, J., Bhattacharyya, I., Farkhondeh-Kish, F., Perez, F. M., Caudle, R. M. & Heft, M. W. (2005) Expression of the receptor of advanced glycation end products in gingival tissues of type 2 diabetes patients with chronic periodontal disease: a study utilizing immunohistochemistry and RT-PCR. *Journal of Clinical Periodontology* **32**, 40–44.
- Kido, J., Bando, M., Hiroshima, Y., Iwasaka, H., Yamada, K., Ohgami, N., Nambu, T., Kataoka, M., Yamamoto, T., Shinohara, Y., Sagawa, I. & Nagata, T. (2012) Analysis of proteins in human gingival crevicular fluid by mass spectrometry. *Journal of Periodontal Research* **47**, 488–499.
- Kim, J. B., Jung, M. H., Cho, J. Y., Park, J. W., Suh, J. Y. & Lee, J. M. (2011) The influence of type 2 diabetes mellitus on the expression of inflammatory mediators and tissue inhibitor of metalloproteinases-2 in human chronic periodontitis. *Journal of Periodontal and Implant Science* **41**, 109–116.
- King, G. L. (2008) The role of inflammatory cytokines in diabetes and its complications. *Journal of Periodontology* **79**, 1527–1534.
- Kolb, H. & Mandrup-Poulsen, T. (2010) The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. *Diabetologia* **53**, 10–20.
- Kurtis, B., Develioglu, H., Taner, I. L., Balos, K. & Tekin, I. O. (1999) IL-6 levels in gingival crevicular fluid (GCF) from patients with non-insulin dependent diabetes mellitus (NIDDM), adult periodontitis and healthy subjects. *Journal of Oral Science* **41**, 163–167.
- Lalla, E., Kaplan, S., Chang, S. M., Roth, G. A., Celenti, R., Hinckley, K., Greenberg, E. & Papapanou, P. N. (2006) Periodontal infection profiles in type 1 diabetes. *Journal of Clinical Periodontology* **33**, 855–862.
- Lalla, E., Lamster, I. B., Drury, S., Fu, C. & Schmidt, A. M. (2000a) Hyperglycemia, glycoxidation and receptor for advanced glycation endproducts: potential mechanisms underlying diabetic complications, including diabetes-associated periodontitis. *Periodontology 2000* **23**, 50–62.
- Lalla, E., Lamster, I. B., Feit, M., Huang, L. & Schmidt, A. M. (1998a) A murine model of accelerated periodontal disease in diabetes. *Journal of Periodontal Research* **33**, 387–399.
- Lalla, E., Lamster, I. B., Feit, M., Huang, L., Spessot, A., Qu, W., Kislinger, T., Lu, Y., Stern, D. M. & Schmidt, A. M. (2000b) Blockade of RAGE suppresses periodontitis-associated bone loss in diabetic mice. *Journal of Clinical Investigation* **105**, 1117–1124.
- Lalla, E., Lamster, I. B. & Schmidt, A. M. (1998b) Enhanced interaction of advanced glycation end products with their cellular receptor RAGE: implications for the pathogenesis of accelerated periodontal disease in diabetes. *Annals of Periodontology* **3**, 13–19.
- Lalla, E. & Papapanou, P. N. (2011) Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. *Nature Reviews Endocrinology* **7**, 738–748.
- Lappin, D. F., Eapen, B., Robertson, D., Young, J. & Hodge, P. J. (2009) Markers of bone destruction and formation and periodontitis in type 1 diabetes mellitus. *Journal of Clinical Periodontology* **36**, 634–641.
- Larsen, C. M., Faulenbach, M., Vaag, A., Ehres, J. A., Donath, M. Y. & Mandrup-Poulsen, T. (2009) Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. *Diabetes Care* **32**, 1663–1668.
- Liu, R., Bal, H. S., Desta, T., Behl, Y. & Graves, D. T. (2006a) Tumor necrosis factor- $\alpha$  mediates diabetes-enhanced apoptosis of matrix-producing cells and impairs diabetic healing. *American Journal of Pathology* **168**, 757–764.
- Liu, R., Bal, H. S., Desta, T., Krothapalli, N., Alyassi, M., Luan, Q. & Graves, D. T. (2006b) Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. *Journal of Dental Research* **85**, 510–514.
- Liu, R., Desta, T., He, H. & Graves, D. T. (2004) Diabetes alters the response to bacteria by enhancing fibroblast apoptosis. *Endocrinology* **145**, 2997–3003.
- Mahamed, D. A., Marleau, A., Alnaeeli, M., Singh, B., Zhang, X., Penninger, J. M. & Teng, Y. T. (2005) G(-) anaerobes-reactive CD4 + T-cells trigger RANKL-mediated enhanced alveolar bone loss in diabetic NOD mice. *Diabetes* **54**, 1477–1486.
- Makiura, N., Ojima, M., Kou, Y., Furuta, N., Okahashi, N., Shizukuishi, S. & Amano, A. (2008) Relationship of *Porphyromonas gingivalis* with glycemic level in patients with type 2 diabetes following periodontal treatment. *Oral Microbiology and Immunology* **23**, 348–351.
- Mandrup-Poulsen, T., Pickersgill, L. & Donath, M. Y. (2010) Blockade of interleukin 1 in type 1 diabetes mellitus. *Nature Reviews Endocrinology* **6**, 158–166.
- Manouchehr-Pour, M., Spagnuolo, P. J., Rodman, H. M. & Bissada, N. F. (1981a) Impaired neutrophil chemotaxis in diabetic patients with severe periodontitis. *Journal of Dental Research* **60**, 729–730.
- Manouchehr-Pour, M., Spagnuolo, P. J., Rodman, H. M. & Bissada, N. F. (1981b) Comparison of neutrophil chemotactic response in diabetic patients with mild and severe periodontal disease. *Journal of Periodontology* **52**, 410–415.
- Martinon, F. (2010) Signaling by ROS drives inflammation and chemotaxis. *European Journal of Immunology* **40**, 616–619.
- Matsumoto, S., Ogawa, H., Soda, S., Hirayama, S., Amaraseena, N., Aizawa, Y. & Miyazaki, H. (2009) Effect of antimicrobial periodontal treatment and maintenance on serum adiponectin in type 2 diabetes mellitus. *Journal of Clinical Periodontology* **36**, 142–148.
- Mealey, B. L. & Oates, T. W. (2006) Diabetes mellitus and periodontal diseases. *Journal of Periodontology* **77**, 1289–1303.
- Naguib, G., Al-Mashat, H., Desta, T. & Graves, D. T. (2004) Diabetes prolongs the inflammatory response to a bacterial stimulus through cytokine dysregulation. *Journal of Investigative Dermatology* **123**, 87–92.
- Nassar, H., Kantarci, A. & van Dyke, T. E. (2007) Diabetic periodontitis: a model for activated innate immunity and impaired resolution of inflammation. *Periodontology 2000* **43**, 233–244.
- Navarro-Sanchez, A. B., Faria-Almeida, R. & Bascones-Martinez, A. (2007) Effect of non-surgical periodontal therapy on clinical and immunological response and glycaemic control in type 2 diabetic patients with moderate periodontitis. *Journal of Clinical Periodontology* **34**, 835–843.
- Nishihara, R., Sugano, N., Takano, M., Shimada, T., Tanaka, H., Oka, S. & Ito, K. (2009) The effect of *Porphyromonas gingivalis* infection on cytokine levels in type 2 diabetic mice. *Journal of Periodontal Research* **44**, 305–310.
- Novaes, A. B. Jr, Gonzalez Gutierrez, F., Grisi, M. F. & Novaes, A. B. (1997) Periodontal disease progression in type II non-insulin-dependent diabetes mellitus patients (NIDDM). Part

- II—Microbiological analysis using the BANA test. *Brazilian Dental Journal* **8**, 27–33.
- O'Connell, P. A., Taba, M., Nomizu, A., Foss Freitas, M. C., Suaid, F. A., Uyemura, S. A., Trevisan, G. L., Novaes, A. B., Souza, S. L., Palioto, D. B. & Grisi, M. F. (2008) Effects of periodontal therapy on glycemic control and inflammatory markers. *Journal of Periodontology* **79**, 774–783.
- Pacios, S., Kang, J., Galicia, J., Gluck, K., Patel, H., Ovaydi-Mandel, A., Petrov, S., Alawi, F. & Graves, D. T. (2012) Diabetes aggravates periodontitis by limiting repair through enhanced inflammation. *FASEB Journal* **26**, 1423–1430.
- Paraskevas, S., Huizinga, J. D. & Loos, B. G. (2008) A systematic review and meta-analysis on C-reactive protein in relation to periodontitis. *Journal of Clinical Periodontology* **35**, 277–290.
- Passoja, A., Knuutila, M., Hiltunen, L., Karttunen, R., Niemela, O., Raunio, T., Vainio, O., Hedberg, P. & Tervonen, T. (2011) Serum interleukin-6 may modulate periodontal inflammation in type 1 diabetic subjects. *Journal of Clinical Periodontology* **38**, 687–693.
- Pickup, J. C. & Crook, M. A. (1998) Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* **41**, 1241–1248.
- Pinducciu, G., Micheletti, L., Piras, V., Songini, C., Serra, C., Pompei, R. & Pintus, L. (1996) Periodontal disease, oral microbial flora and salivary antibacterial factors in diabetes mellitus type 1 patients. *European Journal of Epidemiology* **12**, 631–636.
- Pischon, N., Heng, N., Bernimoulin, J. P., Kleber, B. M., Willich, S. N. & Pischon, T. (2007) Obesity, inflammation, and periodontal disease. *Journal of Dental Research* **86**, 400–409.
- Pradeep, A. R., Raghavendra, N. M., Sharma, A., Patel, S. P., Raju, A., Kathariya, R., Rao, N. S. & Naik, S. B. (2012) Association of serum and crevicular visfatin levels in periodontal health and disease with type 2 diabetes mellitus. *Journal of Periodontology* **83**, 629–634.
- Preshaw, P. M., Alba, A. L., Herrera, D., Jepsen, S., Konstantinidis, A., Makrilakis, K. & Taylor, R. (2012) Periodontitis and diabetes: a two-way relationship. *Diabetologia* **55**, 21–31.
- Preshaw, P. M., Foster, N. & Taylor, J. J. (2007) Cross-susceptibility between periodontal disease and type 2 diabetes mellitus: an immunobiological perspective. *Periodontology 2000* **45**, 138–157.
- Preshaw, P. M. & Taylor, J. J. (2011) How has research into cytokine interactions and their role in driving immune responses impacted our understanding of periodontitis? *Journal of Clinical Periodontology* **38** (Suppl 11), 60–84.
- Ramamurthy, N. S. & Golub, L. M. (1983) Diabetes increases collagenase activity in extracts of rat gingiva and skin. *Journal of Periodontal Research* **18**, 23–30.
- Ramamurthy, N. S., Siegel, M., Iacono, V. J. & Golub, L. M. (1979) Leucocyte response in the gingival crevice of the diabetic rat. *Journal of Periodontal Research* **14**, 289–296.
- Rojo-Botello, N. R., Garcia-Hernandez, A. L. & Moreno-Fierros, L. (2012) Expression of toll-like receptors 2, 4 and 9 is increased in gingival tissue from patients with type 2 diabetes and chronic periodontitis. *Journal of Periodontal Research* **47**, 62–73.
- Ross, J. H., Hardy, D. C., Schuyler, C. A., Slate, E. H., Mize, T. W. & Huang, Y. (2010) Expression of periodontal interleukin-6 protein is increased across patients with neither periodontal disease nor diabetes, patients with periodontal disease alone and patients with both diseases. *Journal of Periodontal Research* **45**, 688–694.
- Rotter, V., Nagaev, I. & Smith, U. (2003) Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor- $\alpha$ , overexpressed in human fat cells from insulin-resistant subjects. *Journal of Biological Chemistry* **278**, 45777–45784.
- Safkan-Seppala, B., Sorsa, T., Tervahartala, T., Beklen, A. & Kontinen, Y. T. (2006) Collagenases in gingival crevicular fluid in type 1 diabetes mellitus. *Journal of Periodontology* **77**, 189–194.
- Sakallioğlu, E. E., Ayas, B., Lutfioğlu, M., Keles, G. C., Acikgoz, G. & Firatlı, E. (2008) Gingival levels of monocyte chemoattractant protein-1 (MCP-1) in diabetes mellitus and periodontitis: an experimental study in rats. *Clinical Oral Investigations* **12**, 83–89.
- Salvi, G. E., Collins, J. G., Yalda, B., Arnold, R. R., Lang, N. P. & Offenbacher, S. (1997a) Monocytic TNF  $\alpha$  secretion patterns in IDDM patients with periodontal diseases. *Journal of Clinical Periodontology* **24**, 8–16.
- Salvi, G. E., Franco, L. M., Braun, T. M., Lee, A., Rutger Persson, G., Lang, N. P. & Giannobile, W. V. (2010) Pro-inflammatory biomarkers during experimental gingivitis in patients with type 1 diabetes mellitus: a proof-of-concept study. *Journal of Clinical Periodontology* **37**, 9–16.
- Salvi, G. E., Kandykaki, M., Troendle, A., Persson, G. R. & Lang, N. P. (2005) Experimental gingivitis in type 1 diabetics: a controlled clinical and microbiological study. *Journal of Clinical Periodontology* **32**, 310–316.
- Salvi, G. E., Lawrence, H. P., Offenbacher, S. & Beck, J. D. (1997b) Influence of risk factors on the pathogenesis of periodontitis. *Periodontology 2000*, **14**, 173–201.
- Salvi, G. E., Yalda, B., Collins, J. G., Jones, B. H., Smith, F. W., Arnold, R. R. & Offenbacher, S. (1997c) Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients. *Journal of Periodontology* **68**, 127–135.
- Sanchez-Cordero, S., Hoffman, H. & Stahl, S. S. (1979) Occurrence of staphylococcus in periodontal pockets of diabetic and nondiabetic adults. *Journal of Periodontology* **50**, 109–113.
- Santacroce, L., Carlaio, R. G. & Bottalico, L. (2010) Does it make sense that diabetes is reciprocally associated with periodontal disease? *Endocrine Metabolic and Immune Disorders-Drug Targets* **10**, 57–70.
- Santana, R. B., Xu, L., Chase, H. B., Amar, S., Graves, D. T. & Trackman, P. C. (2003) A role for advanced glycation end products in diminished bone healing in type 1 diabetes. *Diabetes* **52**, 150, 2–1510.
- Santos, V. R., Lima, J. A., Goncalves, T. E., Bastos, M. F., Figueiredo, L. C., Shibli, J. A. & Duarte, P. M. (2010a) Receptor activator of nuclear factor- $\kappa$ B ligand/osteoprotegerin ratio in sites of chronic periodontitis of subjects with poorly and well-controlled type 2 diabetes. *Journal of Periodontology* **81**, 1455–1465.
- Santos, V. R., Ribeiro, F. V., Lima, J. A., Napimoga, M. H., Bastos, M. F. & Duarte, P. M. (2010b) Cytokine levels in sites of chronic periodontitis of poorly controlled and well-controlled type 2 diabetic subjects. *Journal of Clinical Periodontology* **37**, 1049–1058.
- Saremi, A., Nelson, R. G., Tulloch-Reid, M., Hanson, R. L., Sievers, M. L., Taylor, G. W., Shlossman, M., Bennett, P. H., Genco, R. & Knowler, W. C. (2005) Periodontal disease and mortality in type 2 diabetes. *Diabetes Care* **28**, 27–32.
- Sasaki, T., Ramamurthy, N. S., Yu, Z. & Golub, L. M. (1992) Tetracycline administration increases protein (presumably procollagen) synthesis and secretion in periodontal ligament fibroblasts of streptozotocin-induced diabetic rats. *Journal of Periodontal Research* **27**, 631–639.
- Sastrowijoto, S. H., Hilleman, P., van Steenberg, T. J., Abraham-Inpign, L. & de Graaff, J. (1989) Periodontal condition and microbiology of healthy and diseased periodontal pockets in type 1 diabetes mellitus patients. *Journal of Clinical Periodontology* **16**, 316–322.
- Sastrowijoto, S. H., van der Velden, U., van Steenberg, T. J., Hilleman, P., Hart, A. A., de Graaff, J. & Abraham-Inpign, L. (1990) Improved metabolic control, clinical periodontal status and subgingival microbiology in insulin-dependent diabetes mellitus. A prospective study. *Journal of Clinical Periodontology* **17**, 233–242.
- Sbordone, L., Ramaglia, L., Barone, A., Ciaglia, R. N. & Iacono, V. J. (1998) Periodontal status and subgingival microbiota of insulin-dependent juvenile diabetics: a 3-year longitudinal study. *Journal of Periodontology* **69**, 120–128.
- Sbordone, L., Ramaglia, L., Barone, A., Ciaglia, R. N., Tenore, A. & Iacono, V. J. (1995) Periodontal status and selected cultivable anaerobic microflora of insulin-dependent juvenile diabetics. *Journal of Periodontology* **66**, 452–461.
- Schmidt, A. M., Vianna, M., Gerlach, M., Brett, J., Ryan, J., Kao, J., Esposito, C., Hegarty, H., Hurley, W., Clauss, M., Wang, F., Pan, Y.-C. E., Tsang, T. C. & Stern, D. (1992) Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *Journal of Biological Chemistry* **267**, 14987–14997.
- Schmidt, A. M., Weidman, E., Lalla, E., Yan, S. D., Hori, O., Cao, R., Brett, J. G. & Lamster, I. B. (1996) Advanced glycation endproducts (AGEs) induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *Journal of Periodontal Research* **31**, 508–515.
- Shin, D. S., Park, J. W., Suh, J. Y. & Lee, J. M. (2010) The expressions of inflammatory factors and tissue inhibitor of matrix metalloproteinase-2 in human chronic periodontitis with type 2 diabetes mellitus. *Journal of Periodontal and Implant Science* **40**, 33–38.
- Shultis, W. A., Weil, E. J., Looker, H. C., Curtis, J. M., Shlossman, M., Genco, R. J., Knowler, W. C. & Nelson, R. G. (2007) Effect of periodontitis on overt nephropathy and end-stage renal disease in type 2 diabetes. *Diabetes Care* **30**, 306–311.
- Silva, J. A., Ferrucci, D. L., Peroni, L. A., Abraham, P. G., Salame, A. F., Rossa-Junior, C., Carvalho, H. F. & Stach-Machado, D. R. (2012a) Sequential IL-23 and IL-17 and increased Mmp8 and Mmp14 expression characterize the progression of an experimental model of periodontal disease in type 1 diabetes. *Journal of Cellular Physiology* **227**, 2441–2450.
- Silva, J. A., Lopes Ferrucci, D., Peroni, L. A., de Paula Ishi, E., Rossa-Junior, C., Carvalho, H. F. & Stach-Machado, D. R. (2012b) Periodontal Disease-Associated Compensatory Expression of Osteoprotegerin Is Lost in Type 1 Diabetes Mellitus and Correlates with Alveolar

- Bone Destruction by Regulating Osteoclastogenesis. *Cells Tissues Organs* **196**, 137–150.
- Sima, C., Rhourida, K., Van Dyke, T. E. & Gyurko, R. (2010) Type 1 diabetes predisposes to enhanced gingival leukocyte margination and macromolecule extravasation in vivo. *Journal of Periodontal Research* **45**, 748–756.
- Simpson, T. C., Needleman, I., Wild, S. H., Moles, D. R. & Mills, E. J. (2010) Treatment of periodontal disease for glycaemic control in people with diabetes. *Cochrane Database of Systematic Reviews* Issue 5. Art no. CD004714. doi: 10.1002/14651858. CD004714.pub2.
- Siqueira, M. F., Li, J., Chehab, L., Desta, T., Chino, T., Krothpali, N., Behl, Y., Alikhani, M., Yang, J., Braasch, C. & Graves, D. T. (2010) Impaired wound healing in mouse models of diabetes is mediated by TNF- $\alpha$  dysregulation and associated with enhanced activation of forkhead box O1 (FOXO1). *Diabetologia*, **53**, 378–388.
- Soskolne, W. A. & Klinger, A. (2001) The relationship between periodontal diseases and diabetes: an overview. *Annals of Periodontology* **6**, 91–98.
- Sun, W. L., Chen, L. L., Zhang, S. Z., Wu, Y. M., Ren, Y. Z. & Qin, G. M. (2011) Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal intervention in patients with type 2 diabetes and chronic periodontitis. *Internal Medicine* **50**, 1569–1574.
- Sugano, N., Ikeda, K., Oshikawa, M., Sawamoto, Y., Tanaka, H. & Ito, K. (2004) Differential cytokine induction by two types of Porphyromonas gingivalis. *Oral Microbiology and Immunology* **19**, 121–123.
- Surdacka, A., Ciezka, E., Piorunski-Stolzmann, M., Wender-Ozegowska, E., Korybalska, K., Kawka, E., Kaczmarek, E. & Witowski, J. (2011) Relation of salivary antioxidant status and cytokine levels to clinical parameters of oral health in pregnant women with diabetes. *Archives of Oral Biology* **56**, 428–436.
- Takano, M., Nishihara, R., Sugano, N., Matsumoto, K., Yamada, Y., Takane, M., Fujisaki, Y. & Ito, K. (2010) The effect of systemic anti-tumor necrosis factor- $\alpha$  treatment on Porphyromonas gingivalis infection in type 2 diabetic mice. *Archives of Oral Biology* **55**, 79–384.
- Takeda, M., Ojima, M., Yoshioka, H., Inaba, H., Kogo, M., Shizukuishi, S., Nomura, M. & Amano, A. (2006) Relationship of serum advanced glycation end products with deterioration of periodontitis in type 2 diabetes patients. *Journal of Periodontology* **77**, 15–20.
- Talbert, J., Elter, J., Jared, H. L., Offenbacher, S., Southerland, J. & Wilder, R. S. (2006) The effect of periodontal therapy on TNF- $\alpha$ , IL-6 and metabolic control in type 2 diabetics. *Journal of Dental Hygiene* **80**, 7.
- Taylor, G. W., Burt, B. A., Becker, M. P., Genco, R. J., Shlossman, M., Knowler, W. C. & Pettitt, D. J. (1996) Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *Journal of Periodontology* **67**, 1085–1093.
- Tervonen, T., Oliver, R. C., Wolff, L. F., Bereuter, J., Anderson, L. & Aepli, D. M. (1994) Prevalence of periodontal pathogens with varying metabolic control of diabetes mellitus. *Journal of Clinical Periodontology* **21**, 375–379.
- Thorstenon, H., Dahlen, G. & Hugoson, A. (1995) Some suspected periodontopathogens and serum antibody response in adult long-duration insulin-dependent diabetics. *Journal of Clinical Periodontology* **22**, 449–458.
- Unlu, F., Guneri, P. G., Hekimgil, M., Yesilbek, B. & Boyacioglu, H. (2003) Expression of vascular endothelial growth factor in human periodontal tissues: comparison of healthy and diabetic patients. *Journal of Periodontology* **74**, 181–187.
- Veloso, C. A., Fernandes, J. S., Volpe, C. M., Fagundes-Netto, F. S., Reis, J. S., Chaves, M. M. & Nogueira-Machado, J. A. (2011) TLR4 and RAGE: similar routes leading to inflammation in type 2 diabetic patients. *Diabetes and Metabolism* **37**, 336–342.
- Venza, I., Visalli, M., Cucinotta, M., De Grazia, G., Teti, D. & Venza, M. (2010) Proinflammatory gene expression at chronic periodontitis and peri-implantitis sites in patients with or without type 2 diabetes. *Journal of Periodontology* **81**, 99–108.
- Vieira Ribeiro, F., de Mendonca, A. C., Santos, V. R., Bastos, M. F., Figueiredo, L. C. & Duarte, P. M. (2011) Cytokines and bone-related factors in systemically healthy patients with chronic periodontitis and patients with type 2 diabetes and chronic periodontitis. *Journal of Periodontology* **82**, 1187–1196.
- Watanabe, K., Petro, B. J., Shlimon, A. E. & Unterman, T. G. (2008) Effect of periodontitis on insulin resistance and the onset of type 2 diabetes mellitus in Zucker diabetic fatty rats. *Journal of Periodontology* **79**, 1208–1216.
- Yalda, B., Offenbacher, S. & Collins, J. G. (1994) Diabetes as a modifier of periodontal disease expression. *Periodontology 2000* **6**, 37–49.
- Yan, S. F., Ramasamy, R. & Schmidt, A. M. (2009) Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response. *Journal of Molecular Medicine (Berl)* **87**, 235–247.
- Yoon, M. S., Jankowski, V., Montag, S., Zidek, W., Henning, L., Schluter, H., Tepel, M. & Jankowski, J. (2004) Characterisation of advanced glycation endproducts in saliva from patients with diabetes mellitus. *Biochemical and Biophysical Research Communications* **323**, 377–381.
- Yoshida, T., Flegler, A., Kozlov, A. & Stern, P. H. (2009) Direct inhibitory and indirect stimulatory effects of RAGE ligand S100 on sRANKL-induced osteoclastogenesis. *Journal of Cellular Biochemistry* **107**, 917–925.
- Yu, S., Li, H., Ma, Y. & Fu, Y. (2012) Matrix metalloproteinase-1 of gingival fibroblasts influenced by advanced glycation end products (AGEs) and their association with receptor for AGEs and nuclear factor-kappaB in gingival connective tissue. *Journal of Periodontology* **83**, 119–126.
- Yuan, K., Chang, C. J., Hsu, P. C., Sun, H. S., Tseng, C. C. & Wang, J. R. (2001) Detection of putative periodontal pathogens in non-insulin-dependent diabetes mellitus and non-diabetes mellitus by polymerase chain reaction. *Journal of Periodontal Research* **36**, 18–24.
- Zambon, J. J., Reynolds, H., Fisher, J. G., Shlossman, M., Dunford, R. & Genco, R. J. (1988) Microbiological and immunological studies of adult periodontitis in patients with noninsulin-dependent diabetes mellitus. *Journal of Periodontology* **59**, 23–31.
- Zarco, M. F., Vess, T. J. & Ginsburg, G. S. (2012) The oral microbiome in health and disease and the potential impact on personalized dental medicine. *Oral Diseases* **18**, 109–120.

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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*.