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Oxidative and inflammatory status in Type 2 diabetes patients with periodontitis

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Abstract

Aim: To determine the impact of periodontitis on oxidative/inflammatory status and diabetes control in Type 2 diabetes.

Materials and Methods: A comparative study of 20 Type 2 diabetes patients with periodontitis [body mass index (BMI) 31+5], 20-age/gender-matched, non-periodontitis Type 2 diabetes controls (BMI 29+6) and 20 non-diabetes periodontitis controls (BMI 25+4) had periodontal examinations and fasting blood samples collected. Oxidative stress was determined by plasma small molecule antioxidant capacity (pSMAC) and protein carbonyl levels; inflammatory status by total/differential leucocytes, fibrinogen and high sensitivity C-reactive protein (hsCRP); diabetes status by fasting glucose, HbA1c, lipid profile, insulin resistance and secretion. Statistical analysis was performed using SPSS.

Results: pSMAC was lower ($p = 0.03$) and protein carbonyls higher ($p = 0.007$) in Type 2 diabetes patients with periodontitis compared with those without periodontitis. Periodontitis was associated with significantly higher HbA1c ($p = 0.002$) and fasting glucose levels ($p = 0.04$) and with lower β-cell function (HOMA-β; $p = 0.01$) in diabetes patients. Periodontitis had little effect on inflammatory markers or lipid profiles, but Type 2 diabetes patients with periodontitis had higher levels of hsCRP than those without diabetes ($p = 0.004$) and the lowest levels of HDL-cholesterol of all groups.

Conclusion: Periodontitis is associated with increased oxidative stress and compromised glycaemic control in Type 2 diabetes patients.

Key words: diabetes; inflammation; oxidative stress; periodontitis

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Periodontitis is a common, chronic inflammatory condition that affects the supporting tissues surrounding teeth. The emergence of pathogenic sub-gingival plaque biofilm in a susceptible individual, results in progressive destruction of tooth-supporting tissues and the formation of periodontal pockets between the tooth and surrounding gingival tissues (March & Devine 2011). Susceptibility appears largely due to a phenotype characterized by an exaggerated, ''hyper-inflammatory'' response to the colonizing bacteria (Van Dyke 2007). Progression of untreated periodontitis can result in pain, aesthetic problems, tooth loss and functional difficulties. The incidence of severe chronic periodontitis is 5–10% of populations studied, with incidence and severity reported to be doubled in Type 2 diabetes patients (Löe 1993).

Evidence from a variety of sources demonstrates that periodontitis induces oxidative stress locally within the periodontal tissues (Chapple & Matthews 2007, Chapple et al. 2007). Individual susceptibility to periodontitis may be due, at least in part, to the presence of hyper-active/reactive neutrophils within the peripheral blood, which following chemo-attraction and trafficking to the periodontal tissues, generate increased levels of reactive oxygen species (ROS) both spontaneously and following stimulation of Fc γ-receptors or toll-like receptors on the neutrophil surface by their respective ligands (Fredriksson et al. 2003, Matthews et al. 2007). The elevated levels of ROS generated locally by neutrophils during the inflammatory response to both microbial plaque and plaque-induced tissue damage, is thought to result

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in further collateral host-tissue damage. This is induced both directly, via oxidation of vital tissue components as well as indirectly, via the activation of redox-sensitive gene transcription factors (e.g. nuclear factor kappa B, activating protein-1), which trigger down-stream production of a cascade of pro-inflammatory peptides (Van Dyke 2007) and by increasing cellular senescence (Kurz et al. 2004).

The increased oxidative stress within periodontitis patients does not appear to be confined to the periodontal tissues only. Systemic manifestations of an increased oxidative and inflammatory burden have also been detected in patients with periodontitis providing a potential mechanism whereby periodontal inflammation may impact upon systemic inflammatory status. Reduced plasma small molecule antioxidant capacity (pSMAC; Chapple et al. 1997, D'Aiuto et al. 2010), previously referred to as plasma total antioxidant capacity, and higher plasma levels of cytokines and C-reactive protein (CRP) (Bretz et al. 2005, Gomes-Filho et al. 2011) have been detected in periodontitis patients. A recent meta-analysis suggests that periodontitis may be an independent risk factor for coronary heart disease (Bahekar et al. 2007) and that aggressive treatment of periodontal inflammation improves biomarkers of vascular endothelial function (Tonetti et al. 2007). Interestingly, while it is well established that Type 2 diabetes patients have a significantly elevated risk of periodontitis (Löe 1993, Khader et al. 2006), the presence of periodontitis may exert a converse, negative impact upon cardio-metabolic risk status in Type 2 diabetes patients. Periodontal inflammation has been associated with impaired fasting glucose (Choi et al. 2011) and, in Pima Indians, is an independent predictor of mortality from ischaemic heart disease and diabetic nephropathy (Saremi et al. 2005). Consistent with this, some studies report that periodontal therapy leads to a reduction in HbA1c levels in diabetes (Navarro-Sanchez et al. 2007, Teeuw et al. 2010, Koromantzos et al. 2011) and a more favourable lipid profile in periodontitis (D'Aiuto et al. 2006). However, the nature of the biological relationship between periodontitis and Type 2 diabetes mellitus remains unclear (Janket et al. 2005).

Current evidence also suggests that Type 2 diabetes is associated with a state of increased oxidative stress and that this results in a hyper-inflammatory state (Evans et al. 2003). Hyperglycaemia leads to excess ROS production within the mitochondrial electron transport chain (Brownlee 2001), during AGE formation and following AGE/RAGE interactions, which stimulate NADPH oxidase, principally in neutrophils (Karima et al. 2005). The disruption of the redox balance results in stimulation of cell-signalling pathways associated with inflammation, dysregulation of insulin signalling and development of diabetes complications (Brownlee 2001).

We hypothesize that oxidative stress is a central biological event from which diverse signalling pathways emerge and elevated oxidative stress in association with periodontal inflammation can exacerbate the disease process of Type 2 diabetes when these conditions co-exist. Therefore, the primary aim of this comparative study was to determine the impact of periodontal inflammation on oxidative and inflammatory stress and glycaemic and lipid control in Type 2 diabetes patients.

Materials and Methods

Sixty patients were recruited for this comparative study; 20 subjects within each of the three groups: Type 2 diabetes with periodontitis (DP), Type 2 diabetes with no periodontitis (DNP) and non-diabetes with periodontitis (NDP). While the study was primarily designed to investigate the association between periodontal inflammation and diabetes status by comparison of data from the DP and DNP groups, the addition of the third group of non-diabetes patients with periodontitis also allowed the investigation of the impact of diabetes upon periodontitis by comparison of the data from the DP and NDP groups.

All diabetes patients who participated in this study were diagnosed with Type 2 diabetes according to the WHO classification and were treated by dietary intervention or oral hypoglycaemic agents only. Details of current medications taken by both diabetes groups are provided in Table 1, as are the demographics of each group. None of the patients within the NDP group were taking prescription medication. All study participants were Caucasian except for two of Asian origin (1 DP, 1 DNP). Each study participant gave written, informed consent before their inclusion in the study and completed a detailed, written medical questionnaire. Ethical approval for this study was received from the Cork University Hospitals Ethics Committee (ECM 3 (11) 07/12/04).

Selection criteria

Type 2 diabetes patients were recruited from a local out-patient diabetic clinic over a period of 15 months. Non-diabetes patients with periodontitis were recruited from the waiting list of patients referred to Cork University Dental Hospital for periodontal assessment.

Inclusion criteria for study participation were an age range of 30–70 years inclusive, non-smoker status (by self-report) and, in the periodontitis patients, at least 16 teeth with periodontal pockets >4 mm in at least 6 sites. Exclusion criteria were myocardial infarction/stroke within the last 12 months, unstable angina or other significant cardiac, peripheral vascular, renal, hepatic or endocrine disease, chronic inflammatory conditions, conditions requiring antibiotic prophylaxis, psychiatric disorder, severe physical handicap, carcinoma, immunosuppressive therapy, hospital inpatient, vitamin supplements or antibiotics taken within 3 months or periodontal therapy received within 6 months.

Inclusion criteria for periodontal health in the Type 2 diabetes patients without periodontitis were no evidence of connective tissue loss >2 mm or of supporting bone loss on radiographic investigation (Chapple et al. 1997).

Study design

The study design was a pragmatic comparative study with participants individually matched for both age and gender in the three groups. As data for the oxidative stress markers to be investigated were not available for Type 2 diabetes patients, study numbers could not be determined by power calculations, but were determined using a pragmatic approach by the number of eligible participants that fulfilled the recruitment criteria for the DP group with subsequent individual matching of DNP and NDP ''partners'' for gender and age (± 5 years). Recruitment was completed between 2005 and 2007 with the recruitment period designed to coincide with a full cycle of recall attendance at the diabetes clinic and therefore all current patients attending

Table 1. Clinical and periodontal characteristics of subjects [mean+SD (range)]

	Diabetes, periodontally healthy (DNP; $n = 20$)	Diabetes with periodontitis (DP; $n = 20$)	Non-diabetes periodontitis (NDP; $n = 20$)	p-Value*
Gender (male:female)	14:6	14:6	14:6	NS
Age (years)	55+7	56+7	54+6	NS
BMI (kg/m^2)	28.9+5.5	30.8+5.1	25.0+3.8	0.003‡ 0.024§
Duration of diabetes (years)	5.7+6.9	7.0+4.5	n.a.	NS
Medication				
Anti-inflammatories	20	20	0	–
Oral hypoglycaemics	19	19	0	–
Statins	10	11	0	–
Ace inhibitors	5	7	0	–
β-blockers	3	6	0	–
Channel blockers	2	3	0	–
Diuretics	2	0	0	–
Fruit/vegetable intake (portion/day)	3.6+1.0	3.2+1.2	3.5+1.3	NS
Periodontal pocket depth (mm)	1.8+0.2	3.0+0.6	3.1+0.6	0.003†,§
Percentage plaque	41+19	54+21	43+27	0.03†
Bleeding on probing percentage (BOP%)	22+11	41+19	28+11	0.003† 0.03‡

*Bonferroni adjusted significance levels for comparisons:
†DP *versus* DNP;
‡DP *versus* NDP; and
§DNP *versus* NDP by paired *t*-test.
NS, not significant; BMI, body mass index.

the clinic were assessed. A greater sample size would have required prolonged attendance at the clinic to examine newly diagnosed diabetes patients with attendant logistical difficulties.

Patients with Type 2 diabetes were initially screened within the diabetes out-patient clinic from clinical records followed by verbal examination and finally an intra-oral examination of potentially suitable patients performed by one examiner (E. A.) using mouth mirrors and WHO CPI Probes (CP-8; Hu Friedy, Chicago IL, USA). First, DP were recruited in a sequential manner upon identification and agreement to study participation. Second, DNP were recruited retrospectively from the initial cohort of diabetes patients examined who had not fulfilled the inclusion criteria for periodontitis. This allowed for close matching of DP and DNP pairs. NDP were identified, matched with DP subjects for age and gender and consented to participate in the study.

Study participants were advised of the need to fast for 14 h before their appointment and to consume only water during this time. Verbal recall was used to estimate the average daily consumption of fruit and vegetables with participants being shown the average portion size of the most commonly consumed fruit and vegetables together with a scale from 0.5 to 10 (in 0.5 increments) and asked to indicate their average consumption on the scale. This was included because of the potential impact of phytonutrients such as vitamins A, vitamin C and certain carotenoids on systemic antioxidant capacity (Tonetti et al. 2011, Van der Velden et al. 2011).

Periodontal assessment

Participants within all three groups received a full periodontal examination immediately following the collection of clinical samples. All measurements were made by the same investigator (E. A.). Examination included duplicate measurements of periodontal pocket depths (PPD) using a UNC-PCP15 (Hu Friedy) probe at six sites (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual) per tooth (excluding the third molars) and was measured as the distance from the free gingival margin to the apical extent of the pocket/sulcus. Measurements were rounded to the nearest millimetres. Mean values of PPD per site per patient were calculated. Marginal bleeding on probing (BOP) was assessed following the PPD measurements at four sites per tooth and was assessed 30 s after applying the periodontal probe with a constant force. BOP was recorded as ''1'' (present) if it occurred and ''0'' (absent) if no bleeding occurred and expressed as a percentage of positive sites per patient (BOP%). Similarly, the presence of dental plaque was measured dichotomously at four sites per tooth and an overall percentage of sites with plaque (plaque %) was calculated (O' Leary et al. 1972). Radiographic assessment to determine alveolar bone loss was undertaken as appropriate using bitewing and periapical radiographs.

Clinical samples

Verbal confirmation of fasting status was obtained from participants before clinical sample collection. Five venous blood samples (21 ml total volume) were obtained from the ante-cubital vein using vacuettes (Greiner Bi-one, Stonehouse, UK) containing clotting accelerator, trisodium citrate, EDTA, sodium fluoride or heparin. One heparinized blood sample was centrifuged at 4°C at 1000 *g* for 30 min and the separated plasma aliquoted and stored under liquid N_2 before analysis for pSMAC and protein carbonyls. Previous studies have confirmed the long-term stability of antioxidant activity of plasma samples stored under liquid N_2 (Chapple et al. 1997).

The remaining four blood samples were sent to Cork University Hospital central laboratories. HbA1c levels were

measured by high-performance liquid chromatography; total and differential leucocytes were determined by the Sysmex XE2100 automated haematology analyser (Sysmex, Milton Keynes, UK), fibrinogen by the Ca7000 automated coagulation analyser (Sysmex) and insulin by chemiluminescence (Beckman Coulter DX1, Brea, CA, USA). Fasting plasma glucose was measured by spectrophotometry on an Olympus AU 5400 automated chemistry analyser system (Olympus UK Ltd., London, UK). To determine the fasting lipoprotein profile, serum cholesterol, triglycerides and HDL were also measured spectrophotometrically on an Olympus AU 5400. Results were expressed in mmol/l.

LDL cholesterol was calculated using the formula:

$$\text{LDL} = (\text{Total cholesterol} - \text{HDL} - \text{Triglyceride}/2.18).$$

Determination of body mass index (BMI) and insulin resistance

BMI was derived using body weight (kg) divided by the square of the height (m). Height and weight measurements for the Type 2 diabetes groups were recorded at the diabetes out-patient clinic, and at the periodontal out-patient clinic for the NDP group. Weight was measured with calibrated weighing scales and height with an in situ calibrated wall height measurement scale.

Insulin resistance (in the diabetes groups only) was evaluated by the homoeostasis model assessment (HOMA) which allows the derivation of β-cell secretory function (HOMA-β), and insulin resistance (HOMA-IR) using the formulae:

$$\text{HOMA-IR} = [\text{fasting insulin}(\mu\text{IU/ml})^{*} \text{ fasting glucose}(\text{mmol/l})]/22.5.$$

$$\text{HOMA-}\beta = [20 \times \text{fasting insulin}(\mu\text{IU/ml}) / \text{fasting glucose}(\text{mmol/l}) - 3.5].$$

High sensitivity C-reactive protein (hsCRP)

hsCRP levels in liquid nitrogen-stored plasma were determined by particle-enhanced immunonephelometry using the standard ''CardioPhase hsCRP'' (Dade Behring Holding GmbH, Liederbach, Germany).

Small molecular antioxidant capacity (SMAC)

pSMAC was determined using an enhanced, chemiluminescent assay and a BioOrbit 1250 luminometer (Labtech International, E. Sussex, UK). The assay is based upon the inhibition by a test plasma sample of light output generated by the horseradish-peroxidase catalysed oxidation of luminol (Chapple et al. 1997). Antioxidant capacities of test samples were derived from standard curves produced using the water soluble vitamin E analogue, 6-hydroxy-2,5,7,8, tetramethylchroman-2-carboxylic acid (Trolox) (assay range 0.0625–1.6 nmol) performed both before and after test samples. pSMAC was expressed as a concentration of Trolox™ equivalents (μM Teq/l). The assay measures the combined activities of small molecule antioxidant species (86% of activity is due to vitamin's C, E, A and uric acid) in an in vitro enhanced chemiluminescence assay of biological samples (Maxwell et al. 2006).

Protein carbonyl assay

Oxidative damage of proteins leads to the formation of carbonyl groups, which can be used as an indirect marker of oxidative damage. The IgG group of proteins was isolated from plasma and the carbonyl content of this group was determined by ELISA following the method of Carty et al. (2000). The isolation of IgG proteins eliminates albumin from samples and thus reduces error due to the false positive readings for carbonyl groups given by albumin-glycation products. Also, IgG proteins have a half-life of 2–3 weeks, allowing for the determination of oxidative stress-related markers over a period of time. Carbonyl content was expressed as nmol carbonyl per mg IgG.

Statistical analysis

The primary outcome measures were oxidative stress in plasma as reflected indirectly by decreased pSMAC and directly by increased protein carbonyl levels. Secondary outcome measures were markers of diabetes control, hsCRP and lipid control. Data were analysed using SPSS software, version 13.00 for windows and statistical significance set at $p < 0.05$. Differences in outcome measures between the groups were determined by paired *t*-test, except for hsCRP, where the Wilcoxon signed-rank test was used as the data showed a non-normal distribution (confirmed by Kolmogorov–Smirnov test). Statistical significance for each outcome was corrected for multiple comparisons between groups using the Bonferroni adjustment. Post hoc power calculations ($p = 0.05$) showed that all comparisons had a power > 0.8 except for SMAC (0.68), total cholesterol and triglycerides (0.65), hsCRP (0.63), insulin resistance (0.18) and blood cell counts (0.1–0.24).

Results

Patient screening and selection (Fig. 1)

A total of 949 Type 2 diabetes patients were screened; 742 patients were excluded based on their medical notes and 59 for dental or personal reasons, leaving a total of 149 patients suitable for study participation. Twenty patients were identified as having periodontitis according to study criteria for this group and were recruited together with 20 age and gender matched Type 2 diabetes patients without periodontitis and 20 non-diabetes patients with periodontitis (Table 1). No significant differences in BMI, duration of diabetes and range of medication was found between the diabetes groups. However, both diabetes groups had significantly higher BMI than the non-diabetes periodontitis group ($p < 0.024$). Periodontal status, in terms of pocket depth and percentage plaque, did not differ significantly between periodontitis groups with and without Type 2 diabetes. By contrast, Type 2 diabetes patients with periodontitis had significantly higher levels of BOP than non-diabetes periodontitis patients ($p = 0.03$).

Oxidative status and inflammatory markers

Mean plasma SMAC levels in Type 2 diabetes patients with periodontitis were lower compared with those from diabetes patients without periodontitis ($p = 0.03$) and non-diabetes patients with periodontitis, although the latter difference was not statistically significant (Table 2). This lowered plasma antioxidant capacity in Type 2 diabetes patients with periodontitis was paralleled by a significantly higher level of plasma protein oxidation ($p = 0.007$) compared with that of diabetes patients without periodontitis.

There were no significant differences in fibrinogen levels or total leucocyte,

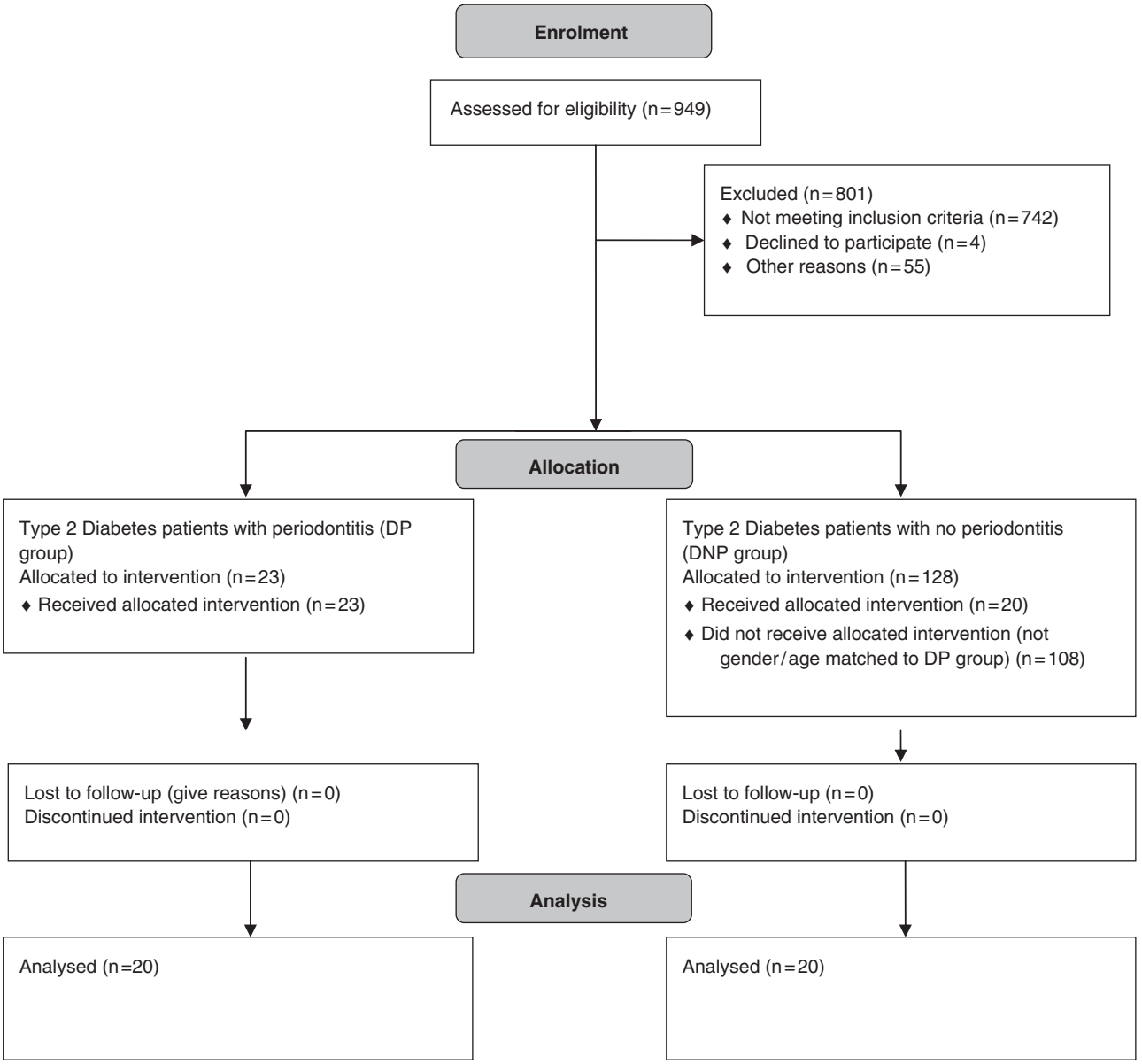

Fig. 1. Flow diagram of Type 2 diabetes patients through study.

neutrophil and lymphocyte counts between patient groups. Median high sensitivity CRP levels did not differ between patients from the two diabetes groups suggesting that periodontitis did not affect this outcome. However, CRP levels in Type 2 diabetes patients with periodontitis were significantly higher compared with those in non-diabetes periodontitis patients ($p = 0.004$; Table 2) suggesting that this outcome was more associated with diabetes rather than periodontitis.

Indicators of glycaemic and lipid control

Significantly higher fasting HbA1c ($p = 0.002$) and glucose ($p < 0.04$) levels were found in Type 2 diabetes patients with periodontitis compared with those from the other two experimental groups (55–73% higher than non-diabetes periodontitis patients; Table 2). This was not the case for the Type 2 diabetes patients without periodontitis whose mean levels of these glycaemic markers were only about 30% higher than those of non-diabetes periodontitis patients.

β-Cell function was significantly depressed in Type 2 diabetes patients with periodontitis compared with that detected in diabetes patients without periodontitis ($p = 0.01$). By contrast, periodontitis had no effect on insulin resistance.

Periodontitis was not associated with differences in plasma lipid status in Type 2 diabetes patients as assessed by levels of total cholesterol, triglycerides, VLDL cholesterol, HDL cholesterol or LDL cholesterol (Table 2). Overall, mean levels of total cholesterol, HDL and LDL cholesterol were higher in non-diabetes patients compared with Type 2 diabetes patients. However, mean levels of triglycerides and VLDL cholesterol tended to be higher and HDL ($p = 0.006$) cholesterol lower in periodontitis patients with Type 2 diabetes compared with non-diabetes patients.

Discussion

Our data demonstrate, for the first time, that Type 2 diabetes patients with

Table 2. Markers of oxidative stress, inflammation, glycaemic control and lipidaemic control [mean+SD (range)]

Marker	*n*	Diabetes, periodontally healthy (DNP)	*n*	Diabetes with periodontitis (DP)	*n*	Non-diabetes periodontitis (NDP)	*p*-Value*
Oxidative stress							
$SMAC_{Plasma}$ (μM Teq/l).	20	523.4+111.1 (342.1–64.8)	20	452.6+100.1 (314.0–85.7)	20	512.8+124.3 (318.6–82.3)	0.03†
Protein carbonyls (nmol/mg IgG)	20	1.99+0.85 (0.49–3.42)	20	2.71+0.94 (1.25–4.15)	–	n.d.	0.007†
Inflammation							
Total leucocytes ($\times 10^9$/l)	19	5.83+1.81 (3.40–11.10)	20	6.23+1.88 (2.40–11.60)	17	6.12+1.80 (3.60–12.30)	NS
Neutrophils ($\times 10^9$/l)	19	3.33+1.38 (1.30–7.27)	20	3.65+1.52 (1.25–8.39)	17	3.71+1.16 (1.97–7.26)	NS
Lymphocytes ($\times 10^9$/l)	19	1.86+0.53 (1.19–3.44)	20	1.94+0.61 (0.85–3.00)	17	1.85+0.74 (1.05–4.09)	NS
Fibrinogen (g/l)	16	3.13+0.80 (2.00–4.00)	17	3.12+0.72 (2.20–4.30)	16	2.82+0.58 (2.00–4.40)	NS
hsCRP§ (mg/l)	20	1.75 (0.70–4.02)	18	1.94 (1.30–3.50)	20	1.23 (0.81–2.02)	0.004‡
Glycaemic control							
HbA1c (%, mmol/mol)	20	6.8(51)+1.2(10) 5.1(32)–0.1(87)	20	8.4(68)+1.5(7) 5.9(41)–10.6(92)	20	5.4(36)+0.2(21) 5.1(32)–5.8(40)	0.002† 0.002‡
Fasting glucose (mmol/l)	20	6.78+1.46 (4.80–10.10)	20	8.61+2.90 (3.80–14.10)	20	5.04+0.43 (4.30–5.60)	0.04† 0.002‡
HOMA-β	19	60.32+27.80 (19.70–120.90)	19	36.58+20.19 (6.50–88.30)	–	n.a.	0.01†
HOMA-IR	19	1.34+1.06 (0.50–4.40)	19	1.14+0.55 (0.40–2.10)	–	n.a.	NS
Lipidaemic control							
Total cholesterol (mmol/l)	20	4.22+0.81 (3.1–5.8)	19	4.55+1.02 (2.7–6.2)	17	5.37+2.20 (3.6–11.7)	NS
Triglycerides (mmol/l)	20	1.73+0.76 (0.79–4.05)	19	1.99+0.90 (0.60–3.70)	17	1.40+0.83 (0.48–3.41)	NS
VLDL Cholesterol (mmol/l)	15	0.71+0.21 (0.4–1.1)	15	0.93+0.44 (0.3–1.7)	16	0.60+0.41 (0.3–1.6)	NS
HDL Cholesterol (mmol/l)	15	1.30+0.32 (0.92–2.01)	15	1.13+0.26 (0.86–1.76)	16	1.54+0.49 (0.86–2.81)	0.006‡
LDL Cholesterol (mmol/l)	15	2.20+0.63 (1.5–3.7)	15	2.42+0.69 (1.3–3.5)	16	3.54+1.74 (1.2–9.5)	0.026‡

*Bonferroni adjusted significance levels for comparisons:
†DP *versus* DNP; and
‡DP *versus* NDP by paired t-test except for hsCRP (Wilcoxon).
§Median (interquartile range).
NS, not significant; hsCRP, high sensitivity C-reactive protein; SMAC, small molecular antioxidant capacity.

periodontitis have augmented plasma biomarkers of oxidative stress as evidenced by the finding of reduced pSMAC combined with increased levels of protein carbonyls, a measure of protein oxidation. In addition, the data show that Type 2 diabetes patients with periodontitis have impaired glycaemic status and exhibit significantly lowered β-cell function and higher levels of HbA1c and fasting glucose than matched patients without periodontitis.

It is important to understand that assays formerly regarded as measuring total antioxidant capacity, are limited by an inability to measure antioxidant enzyme systems, protein antioxidants and the contribution of small molecule antioxidants as co-factors in the complex antioxidant biology of mammals in vivo (Sies 2007). Therefore the SMAC data reported here are from an assay validated and characterized for plasma analyses (Chapple et al. 1997, Maxwell et al. 2006). The results for pSMAC in the non-diabetes periodontitis group were similar to data in studies by our group, which interestingly, did not detect any significant differences in pSMAC between periodontally healthy and diseased, *non-diabetes* patients (Chapple et al. 1997). However, signs of enhanced oxidative stress in Type 2 diabetes patients with periodontitis, as measured by both markers used within this study suggests that periodontitis has a negative effect on the already compromised oxidative status of Type 2 diabetes patients (Brownlee 2001, Evans et al. 2003). Indeed, previous work has shown a reduction in lipid peroxidation, another marker of oxidative stress, in Type 2 diabetes patients following periodontal therapy (Sonoki et al. 2006).

Compromised glycaemic control (fasting glucose and HbA1c levels) in association with periodontitis was identified within the Type 2 diabetes patients in this study and supports previously published data suggesting compromised glucose metabolism in relation to periodontitis (Choi et al. 2011). Although our data do not provide any evidence that periodontitis is associated with altered insulin resistance they do, for the first time, demonstrate compromised β-cell function in association with

periodontitis in Type 2 diabetes patients. Oxidative stress has been implicated in β-cell dysfunction in diabetes (Evans et al. 2003). Our combined finding of increased oxidative stress and reduced β-cell function in Type 2 diabetes patients with periodontitis supports this possibility and suggests that periodontitis amplifies oxidative stress pathways responsible for β-cell dysfunction.

Oxidative stress leads to an up-regulation of pro-inflammatory pathways implicated in the pathogenesis of both periodontitis and diabetes (Brownlee 2001, Evans et al. 2003, Chapple et al. 2007). One possible source of the oxidative stress identified in Type 2 diabetes patients with periodontitis may be hyperactive/reactive neutrophils as both periodontitis and diabetes are associated with neutrophil priming and enhanced ROS release, correlating with both severity of periodontitis and glycaemic control (Fredriksson et al. 2003, Karima et al. 2005, Chapple & Matthews 2007, Chapple et al. 2007, Matthews et al. 2007). It may be hypothesized that hyperactive/reactive neutrophils releasing high levels of extracellular ROS could potentially overwhelm antioxidant defences and contribute to β-cell dysfunction. Further work is required to explore the impact of periodontal inflammation on insulin resistance and β-cell function, specifically along the oxidative stress/inflammation axis.

While our data show that periodontitis is associated with a systemic increase in oxidative stress in Type 2 diabetes patients, evidence that markers of inflammation were similarly affected was equivocal. Neither the total or the differential white blood cell counts, nor fibrinogen levels differed between patient groups, however, post hoc power calculations showed that the study was under-powered for these outcomes. Significantly higher levels of hsCRP were detected in Type 2 diabetes patients with periodontitis compared with non-diabetes periodontitis patients and both diabetes groups had higher mean levels than the non-diabetes group. These data agree with reports that diabetes is associated with increased hsCRP (Barzilay et al. 2001) and suggest that periodontitis further augments this, when Type 2 diabetes and periodontitis co-exist. An association between hsCRP and periodontitis has been reported in Type 2 diabetes patients (Chen et al. 2010) with periodontal therapy reported to reduce CRP in this patient group (D'Aiuto et al. 2006). Furthermore, markers of oxidative stress have been identified as determinants of CRP levels (Abramson et al. 2005) suggesting that the enhanced oxidative stress associated with periodontitis in the Type 2 diabetes patients in this study might have exerted an additional pro-inflammatory effect. That there was no significant difference between hsCRP levels, or the other markers of inflammation measured, between Type 2 diabetes patients with and without periodontitis, is likely to be a reflection of the effects of the anti-inflammatory medications taken by diabetes patients.

Longitudinal studies in systemically healthy periodontitis patients have demonstrated that successful periodontal therapy produces a lower risk plasma lipid profile (D'Aiuto et al. 2006). No significant differences in lipid levels were observed between the Type 2 diabetes groups. Lower mean levels of total and LDL cholesterol were found in both diabetes groups compared with the non-diabetes group but this reduction can probably be attributed to the statin medications taken exclusively by the diabetes groups. However, the lowest HDL and highest triglyceride levels were found in Type 2 diabetes patients with periodontitis and were, for HDL, significantly different from non-diabetes periodontitis patient levels. Statins have modest effects on HDL and triglyceride levels and our data suggest that statin medication did not completely negate the possible dyslipidaemic effect of periodontal inflammation in the Type 2 diabetes patients studied. As low HDL levels and elevated hsCRP are known cardiovascular risk factors, the impact of periodontitis on oxidative stress/inflammatory pathways, insulin production and cardiovascular health needs to be further explored in Type 2 diabetes mellitus.

The principal limitation of this study relates to the small sample size, as evidenced by the low post hoc power that was observed with several outcome measures and was a consequence of strict inclusion criteria necessitating the initial screening of 949 diabetes patients to provide 20 patients for the DP group. Furthermore, exclusion of smokers and tight matching for gender, age and periodontal status to reduce potential confounding factors limited expansion of group numbers. These restrictions at the recruitment stage, to control for potential confounders, limits the generalizations that can be drawn from this study.

Other variables that potentially affect data interpretation are BMI and medications (including statins) that can affect oxidative status; fruit/vegetable intake that can influence antioxidant micronutrient levels; and diabetes duration that can affect glycaemic control. Post-recruitment analysis found no significant differences between the two diabetes study groups for any of these factors and all participants were lifelong non-smokers. Furthermore, as the medication regimes did not differ between diabetes groups, this should not be a factor in interpreting potential effects of periodontitis on diabetes, although some effects might be masked and not detected.

In conclusion, while the data reported here need to be further investigated in large cohort studies and in randomized intervention studies, they do provide evidence that periodontitis has a negative influence on oxidative stress and metabolic status in Type 2 diabetes, and highlight the need to inform Type 2 diabetes patients of the importance of oral health in their overall metabolic and cardiovascular risk management.

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References

Abramson, J. L., Hooper, W. C., Jones, D. P., Ashfaq, S., Rhodes, S. D., Weintraub, W. S., Harrison, D. G., Quyyumi, A. A. & Vaccarino, V. (2005) Association between novel oxidative stress markers and C-reactive protein among adults without clinical coronary heart disease. *Atherosclerosis* **178**, 115–121.

Bahekar, A. A., Singh, S., Saha, S., Molnar, J. & Arora, R. (2007) The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *American Heart Journal* **154**, 830–837.

Barzilay, J. I., Abraham, L., Heckbert, S. R., Cushman, M., Kuller, L. H., Resnick, H. E. & Tracy, R. P. (2001) The relation of markers of inflammation to the development of glucose disorders in the elderly: the cardiovascular health study. *Diabetes* **50**, 2384–2389.

Bretz, W. A., Weyant, R. J., Corby, P. M., Ren, D., Weissfeld, L. & Kritchevsky, S. B. (2005) Inflammatory markers, periodontal diseases, and periodontal infections in an elderly population. *Journal of American Geriatrics Society* **53**, 1532–1537.

Brownlee, M. (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813–820.

Carty, J. L., Bevan, R., Waller, H., Mistry, N., Cooke, M., Lunec, J. & Griffiths, H. R. (2000) The effects of vitamin C supplementation on protein oxidation in healthy volunteers. *Biochemical and Biophysical Research Communication* **273**, 729–735.

Chapple, I. L. C., Brock, G. R., Milward, M. R., Ling, N. & Matthews, J. B. (2007) Compromised GCF total antioxidant capacity in periodontitis: cause or effect? *Journal of Clinical Periodontology* **34**, 103–110.

Chapple, I. L. C., Mason, G. M., Matthews, J. B., Thorpe, G. H. G., Maxwell, S. R. J. & Whitehead, T. (1997) Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. *Annals of Clinical Biochemistry* **34**, 412–421.

Chapple, I. L. C. & Matthews, J. B. (2007) The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontology 2000* **43**, 160–232.

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.

Choi, Y. H., McKeown, R. E., Mayer-Davis, E. J., Liesem, A. D., Song, K. B. & Merchant, A. T. (2011) Association between periodontitis and impaired fasting glucose and diabetes. *Diabetes Care* **34**, 381–386.

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.

D'Aiuto, F., Parkar, M., Nibali, L., Suvan, J., Lessem, J. & Tonetti, M. S. (2006) Periodontal infections cause changes in traditional and novel cardiovascular risk factors: results from a randomized controlled clinical trial. *American Heart Journal* **151**, 977–984.

Evans, J. L., Goldfine, I. D., Maddux, B. A. & Grodsky, G. M. (2003) Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* **52**, 1–8.

Fredriksson, M. I., Gustaffson, A. K., Bergstrom, K. G. & Asman, B. E. (2003) Constitutionally hyperreactive neutrophils in periodontitis. *Journal of Periodontology* **74**, 219–224.

Gomes-Filho, I. S., Freitas Coelho, J. M., Seixas da Cruz, S., Passos, J. S., Teixeira de Freitas, C. O., Aragão Farias, N. S., Amorim da Silva, R., Silva Pereira, M. N., Lima, T. L. & Barreto, M. L. (2011) Chronic Periodontitis and C-reactive Protein Levels. *Journal of Periodontology* **82**, 969–978.

Janket, SJ., Wightman, A., Baird, AE., Van Dyke, TE. & Jones, JA. (2005) Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. *Journal of Dental Research* **84**, 1154–1159.

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* **72**, 868–870.

Khader, Y. S., Dauod, A. S., El-Qaderi, S. S., Alkafajei, A. & Batayha, W. Q. (2006) Periodontal status of diabetics compared with nondiabetics: a meta-analysis. *Journal of Diabetes and its Complications* **20**, 59–68.

Koromantzos, P. A., Makrilakis, K., Dereka, X., Katsilambros, N., Vrotsos, I. A. & Madianos, P. N. (2011) A randomized, controlled trial on the effect of non-surgical periodontal therapy in patients with type 2 diabetes. Part I: effect on periodontal status and glycaemic control. *Journal of Clinical Periodontology* **38**, 142–147.

Kurz, D. J., Decary, S., Hong, Y., Trivier, E., Akhmedov, A. & Erusalimsky, J. D. (2004) Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *Journal of Cellular Science* **117**, 2417–2426.

Loe, H. (1993) Periodontal Disease. The sixth complication of diabetes mellitus. *Diabetes Care* **16**, 329–334.

March, P. D. & Devine, D. (2011) How is the development of dental biofilms influenced by the host? *Journal of Clinical Periodontology* **38** (S11), 28–35.

Matthews, J. B., Wright, H. J., Roberts, A., Cooper, P. R. & Chapple, I. L. C. (2007) Hyperactivity and reactivity of peripheral blood neutrophils in chronic periodontitis. *Clinical and Experimental Immunology* **147**, 255–264.

Maxwell, S., Dietrich, T. & Chapple, I. L. C. (2006) Prediction of serum total antioxidant activity from the concentration of individual serum antioxidants. *Clinica Chimica Acta* **372**, 188–194.

Navarro-Sanchez, AB., 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.

O'Leary, T. J., Drake, R. B. & Naylor, J. E. (1972) The plaque control record. *Journal of Periodontology* **43**, 38.

Saremi, A., Nelson, R. G., Tulloch-Reid, M., Hanson, R. L., Sievens, 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.

Sies, H. (2007) Total antioxidant capacity: appraisal of a concept. *Journal of Nutrition* **137**, 1493–1495.

Sonoki, K., Nakashima, S., Takata, Y., Naito, T., Fujisawa, K., Ootsubo, T., Wakisaka, M., Iwase, M., Iida, M. & Yokota, M. (2006) Decreased lipid peroxidation following periodontal therapy in type 2 diabetic patients. *Journal of Periodontology* **77**, 1907–1913.

Teeuw, W. J., Gerdes, V. E. & Loos, B. G. (2010) Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and meta-analysis. *Diabetes Care* **33**, 421–427.

Tonetti, M., D'Aiuto, F., Nibali, L., Donald, A., Storry, C., Parkar, M., Suvan, J., Hingorani, A. D., Vallance, P. & Deanfield, J. (2007) Treatment of periodontitis and endothelial dysfunction. *New England Journal of Medicine* **356**, 911–920.

Tonetti, M. S., Chapple, I. L. & Working Group 3 of Seventh European Workshop on periodontology. (2011) Biological approaches to the development of novel periodontal therapies – consensus of the Seventh European Workshop on Periodontology. *Journal of Clinical Periodontology* **38** (Suppl. 11), 114–118.

Van der Velden, U., Kuzmanova, D. & Chapple, I. L. (2011) Micronutritional approaches to periodontal therapy. *Journal of Clinical Periodontology* **38** (Suppl. 11), 142–158.

Van Dyke, T. E. (2007) Cellular and molecular susceptibility determinants for periodontitis. *Periodontology 2000* **45**, 10–13.

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Clinical Relevance

Scientific rationale for the study: Oxidative stress is involved in the pathology of both periodontitis and Type 2 diabetes. The co-existence of both diseases may augment the pathological effects of oxidative stress.

Principal findings: Periodontitis was associated with increased oxidative stress and more compromised glycaemic control in Type 2 diabetes patients. Lower insulin secretion may contribute to the impairment of glycaemic control as periodontitis was also associated with reduced β-cell function.

Practical implications: Diagnosis and treatment of periodontitis is essential in Type 2 diabetes patients to minimize the potentially pathogenic effects of cumulative oxidative stress upon β-cell function and glycaemic control.