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# Experimental gingivitis in type 1 diabetics: a controlled clinical and microbiological study

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***Abstract***

**Objective:** To monitor clinical and microbiological changes during experimental gingivitis in type 1 diabetics and non-diabetics.

**Materials and Methods:** Nine type 1 diabetics with good/moderate metabolic control and nine age-gender matched non-diabetics were recruited. Probing pocket depths in all subjects did not exceed 4 mm and none were affected by attachment loss. According to the original model, an experimental 3-week plaque accumulation resulting in experimental gingivitis development and a subsequent 2-week period of optimal plaque control were staged. Subgingival plaque samples were collected at days 0, 21 and 35 from one site per quadrant, pooled and analysed using checkerboard DNA–DNA hybridization.

**Results:** Diabetics (mean age 25.6 ± 5.8 standard deviation (SD), range 16–35 years) had a mean $HbA_{1c}$ level of 8.1 ± 0.7% (SD), while non-diabetics (mean age 24.8 ± 5.7 (SD), range 15–36 years) were metabolically controlled ($HbA_{1c} \leqslant 6.5\%$). Between Days 0, 21 and 35, no statistically significant differences in mean plaque and gingival index scores were observed between diabetics and non-diabetics. At days 7 and 21, however, diabetics showed statistically significantly higher percentages of sites with gingival index scores $\geqslant 2$ compared with non-diabetics. Mean DNA probe counts of the red and orange complex species increased significantly ($p < 0.05$) between days 0 and 21 and decreased significantly ($p < 0.05$) between days 21 and 35 in both groups.

**Conclusion:** Both diabetics and non-diabetics react to experimental plaque accumulation with gingival inflammation. Type 1 diabetics, however, develop an earlier and higher inflammatory response to a comparable bacterial challenge.

Key words: bacterial challenge; experimental gingivitis; gingival inflammation; gingivitis; subgingival microbiota; type 1 diabetes

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Type 1 diabetes mellitus is a systemic autoimmune disease characterized by insufficient insulin secretion of the pancreatic $\beta$ cells. This metabolic disorder involves abnormal regulation of both glucose and lipid metabolisms resulting in hyperglycemia and hyperlipidemia. Major systemic complications of uncontrolled diabetes such as retinopathy, nephropathy, neuropathy, vascular and cardiovascular disorders are the result of a prolonged hyperglycemic and hyperlipidemic state. To this list of long-term diabetic complications, periodontal diseases have been added as the sixth complication (Löe 1993).

Periodontal diseases are multifactorial diseases characterized by a chronic inflammatory process initiated by the presence of a Gram-negative anaerobic bacterial infection (Haffajee & Socransky 1994). Although chronic periodontitis affects an estimated 7–13% (moderate–severe) to 22–40% (mild) of the adult population, respectively (Papapanou 1996, Norderyd & Hugoson 1998, Albandar et al. 1999), studies have suggested that the bacterial challenge accounts for a relatively small proportion (around 20%) of the variance in disease expression (Grossi et al. 1994). On the other hand, studies involving adult twins have demonstrated that host factors account for about 50% of the variance in disease expression (Michalowicz et al. 1991). Although bacterial biofilms are an essential prerequisite for periodontal disease development, a susceptible host is required. Hence, a new concept of periodontal disease pathogenesis has been proposed (Page et al. 1997). In this recent concept, the model of a biofilm-

dominated process was extended to one in which the host response emerged as the dominant effect modifier in periodontal disease expression. Data indicated that subjects exhibiting generalized severe ''chronic'' periodontitis may present with an exaggerated immunoinflammatory response to the bacterial challenge (Salvi et al. 1998). Acquired risk factors, such as diabetes mellitus, may account for a hyperinflammatory host response to the bacterial challenge (Salvi et al. 1997a, b), thereby influencing periodontal disease susceptibility, progression and outcome of therapy.

The association between diabetes mellitus and periodontal diseases has long been debated on the basis of conflicting results. On one hand, several reports indicated little or no association between diabetes and periodontal disease status (Barnett et al. 1984, Tervonen & Knuuttila 1986, Rosenthal et al. 1988, Hayden & Buckley 1989, Sandholm et al. 1989, Sastrowijoto et al. 1990). Others, however, including large epidemiological population-based surveys, tended to support a higher prevalence and severity of periodontal diseases in diabetic patients compared with non-diabetic controls (Hugoson et al. 1989, Emrich et al. 1991, Katz et al. 1991, Tervonen & Oliver 1993, Oliver & Tervonen 1993, 1994, Seppälä & Ainamo 1993, 1994, Grossi et al. 1994). This relationship appears to be correlated with the status of metabolic control as indicated by blood glucose and glycosylated haemoglobin levels, the onset and duration of the diabetic condition and the presence of medical complications. Moreover, the prevalence of periodontal disease increased among diabetics after puberty and with an increasing age of the population (Cianciola et al. 1982, Gusberti et al. 1983, Rylander et al. 1987, Hugoson et al. 1989, de Pommereau et al. 1992, Karjalainen & Knuuttila 1996). This susceptibility, however, did not correlate with increased levels of plaque and calculus accumulations (Rosenthal et al. 1988, Seppälä et al. 1993). Collectively, these studies support the concept of a mutual relationship between the two diseases, particularly in subjects with poorly controlled diabetes mellitus.

Therefore, the aim of the present clinical experiment was to monitor clinical and microbiological changes occurring during a 21-day period of undisturbed plaque accumulation and a subsequent 14-day period of meticulous plaque control in type 1 diabetics and to compare these changes to those of age–gender matched non-diabetics.

## Materials and Methods

The study was designed and conducted as a controlled 35-day experimental gingivitis following the model proposed by Löe et al. (1965). The study protocol was submitted to and approved by the Ethical Committee of the Canton of Berne, Switzerland.

### Patient selection

Type 1 diabetics attending on a regular basis the Department of Endocrinology and Diabetology of the University Hospital (Inselspital) of the University of Berne were screened and recruited. The diagnosis of type 1 diabetes was based on the laboratory and clinical criteria routinely applied in the Department of Endocrinology and Diabetology of the University of Berne. Subject recruitment and matching was performed by one examiner. Assessment of clinical parameters as well as microbiological sampling and processing was carried out by another examiner blinded to the medical status of the patient. In order to be enrolled, diabetics had to meet the following inclusion criteria:

- Age $\leqslant 40$ years.
- Confirmed diagnosis of diabetes mellitus type 1.
- Insulin therapy $\geqslant 12$ months.
- Good/moderate metabolic control as determined by mean glycosylated haemoglobin level of $\leqslant 9.5\%$ in the past 12 months.
- No diabetes-related systemic complications.
- No other relevant medical conditions.
- A dentition with a minimum of 24 permanent teeth.
- Pocket probing depth $\leqslant 4$ mm at six sites/tooth.
- Willingness to comply with all study requirements and signing informed consent.

Patients were excluded for one of the following conditions:

- Diabetes type 2.
- Smoking $>5$ cigarettes/day.
- Antibiotic therapy within 3 months of the baseline examination.
- Chronic medication known to affect the periodontal status within 4 weeks of the baseline examination.
- Pregnant or lactating females.
- Uncontrolled type 1 diabetes.
- Periodontal attachment loss anywhere in the dentition.
- Alcohol or drug abuse.
- Incipient carious lesions which could potentially exacerbate during a period of oral hygiene abstinence.
- Stomatological diseases.

Age–gender matched non-diabetics (mean glycosylated haemoglobin level $\leqslant 6.5\%$) subjects were recruited from the pool of the Department of Periodontology and Fixed Prosthodontics of the University of Berne.

### Clinical assessments and procedures

After a prophylaxis procedure, including instruction in optimal oral hygiene practices and a thorough scaling and polishing of the entire dentition, diabetic and non-diabetic subjects were asked to perform optimal tooth cleaning procedures for a period of 3–4 weeks. The ability to perform proper plaque control was assessed for each patient before entering the experimental phase. At the end of the pre-experimental phase, subjects were evaluated for the presence of plaque and gingivitis, the objective being the achievement of low plaque (PlI) and gingival index (GI) scores at baseline compatible with gingival health.

Subsequently, the subjects were asked to abstain from all oral hygiene practices for a period of 21 days during which plaque was allowed to accumulate on all tooth surfaces. Following this period of no-oral-hygiene, all subjects resumed their optimal mechanical plaque control practices to reach pre-experimental levels of oral cleanliness and gingival health. At baseline (day 0), after 7, 14 and 21 days of undisturbed plaque accumulation and 14 days following re-institution of oral hygiene practices (i.e. day 35), the following clinical parameters were assessed at six sites of each tooth in the entire dentition:

- Supragingival plaque accumulation according to the criteria of the PI system Silness & Löe 1964).
- Gingival health or inflammation according to the criteria of the GI system (Löe & Silness 1963).

Third molars were excluded from the assessments.

#### Subgingival bacterial sampling and analysis

After removal of supragingival plaque and isolation of the area with cotton rolls, subgingival plaque samples were collected with individual sterile Gracey curettes from pre-determined sites (i.e. disto-lingual sites of first molars) in each quadrant. The sites for plaque sampling were selected at baseline. Plaque samples were collected at baseline and again at Days 21 and 35. The four subgingival samples were pooled in an Eppendorf tube containing 600 µl of TE solution (10 mM tris-HCl, 1 mM EDTA, pH 7.6) (Syed et al. 1980). After dispersion, 400 µl of 0.5 M NaOH was added.

Counts of 40 subgingival species were determined in each pooled plaque sample using a modification (Haffajee et al. 1997) of the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994). The samples were lysed and the DNA placed in lanes on a nylon membrane using a minislot device (Immunetics, Cambridge, MA, USA). After fixation of the DNA to the membrane, the membrane was placed in a miniblotter 45 (Immunetics) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 40 subgingival bacterial species were hybridized in individual lanes of the miniblotter. After hybridization, the membranes were washed at high stringency and the DNA probes detected using antibody to digoxigenin conjugated with alkaline phosphatase and applying chemifluorescence detection. The probes and their source strains have been described by Ximénez-Fyvie et al. (2000). Signals were detected using AttoPhos substrate (Amersham Life Science, Arlington Heights, IL, USA) and a Storm Fluorimager 840 (Molecular Dynamics, Sunnyvale, CA, USA). Two lanes in each run contained standards at concentrations of $10^5$ and $10^6$ cells of each species. Signals were evaluated using the Storm Fluorimager 840 (Amersham Biosciencies, Piscataway, NJ, USA) and converted to absolute counts by comparison with the standards on the same membrane.

The outline of the experimental procedures is summarized in Fig. 1.

#### Statistical analysis

The null hypothesis of no significant clinical and microbiological differences during an experimental gingivitis period between diabetic patients with good/moderate metabolic control and non-diabetic controls was tested. Clinical data are presented as mean values ± 1 standard deviation (SD). Paired and unpaired *t*-tests were used for differences in PlI and GI scores. Odds ratios (OR) were estimated by the Mantel–Haenszel test. Differences in mean total DNA probe counts and relative proportions of the complex species were tested using the Wilcoxon rank sum test. The level of statistical significance was set at $\alpha = 0.05$.

## Results

All 18 subjects entered into the study completed the final examination at Day 35.

As shown in Table 1, there were no significant differences between diabetics and non-diabetics with respect to age, race, gender and the number of teeth present. The mean age was 25.6 ± 5.8 years (range 16–35) for the diabetic and 24.8 ± 5.7 years (range 15–36) for the non-diabetic cohorts, respectively. The mean percentage (%) of glycosylated haemoglobin ($HbA_{1c}$) amounted to 8.1 ± 0.7% (SD) in the diabetics and to ⩽6.5% in all non-diabetic subjects.

Figure 2 illustrates mean full-mouth PlI scores ± SD at baseline (day 0) and days 7, 14, 21 and 35 in diabetics (D) and non-diabetics (ND). Both diabetics and non-diabetics displayed a statistically significant increase ($p < 0.05$) in the mean PlI score during 3 weeks of undisturbed plaque accumulation, i.e. from baseline (D: 0.2 ± 0.1; ND: 0.1 ± 0.1) to day 21 (D: 2.0 ± 0.1; ND: 1.9 ± 0.3). Within both groups, a similar statistically significant decrease ($p < 0.05$) in the mean PlI score was observed after re-institution of mechanical plaque control, i.e. from days 21 to 35 (D: 0.1 ± 0.1; ND: 0.1 ± 0.05). No statistically significant differences, however, were observed when comparing the mean PlI of diabetics to those of non-diabetics at any observation periods.

Figure 3 shows mean full-mouth GI scores ± SD at baseline (day 0) and days 7, 14, 21 and 35 in diabetics and non-diabetics. No statistically significant difference was found at day 0 between the two groups. Both diabetics and non-diabetics displayed a statistically significant increase ($p < 0.05$) in the mean GI score from baseline (D: 0.3 ± 0.3; ND: 0.2 ± 0.1) to Day 21 (D: 1.4 ± 0.1; ND: 1.2 ± 0.1). Within both groups, a similar statistically significant

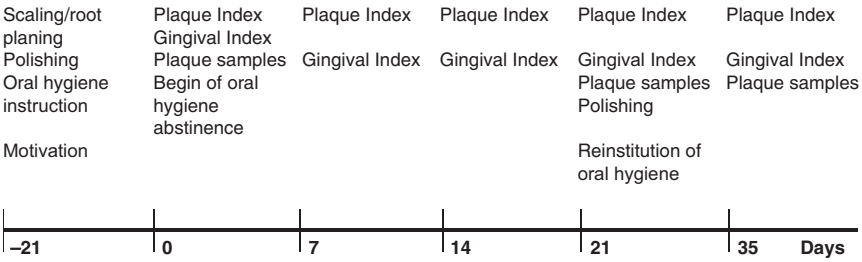


*Fig. 1.* Study design of experimental gingivitis (Löe et al. 1965).

*Table 1.* Demographic data of both type 1 diabetics and matched non-diabetic controls

| | Type 1 diabetics | Non-diabetics |
|---|---|---|
| *N* | 9 | 9 |
| Age (years) ± SD | 25.6 ± 5.8 | 24.8 ± 5.7 |
| Age range (years) | 16–35 | 15–36 |
| Male/female | 3/6 | 3/6 |
| Race | Caucasian | Caucasian |
| Teeth present ± SD | 27.0 ± 1.4 | 27.6 ± 1.3 |
| Mean $HbA_{1c}$(%) | 8.1 ± 0.7 | ⩽6.5 |
| Duration of diabetes (years) ± SD | 9.0 ± 5.3 | – |

SD, standard deviation.

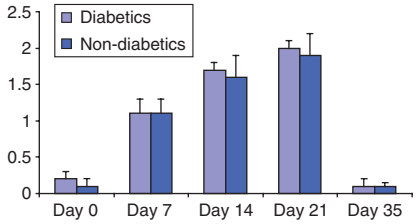


*Fig. 2.* Mean full-mouth plaque index scores at baseline (day 0) and days 7, 14 and 21 of experimental plaque accumulation and at day 35 (after 2 weeks of re-institution of oral hygiene practices). No statistically significant differences were found between diabetics and non-diabetics.

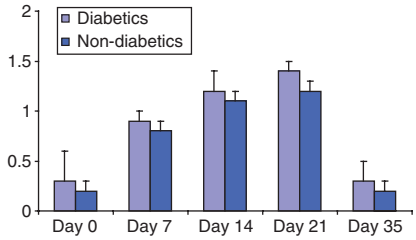


*Fig. 3.* Mean full-mouth gingival index scores at baseline (day 0) and days 7, 14 and 21 of experimental plaque accumulation and at Day 35 (after 2 weeks of re-institution of oral hygiene practices). Statistically significant differences were found between diabetics and non-diabetics after 7 ($p<0.05$) and 21 days ($p<0.01$).

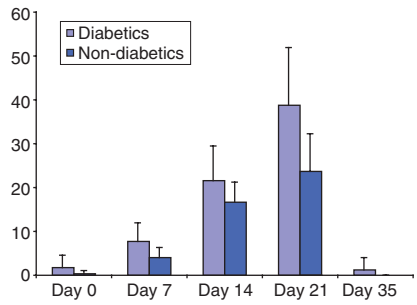


*Fig. 4.* Mean percentages of sites with gingival index scores $\geqslant 2$ at baseline (day 0) and days 7, 14 and 21 of experimental plaque accumulation and at day 35 (after 2 weeks of re-institution of oral hygiene practices). Statistically significant differences were found between diabetics and non-diabetics after 7 ($p<0.05$) and 21 days ($p<0.05$).

decrease ($p<0.05$) in GI scores was observed during the resolution period of gingival inflammation, i.e. from days 21 to 35 (D: $0.3 \pm 0.2$; ND: $0.2 \pm 0.1$). After 7 (D: $0.9 \pm 0.1$; ND: $0.8 \pm 0.1$) ($p<0.05$) and 21 (D: $1.4 \pm 0.1$; ND: $1.2 \pm 0.1$) ($p<0.01$) days, however, diabetics displayed statistically significant higher mean GI scores compared with non-diabetic controls.

Figure 4 illustrates mean% $\pm$ SD of sites with GI scores $\geqslant 2$ at baseline (day 0) and days 7, 14, 21 and 35 in diabetics and non-diabetics. In both diabetics and non-diabetics, a statistically significant increase ($p<0.05$) in the % of bleeding gingival sites was observed from baseline (D: $1.8 \pm 2.8$; ND: $0.3 \pm 0.7$) to day 21 (D: $38.8 \pm 13.2$; ND: $23.6 \pm 8.7$). Within both groups, a comparable statistically significant decrease ($p<0.05$) in the % of GI scores $\geqslant 2$ was observed from days 21 to 35 (D: $1.2 \pm 2.9$; ND: $0.0 \pm 0.0$). After 7 (D: $7.8 \pm 4.1$; ND: $4.0 \pm 2.4$) ($p<0.05$) and 21 (D: $38.8 \pm 13.2$; ND: $23.6 \pm 8.7$) ($p<0.05$) days of development of gingival inflammation, however, diabetics displayed statistically significant higher % of bleeding gingival sites. After 21 days, diabetics were 16 times more likely to display a substantial % of bleeding sites (i.e. $\geqslant 35\%$) compared with non-diabetics (OR 16, 95% confidence interval (CI): 1.3–194.0, $p<0.03$).

Figure 5 shows total DNA probe counts $\times 10^5$ from pooled subgingival plaque samples collected at baseline (day 0) and days 21 and 35. No statistically significant differences were detected between diabetics and non-diabetics at baseline and at days 21 and 35. In both diabetics and non-diabetics, a statistically significant increase ($p<0.05$) in total DNA probe counts was observed from baseline (D: $9.5 \times 10^4 \pm 6.7 \times 10^4$; ND: $5.0 \times 10^4 \pm 4.7 \times 10^4$) to day 21 (D: $15.5 \times 10^4 \pm 6.2 \times 10^4$; ND: $16.2 \times 10^4 \pm 4.8 \times 10^4$).

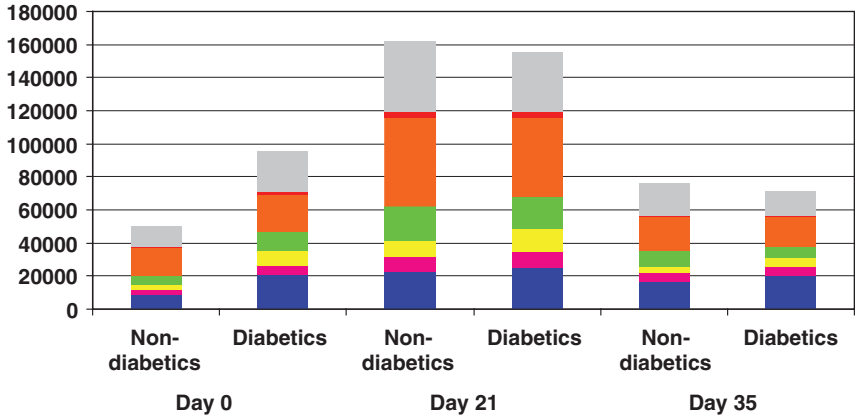


*Fig. 5.* Mean total DNA bacterial counts at baseline (day 0) and day 21 of experimental plaque accumulation and at day 35 (after 2 weeks of re-institution of oral hygiene practices). No statistically significant differences were found between diabetics and non-diabetics.

In particular, diabetics showed from baseline to Day 21 a statistically significant increase ($p<0.05$) in DNA probe counts of the red and orange complex species. In non-diabetics, a statistically significant increase ($p<0.05$) was noted from baseline to day 21 for the red, orange, green and blue complex species. Within both groups, a statistically significant decrease ($p<0.05$) in total DNA probe counts was observed from days 21 to 35 (D: $7.1 \times 10^4 \pm 3.5 \times 10^4$; ND: $7.5 \times 10^4 \pm 5.4 \times 10^4$). At day 35, samples from diabetics harboured significantly fewer ($p<0.05$) DNA probe counts of red, orange, green and yellow complex species compared with day 21. In non-diabetics, a significant decrease ($p<0.05$) in DNA probe counts from days 21 to 35 was observed for red, orange and green complex species.

Figure 6 illustrates the proportions of bacterial complex species from subgingival plaque samples collected at baseline (day 0) and days 21 and 35. No statistically significant differences were detected between diabetics and non-diabetics at baseline and at days 21 and 35. From baseline to day 21, a statistically significant ($p<0.05$) increase was observed for orange complex species in diabetics and for red complex species in non-diabetics, respectively. Concomitantly, in both diabetics and non-diabetics, a statistically significant ($p<0.05$) decrease was noted for blue complex species. From days 21 to 35, a statistically significant ($p<0.05$) decrease was noted for red complex species in diabetics and for red and orange complex species in non-diabetics, respectively. During the same time period, a statistically significant ($p<0.05$) increase in blue

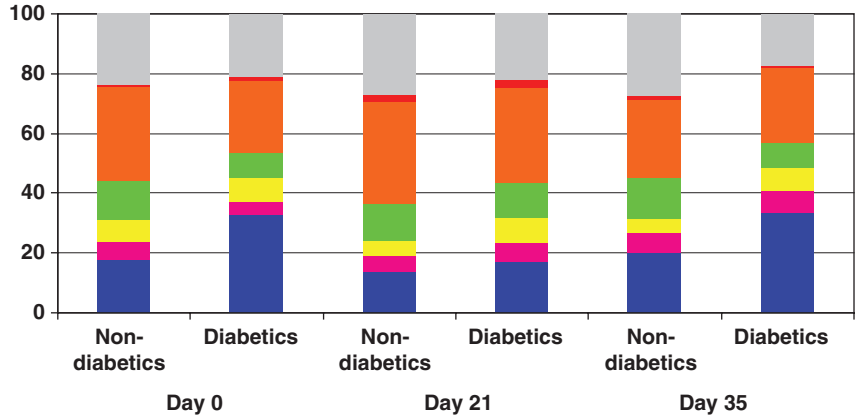


*Fig. 6.* Mean DNA proportions of bacterial complexes at baseline (day 0) and day 21 of experimental plaque accumulation and at day 35 (after 2 weeks of re-institution of oral hygiene practices). No statistically significant differences were found between diabetics and non-diabetics.

complex species was observed in both groups.

## Discussion

The present clinical experiment has demonstrated that, irrespective of the diabetic status, a cause and effect relationship was established between the bacterial challenge and the host response as visualized by the development of gingival inflammation resulting from undisturbed plaque accumulation. In that respect, the results fully confirmed those of the original model (Löe et al. 1965). The elimination of the bacterial biofilm resulted in complete resolution of gingivitis and the establishment of gingival health. Owing to the fact that pre-experimental levels of gingival health were reached by the mere control of the bacterial challenge in both diabetics and non-diabetics, the basic concepts for prevention of gingival and periodontal disease apply to type 1 diabetics as well.

Nevertheless, the development of experimental gingivitis in diabetics appeared at an earlier time point and was more pronounced than that observed in non-diabetic controls. Since no differences were identified in the bacterial amount and composition of the two groups, this clearly indicates a hyperinflammatory response to the same bacterial challenge in young type 1 diabetics with good/moderate metabolic control. Similar results of a higher inflammatory response during experimental plaque accumulation in systemically healthy elderly (e.g. $>65$ years) compared with young healthy adults were reported by Holm-Pedersen et al. (1975). However, in that study, the influence of a more accentuated plaque accumulation in the elderly group cannot be ignored.

Previous studies have provided evidence that the composition of the subgingival biofilm of periodontally healthy and diseased sites did not significantly differ between diabetics and non diabetics (Sastrowijoto et al. 1989, 1990, Mandell et al. 1992, Sbordone et al. 1995). In the present study, PlI scores did not significantly differ between the two groups at any time point of the experimental protocol. Moreover, no significant differences were found with respect to the composition of the subgingival biofilm between diabetics and non-diabetics at baseline, Days 21 and 35. During the period of undisturbed plaque accumulation (i.e. days 0–21), a significant increase in total DNA probe counts was observed in both groups. In particular, the significant increase in the red and orange complex species reflected a shift towards more Gram-negative anaerobic bacterial proportions. This altered microbiota resembled that observed in chronic periodontitis subjects (Ximénez-Fyvie et al. 2000). Clinically, this microbial shift was accompanied by a transition from gingival health to gingivitis. After re-institution of oral hygiene practices (i.e. from days 21 to 35), a reverse shift of the microbiota towards more Gram-positive facultative aerobic proportions was observed. Specifically, a decrease in proportions of red and orange complex species was accompanied by a significant increase in proportions of blue complex species in both groups. This may reflect the re-establishment of a microbiota compatible with gingival health (Ximénez-Fyvie et al. 2000). This transient shift of the subgingival microbiota during the experimental period from a health-associated to a disease-associated microbiota and back to a health-associated microbiota following re-institution of optimal oral hygiene appears to be the consequence rather than the cause of the immunoinflammatory response. Such longitudinal sequences have been documented following the placement of overhanging fillings into the subgingival environment of otherwise healthy young volunteers (Lang et al. 1983). Gingival bleeding preceded the peak levels of the colonization of the area with Gram-negative anaerobic bacteria. The fact that gingival inflammation, as defined by bleeding on marginal probing, preceded peak levels of Gram-negative anaerobic bacteria was based on the hypothesis that bacterial nutrients could be provided by bleeding sites associated with inflamed gingival tissues.

Previously, several studies reported that, despite similar plaque levels, diabetics presented an increased frequency and severity of gingival inflammation compared with non-diabetics (Bernick et al. 1975, Cianciola et al. 1982, Ervasti et al. 1985, Sandholm et al. 1989, Katz et al. 1991, de Pommereau et al. 1992, Pinson et al. 1995).

In the present study, the level of metabolic control in the diabetics was good-to-moderate ($HbA_{1C}$ $8.1 \pm 0.7\%$). In the diabetic group, however, statistically significant higher mean GI scores were reached after 7 and 21 days. At day 21, diabetics exhibit a higher % of bleeding sites compared to the non-diabetic controls. In diabetics, this reflects an OR of 16 (95% CI: 1.3–194.0, $p < 0.03$), i.e. a 16 times higher probability of reacting to a 3-week undisturbed bacterial challenge with substantial bleeding (i.e. 35% with GI $\geqslant 2$) compared with non-diabetic controls. The findings of the present study are in disagreement to those of Ervasti et al. (1985) who found that the proportion of bleeding surfaces was significantly smaller in adult diabetics compared with non-diabetic controls. However, a true comparison of the present longitudinal study with the latter cross-sectional survey may not be appropriate. Nevertheless, explanations for the discrepancies in the results may be attributable to the different age cohorts studied in the two studies or – more likely – to the various

definitions of metabolic control. The present study incorporated diabetics with metabolic control that was considered good-to-moderate with a mean $HbA_{1c}$ of 8.1%, while the latter study considered an $HbA_{1c} \leqslant 10$ to be a good metabolic control in their patients. If the metabolic control was poor as defined by an $HbA_{1c} \geqslant 12$, the bleeding tendencies were higher than those of the well and moderately controlled diabetics and non-diabetic controls. Similar findings were reported (Gislén et al. 1980) in another cross-sectional survey in which metabolic control had a significant impact on gingival conditions. In that study there were no differences in the GI between diabetic and healthy young subjects in the presence of low plaque scores (PlI 0–0.69). However, in the presence of substantial amounts of plaque (PlI 0.70–1.39 and PlI 1.40–2.00) only the young type 1 diabetics with poor metabolic control ($HbA_{1c} \geqslant 160$) exhibited statistically significantly higher GI scores compared with the non-diabetic controls.

In other studies, however, no influence of the metabolic control on the gingival conditions was found in young diabetics (Sandholm et al. 1989, de Pommereau et al. 1992).

In the present longitudinal study, good/moderate levels of $HbA_{1C}$ in the diabetic population were assured and, hence, a relationship between the metabolic control and the amount of gingival bleeding can be excluded. On the other hand, young well-controlled type 1 diabetics still seem to be more susceptible to the initiation of gingival inflammation upon the bacterial challenge.

In conclusion, both diabetics and non-diabetics reacted to experimental plaque accumulation with gingival inflammation. Young type 1 diabetics, however, developed an earlier and significantly higher inflammatory response to a comparable bacterial challenge than that of age and gender matched non-diabetic controls. Analysis of gingival crevicular fluid inflammatory mediators will provide further insight into the development of a different inflammatory reaction between diabetics and non-diabetics.

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## References

Albandar, J. M., Brunelle, J. A. & Kingman, A. (1999) Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. *Journal of Periodontology* **70**, 13–29.

Barnett, M. L., Baker, R. L., Yancey, J. M., MacMillan, D. R. & Kotoyan, M. (1984) Absence of periodontitis in a population of insulin dependent diabetes mellitus (IDDM) patients. *Journal of Periodontology* **55**, 402–405.

Bernick, S. M., Cohen, D. W., Baker, R. L. & Laster L, . (1975) Dental disease in children with diabetes mellitus. *Journal of Periodontology* **46**, 241–245.

Cianciola, L., Park, B. H., Bruck, E., Mosovich, L. & Genco, R. J. (1982) Prevalence of periodontal disease in insulin-dependent diabetes mellitus (juvenile diabetes). *Journal of the American Dental Association* **104**, 653–660.

de Pommereau, V., Dargent, P. C., Robert, J. J. & Brion, M. (1992) Periodontal status in insulin dependent diabetic adolescents. *Journal of Clinical Periodontology* **19**, 628–632.

Emrich, L. J., Shlossman, M. & Genco, R. J. (1991) Periodontal disease in non-insulin dependent diabetes mellitus. *Journal of Periodontology* **62**, 123–131.

Ervasti, T., Knuuttila, M. L. E., Pohjamo, L. & Haukipuro, K. (1985) Relation between control of diabetes mellitus and gingival bleeding. *Journal of Periodontology* **56**, 154–157.

Gislén, G., Nilsson, K. O. & Matsson, L. (1980) Gingival inflammation in diabetic children related to degree of metabolic control. *Acta Odontologica Scandinavica* **38**, 241–246.

Grossi, S. G., Zambon, J. J., Ho, A. W., Koch, G., Dunford, R. G., Machtei E, . E., Norderyd, O. M. & Genco, R. J. (1994) Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *Journal of Periodontology* **65**, 260–267.

Gusberti, F. A., Syed, S. A., Bacon, G., Grossman, N. & Loesche, W. J. (1983) Puberty gingivitis in insulin-dependent diabetic children. I. Cross-sectional observations. *Journal of Periodontology* **54**, 714–720.

Haffajee, A. D., Cugini, M. A., Dibart, S., Smith, C., Kent, R. L. & Socransky, S. S. (1997) The effect of SRP on the clinical and microbiological parameters of periodontal diseases. *Journal of Clinical Periodontology* **24**, 324–334.

Haffajee, A. D. & Socransky, S. S. (1994) Microbial etiological agents of destructive periodontal diseases. *Periodontology 2000* **5**, 78–111.

Hayden, P. & Buckley, L. A. (1989) Diabetes mellitus and periodontal disease in an Irish population. *Journal of Periodontal Research* **24**, 298–302.

Holm-Pedersen, P., Agerbaek, N. & Theilade, E. (1975) Experimental gingivitis in young and elderly individuals. *Journal of Clinical Periodontology* **2**, 14–24.

Hugoson, A., Thorstensson, H., Falk, H. & Kuylenstierna, J. (1989) Periodontal conditions in insulin-dependent diabetics. *Journal of Clinical Periodontology* **16**, 215–223.

Karjalainen, K. M. & Knuuttila, M. L. (1996) The onset of diabetes and poor metabolic control increases gingival bleeding in children and adolescents with insulin-dependent diabetes mellitus. *Journal of Clinical Periodontology* **23**, 1060–1067.

Katz, P. P., Wirthlin, M. R., Szpunar, S. M., Selby, J. V., Sepe, S. J. & Showstack, J. A. (1991) Epidemiology and prevention of periodontal disease in individuals with diabetes. *Diabetes Care* **14**, 375–385.

Lang, N. P., Kiel, R. A. & Anderhalden, K. (1983) Clinical and microbiological effects of subgingival restorations with overhanging or clinically perfect margins. *Journal of Clinical Periodontology* **10**, 563–578.

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

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* **21**, 533–551.

Löe, H., Theilade, E. & Börglum Jensen, S. (1965) Experimental gingivitis in man. *Journal of Periodontology* **36**, 5–15.

Mandell, R. L., DiRienzo, J., Kent, R., Joshipura, K. & Haber, J. (1992) Microbiology of healthy and diseased periodontal sites in poorly controlled insulin-dependent diabetics. *Journal of Periodontology* **63**, 274–279.

Michalowicz, B. S., Aeppli, D., Virag, J. G., Klump, D.G, Hinrichs, J. E., Segal, N. S., Bouchard, T. J. Jr & Pihlstrom, B. L. (1991) Periodontal findings in adult twins. *Journal of Periodontology* **62**, 293–299.

Norderyd, O. & Hugoson, A. (1998) Risk of severe periodontal disease in a Swedish adult population. A cross-sectional study. *Journal of Clinical Periodontology* **25**, 1022–1028.

Oliver, R. C. & Tervonen, T. (1993) Periodontitis and tooth loss: comparing diabetics with the general population. *Journal of the American Dental Association* **124**, 71–76.

Oliver, R. C. & Tervonen, T. (1994) Diabetes – a risk factor for periodontitis in adults? *Journal of Periodontology* **65**, 530–538.

Page, R. C., Offenbacher, S., Schroeder, H. E., Seymour, G. J. & Kornman, K. S. (1997) Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. *Periodontology 2000* **14**, 216–248.

Papapanou, P. N. (1996) Periodontal diseases: epidemiology. *Annals of Periodontology* **1**, 1–36.

Pinson, M., Hoffman, W. H., Garnick, J. J. & Litaker, M. S. (1995) Periodontal disease and type 1 diabetes mellitus in children and adolescents. *Journal of Clinical Peridontology* **22**, 118–123.

Rosenthal, I. M., Abrams, H. & Kopczyk, R. A. (1988) The relationship of inflamatory periodontal disease to diabetic status in insulin dependent diabetes mellitus patients. *Journal of Clinical Periodontology* **15**, 425–429.

Rylander, H., Ramberg, P., Blohme, G. & Lindhe, J. (1987) Prevalence of periodontal disease in young diabetics. *Journal of Clinical Periodontology* **14**, 38–43.

Salvi, G. E., Brown, E. C., Fujihashi, K., Kiyono, H., Smith, F. W., Beck, J. D. & Offenbacher, S. (1998) Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. *Journal of Periodontal Research* **33**, 212–225.

Salvi, G. E., Collins, J. G., Yalda, B., Arnold, R. R., Lang, N. P. & Offenbacher, S. (1997a) Monocytic TNFα secretion patterns in IDDM patients with periodontal diseases. *Journal of Clinical Periodontology* **24**, 8–16.

Salvi, G. E., Yalda, B., Collins, J. G., Jones, B. H., Smith, F. W., Arnold, R. R. & Offenbacher, S. (1997b) Inflammatory mediator response as a potential marker for periodontal diseases in IDDM patients. *Journal of Periodontology* **68**, 127–135.

Sandholm, L., Swanljung, O., Rytomaa, I., Karpio, E. A. & Maenpaa, J. (1989) Periodontal status of finnish adolescents with insulin-dependent diabetes mellitus. *Journal of Clinical Periodontology* **16**, 617–620.

Sastrowijoto, S. H., Hillemans, P., van Steenbergen, T. J., Abraham-Inpijn, 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 Steenbergen, T. J., Hillemans, P., Hart, A.A, de Graaff, J. & Abraham-Inpijn, L. (1990) Improved metabolic control, clinical periodontal status and subgingival microbiology in insulin-dependent diabetes mellitus. *Journal of Clinical Periodontology* **17**, 232–242.

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.

Seppälä, B. & Ainamo, J. (1994) A site-by-site follow-up study on the effect of controlled *versus* poorly controlled insulin-dependent diabetes mellitus. *Journal of Clinical Periodontology* **21**, 161–165.

Seppälä, B., Seppälä, M. & Ainamo, J. (1993) A longitudinal study on insulin-dependent diabetes mellitus and periodontal disease. *Journal of Clinical Periodontology* **20**, 161–165.

Silness, J. & Löe, H. (1964) Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal disease. *Acta Odontologica Scandinavica* **22**, 121–135.

Socransky, S. S., Smith, C., Martin, L., Paster, B. J., Dewhirst, F. E. & Levin, A. E. (1994) ''Checkerboard'' DNA–DNA hybridization. *Biotechniques* **17**, 788–793.

Syed, S. A., Svanberg, M. & Svanberg, G. (1980) The predominant cultivable dental plaque flora of beagle dogs with gingivitis. *Journal of Periodontal Research* **15**, 123–136.

Tervonen, T. & Knuuttila, M. (1986) Relation of diabetes control to periodontal pocketing and alveolar bone level. *Oral Surgery Oral Medicine Oral Pathology* **61**, 346–349.

Tervonen, T. & Oliver, R. C. (1993) Long-term control of diabetes mellitus and periodontitis. *Journal of Clinical Periodontology* **20**, 431–435.

Ximénez-Fyvie, L. A., Haffajee, A. D. & Socransky, S. S. (2000) Microbial composition of supra-and subgingival plaque in subjects with adult periodontitis. *Journal of Clinical Periodontology* **27**, 722–732.

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