DEPARTMENT OF ORAL MEDICINE
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PERIODONTAL DISEASE IN INSULIN–DEPENDENT DIABETICS

BEDIA SAFKAN–SEPPÄLÄ

Academic dissertation

To be publicly discussed, with the assent of the Faculty of Medicine of the University of Helsinki, in the main auditorium of the Institute of Dentistry on February 2, 2001, at 12 noon.

Helsinki 2001
Key words: connective tissue, gingiva, periodontal disease; cross-sectional study, longitudinal study; diabetes mellitus, insulin-dependent; dark field microscopy, electron microscopy.

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The aim of this investigation was to relate the effects of the metabolic control of adult insulin–dependent diabetes mellitus to the periodontal health of these patients. It has been proposed that long–term poorly controlled insulin–dependent diabetic (PIDD) subjects are at risk for periodontal disease. We compared the periodontal health status of PIDD subjects with controlled insulin–dependent diabetic (CIDD) subjects in cross–sectional and longitudinal studies. The proportional distributions of bacterial morphotypes, especially spirochetes, were also studied. Histopathological features of gingival tissues were evaluated in 19 PIDD, 10 CIDD, and 10 non–diabetic control subjects.

At baseline examination, 55 PIDD and 41 CIDD subjects were examined at the III Department of Medicine, University of Helsinki, and at the Helsinki Health Centre. The subjects were mostly long–term (≥ 10 years) insulin–dependent diabetics with an age–range from 17 to 65 years. Mean glycosylated hemoglobin and blood glucose levels were significantly higher in PIDD than CIDD subjects at baseline (p < 0.001, t–test). Of 71, 38 subjects participated in the one–year and 22 in the two–year longitudinal study.

Tooth loss was greater in the PIDD than CIDD subjects in the cross-sectional and longitudinal study. The two–year longitudinal study revealed that PIDD subjects experienced clearly more gingival inflammation and bleeding after probing than did CIDD subjects. Despite similar oral hygiene conditions, the PIDD subjects exhibited at baseline and at two follow–up examinations more gingival recession than the CIDD subjects (p < 0.05, χ²–test), more loss of attachment (p < 0.01, χ²–test), and more alveolar bone loss (p < 0.001, χ²–test), especially in the molar and lower incisor areas. However, no statistically significant differences were detected regarding mean probing depths (p > 0.05, t–test).

These data may suggest that poorly controlled diabetes accelerates the progression of periodontitis lesion in the active phase of the disease. According to the histological findings, increased plasma cell numbers with less collagen and fewer fibroblasts were detectable in the gingival connective tissue of PIDD subjects than in the metabolically balanced CIDD and healthy non–diabetic control subjects.

The composition of the subgingival microflora in 106 analyzed sites, comprising 55 healthy (probing depth < 4mm) and 51 diseased (probing depth ≥ 4mm) periodontal sites, revealed that the mean
spirochete and motile rod percentages in deep pockets were significantly higher in PIDD than in CIDD subjects \( (p < 0.01, p < 0.001, \chi^2\text{-test}) \).

Overall, these results indicate that long–term metabolic control is of significant importance to insulin–dependent diabetic subjects and their periodontal health.

**List of Original Publications**

The investigation is based on the following articles referred to by Roman numerals in the text.


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<tr>
<td>AGEs</td>
<td>advanced glycation end–products</td>
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<td>BGL</td>
<td>blood glucose level (mmol/l)</td>
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<td>CIID</td>
<td>controlled insulin–dependent diabetes</td>
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<td>CLR</td>
<td>cumulative logistic regression analysis</td>
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<td>DM</td>
<td>diabetes mellitus</td>
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<td>GAG</td>
<td>glycosaminoglycan</td>
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<td>GCF</td>
<td>gingival crevicular fluid</td>
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<tr>
<td>GHbA1</td>
<td>glycosylated hemoglobin A$_1$ (%)</td>
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<td>GHbA$_{1c}$</td>
<td>glycosylated hemoglobin A$_{1c}$ (%)</td>
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<td>HLA</td>
<td>human leukocyte antigens</td>
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<td>ICT</td>
<td>infiltrated connective tissue</td>
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<td>IDDM</td>
<td>insulin–dependent diabetes mellitus</td>
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<td>IL–1β</td>
<td>interleukin–1β</td>
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<td>IL–8</td>
<td>interleukin–8</td>
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<td>LPS</td>
<td>lipopolysaccharide</td>
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<td>N$_V$</td>
<td>numeric density data</td>
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<td>NCT</td>
<td>non–infiltrated connective tissue</td>
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<td>NIDDM</td>
<td>non–insulin–dependent diabetes mellitus</td>
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<td>OLR</td>
<td>ordinary logistic regression analysis</td>
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<td>PAS</td>
<td>Periodic Acid Schiff</td>
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<td>PGE$_2$</td>
<td>prostaglandin–E2</td>
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<td>PIDD</td>
<td>poorly controlled insulin–dependent diabetes</td>
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<td>PMN</td>
<td>polymorphonuclear neutrophil</td>
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<tr>
<td>$t$–test</td>
<td>Student’s $t$–test</td>
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<td>TNF–α</td>
<td>tumor necrosis factor–α</td>
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<td>$V_V$</td>
<td>volumetric density data</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>ZIDAS</td>
<td>Zeiss Interactive Digital Analysis System</td>
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<td>$\chi^2$</td>
<td>chi$^2$–test</td>
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1. Introduction

The disease diabetes mellitus (DM) comprises a heterogeneous group of pathological conditions characterized by altered glucose metabolism (Bennet 1990). The exact molecular etiopathogenesis of insulin–dependent diabetes mellitus (IDDM) is currently unknown, but an infection – evidently viral – in genetically susceptible individuals has been suggested (Yoon 1991, Dennison et al. 1996, Åkerblom et al. 1997, Herrath et al. 1997, Herrath & Holz 1997). Non–insulin–dependent diabetes mellitus (NIDDM) is the most common type of diabetes, often associated with obesity suggesting a genetic defect in insulin secretion, insulin resistance, or impaired glucose tolerance (Watkins et al. 1996, Groop & Tuomi 1997).

The incidence of IDDM in Finland is among the highest in the world. Since 1953 it has increased almost four–fold from 12 to 45/100 000/year. At the end of 1996, 3.3% of the whole population, i.e., 30 000 subjects, were registered as IDDM and 140 000 as NIDDM patients (Tuomilehto & Reunanen 1997). Of all those with diabetes in Finland, 25% have clearly poor (GHbA1c ≥ 10%), 45% poor (GHbA1c ≥ 9%), 17% moderate (GHbA1c 7.1–8.9 %), and only 13% good (GHbA1c ≤ 7%) metabolic control (Kangas 1993).

Altered host defence and inflammatory cell mediator responses have been frequently demonstrated in diabetes, especially in patients with poor metabolic control (Casey 1990). In poorly controlled IDDM subjects, increased non–enzymatic glycosylation may dramatically interfere with host defence molecular functions and host–microbial interactions such as microbial adherence (Ueta et al. 1993, Marova et al. 1995, Perschel et al. 1995, Schubert & Heesemann 1995). Regarding the oral defence mechanisms, the decreased salivary fluid flow in poorly controlled IDDM subjects (Harrison & Bowen 1987, Field et al. 1997) may result in a selected bacterial and fungal microflora (Trieger & Boguslaw 1990, Thorstensson 1995, Karjalainen et al. 1996).

DM subjects are 2.3 times more likely to be afflicted with periodontal disease than non–diabetic subjects (Grossi et al. 1994), and several cross–sectional studies of the association between DM and periodontal diseases have been published (Glavind et al. 1968, Ervasti et al. 1985, Sandholm et al. 1989b, Emrich et al. 1991, Tervonen & Oliver 1993, Thorstensson 1995, Taylor et al. 1996). Nevertheless, only a few longitudinal studies have succeeded in calculating the amount of annual loss of attachment and loss of alveolar bone in IDDM subjects (Tervonen et al. 1991b, Westfelt et al. 1996, Firatli 1997, Taylor & Becker 1998). Long–duration and poor metabolic
control may be potential risk factors for periodontal disease progression, especially considering the vascular complications, selected micro–organisms, as well as the destructive inflammatory reaction of the tooth–supporting tissues. The contribution of these factors to the development of periodontal disease needs to be evaluated critically and thoroughly.

2. Review of the literature

2.1. General aspects of diabetes mellitus. DM comprises a heterogeneous disease group and is according to WHO (1985) divided mainly into two groups.

Type I, or IDDM, is characterized by the destruction of Langerhans’ insulin–producing beta cells. An autoimmune destruction triggered by environmental factors in genetically susceptible individuals leads to the destruction of these beta cells (Åkerblom et al. 1997). Specific HLA haplotypes have also been associated with IDDM (Tuomilehto & Reunanen 1997). Genetic screening appears essential in the identification of individuals at increased risk for IDDM (Åkerblom et al. 1997).

Type II, or NIDDM, also called adult–onset diabetes, is a group of genetically determined diseases controlled by diet and/or hypoglycemic agents and/or exogenous insulin (Groop & Tuomi 1997). NIDDM patients cannot compensate for insulin resistance at hyperglycemic levels by increasing insulin secretion (Groop & Tuomi 1997). Uncontrolled IDDM and NIDDM subjects have similar clinical long–term manifestations with retinopathy, angiopathy, neuropathy, nephropathy, and other severe diabetic complications, such as limb amputations (DCCT 1993, Sowers & Epstein 1995). These complications can be triggered and aggravated during the course of an infection (Perschel et al. 1995). On the other hand, an infection may disrupt the metabolic balance in diabetes, resulting in a need for higher insulin doses (Perschel et al. 1995).

Metabolic control, an important variable regarding diabetic complications (DCCT 1993, DCCT 1997), can be determined from blood glucose levels (BGL, mmol/l), and from glycosylated hemoglobin measurements known as GHbA₁c. Realistic DM treatment aims at GHbA₁c levels < 7.5% with reference levels for GHbA₁c between 4 and 6%. Reference levels for BGL are 3.5 to 5.5 mmol/l with fasting BGL < 7.8 mmol/l and a two-hour glucose–tolerance test < 10 mmol/l (Yki–Järvinen 1999). According to the World Health Organization (WHO), a new classification and diagnosis of DM regarding
fasting blood glucose is suggested to be lowered to 6.1 mmol/l (Alberti & Zimmet 1998). Most of the diabetic subjects having their yearly medical examination at the health care center or the outpatient clinic at the Helsinki University hospital may be grouped by the specialist/endocrinologist into two categories describing their metabolic control (Luukkanen & Ristimäki 1991). Of importance for their grouping of diabetics according to the metabolic control is that the longitudinal pre–study evaluation of metabolic control is based upon GHbA1 or GHbA1c measurements, duration of diabetes, age and sex, occurrence of diabetic complications, insulin resistance, glucose tolerance, and medication for diabetes. The primary source of care in Finland for diabetic subjects aged 15 to 64 years is the health care center for 54%, while 34% used hospital outpatient care, 8% the services of private physicians, and 4% the occupational health care system (Kangas 1997).


2.3. Periodontal studies.

2.3.1. Clinical cross-sectional studies. DM and periodontal disease have been extensively studied with varying results (see reviews by Yalda et al. 1994, Genco 1996, The Research, Science and Therapy Committee of the American Academy of Periodontology, Dennison et al. 1996), due to great varieties in grouping populations of diabetic subjects, and due to distinctive epidemiological methods in
recording periodontal disease. However, high prevalences of periodontal disease among patients with diabetes have been found in a number of studies (Ray & Orban 1950, Wolf 1977, Cianciola et al. 1982, Shlossman et al. 1990, Emrich et al. 1991, Tervonen & Oliver 1993, Grossi et al. 1994, Pinson et al. 1995, Taylor et al. 1996). Diabetic children and adolescents with poor metabolic control and/or organ complications, exert a tendency towards higher gingival index scores than those without diabetes who are of the same age (Gislén et al. 1980, Gusberti et al. 1983, Harrison & Bowen 1987, Rylander et al. 1987, Novaes Jr. et al. 1991, de Pommereau et al. 1992). Mattila et al. (1989) and DeStefano et al. (1993) have demonstrated an association between periodontal and cardiovascular diseases, and according to Thorstensson (1995), also diabetic nephropathy, including microalbuminuria, is associated with severe periodontal disease in adult long–term IDDM.

Glavind et al. (1968) showed that patients with a diabetes history longer than 10 years had greater loss of periodontal structures than did those with a history less than 10 years. They suggested that diabetic patients with retinal changes also show greater attachment loss than do those with no complications, and this finding has been verified by several investigators. Other diabetic complications related to the kidneys, blood circulation, and nephropathy have also been associated with periodontal disease (von Heinrich 1980, Rylander et al. 1987, Bačić et al. 1988, Willershausen–Zönnchen & Hamm 1988, Karjalainen et al. 1994).

Case reports have shown rapid periodontal destruction to occur in adults with poorly controlled diabetes and elevated blood glucose levels (Bartolucci & Parkes 1981, Ainamo et al. 1990), often associated with increased amounts of dental calculus and attachment loss (Tervonen & Oliver 1993). Insulin–dependent diabetic patients, irrespective of the duration of their disease, have been reported to show a higher prevalence of gingivitis than those without diabetes (Hugoson et al. 1989), and among 40– to 49–year–old long–duration diabetic subjects, significantly more periodontitis lesions (pocket depths \( \geq 6 \) mm) and alveolar bone loss were found in comparison to non–diabetic subjects (Bačić et al. 1988, Thorstensson & Hugoson 1993). Heavy tobacco smoking also seems to result in pocket formation, loss of attachment, increased risk for periopathogens, and vascular changes (Bergström & Preber 1994, Grossi et al. 1996, Zambon et al. 1996).
2.3.2. Clinical longitudinal studies. The literature published on cross-sectional studies on periodontal disease and diabetes mellitus provides limited information on the relation between DM and periodontal disease. Williams and Mahan (1960) suggested that diabetic subjects receiving periodontal treatment showed significant reduction in insulin requirements. Miller et al. (1992) observed in a study of 9 insulin–dependent patients that metabolic balance can be beneficially modified by controlling their periodontal inflammation. However, Tervonen et al. (1991a) did not during their short follow–up study of three to four months find significant differences in gingival bleeding or periodontal pockets after oral–hygiene instruction, scaling and root planing in 34 IDDM subjects and 45 healthy controls. In a five–year follow–up of 20 diabetic and 20 control subjects attending dental treatment every three month, no significant correlation could be found between changes in bleeding on probing, probing depth, and mean long–term GHbA$_1c$ values (Westfelt et al. 1996). However, in a 12–month longitudinal study, Tervonen and Karjalainen (1997) found in 8 adult type I poorly controlled diabetic patients a faster recurrence of deepened pockets than in 13 with controlled diabetes and 10 without diabetes. Taylor et al. (1996) suggested, based on a 2–year longitudinal study on 80 NIDDM subjects, that severe periodontitis is a risk factor for poor glycemic control. Grossi et al. (1996) have demonstrated in a one–year longitudinal study in 85 Pima Indians with poorly controlled NIDDM that an improved periodontal condition with a significant gain in attachment levels and subsequent reduction of periodontal inflammation results concomitantly in a significant reduction of glycosylated hemoglobin. 

Firatli (1997) and Novaes et al. (1997) showed that without periodontal treatment, bone or attachment loss occurred more rapidly in IDDM subjects than in non–diabetic subjects. Smith et al. (1996) studied 18 IDDM patients during two months, but could find no significant differences in their glycosylated hemoglobin A$_1c$ levels before and after non–surgical periodontal therapy. This finding seemed to be in agreement with that of Alridge et al. (1995) in their study of 27 IDDM subjects, who, when re–examined after two months, demonstrated no significant improvement in their immediate glycosylated hemoglobin levels, spite the fact that all periodontal parameters of these subjects decreased during the study.

Of importance to metabolic control seems to be the long–term effect of periodontal treatment with strictly controlled plaque conditions in IDDM and NIDDM subjects. Thus, longitudinal site–by–site
studies may, under well-defined medical conditions, offer new information about the relation between diabetes mellitus and periodontal diseases.

2.4. **Microbiological studies.** The etiology of periodontal disease in hyperglycemic IDDM subjects might at least partially be explained by the selected pathogen microflora in the diseased periodontal pocket (Trieger & Boguslaw 1990). Elevated glucose in saliva (Harrison & Bowen 1987, Field et al. 1997) and gingival crevicular fluid (Ficara et al. 1975), as well as decreased salivary fluid flow (Harrison & Bowen 1987, Field et al. 1997), may influence the plaque bacterial populations by favoring the growth of some bacterial species at the expense of others (Trieger & Boguslaw 1990). It may also result in increased non-enzymatic glycosylation of proteins (inflammatory mediators, immunoglobulins and other host–response mediators) and cells involved in the oral cavity/body defense reactions against inflammation and infections and lead to modifications in their functions involving defense against infections (Morinushi et al. 1989, Casey 1990).

It has been well documented that *Porphyromonas gingivalis* can invade oral epithelial cells (Sandros et al. 1993), and this microbe was found in IDDM patients to be their most frequently present periodontal pathogen (Grossi et al. 1994, Thorstensson 1995). *P. gingivalis* and *Treponema denticola* as whole bacterial cells as well as their potential virulence factors (proteinases surface proteins, lipopolysaccharides (LPSs)) can activate neutrophil matrix metallo- and serine proteinases during phagocytosis (Ding et al. 1996, Ding et al. 1997).

Earlier cross-sectional dark field microscopy studies on the percentage distribution of subgingival bacterial morphotypes have demonstrated that spirochetes and motile bacteria residing at the most apical level of the periodontal pocket are associated with periodontal disease (Listgarten & Helldén 1978, Lindhe et al. 1980, Listgarten & Levin 1981, Armitage et al. 1982, Tanner et al. 1984, Zappa et al. 1986, Slots & Listgarten 1988, Omar et al. 1990). Hyperglycemia and an abnormal host–defence mechanism may result, in adult long-term IDDM, in growth of selected pathogenic micro–organisms such as spirochetes and motile rods.

A few studies (Gusberti et al. 1983, Mashimo et al. 1983, Mandell et al. 1992) on the distribution of spirochetes and motile bacteria in the subgingival microflora of PIDD and CIDD subjects suggest a relation between poor metabolic control and severe alveolar bone loss. According to Sandholm et al. (1989a), 85 subjects with IDDM, aged 12 to 18 years, had almost identical proportions of spirochetes and
flagellated bacteria as 85 non–diabetic age– and sex–matched control subjects. Sastrowijoto et al. (1990) could find neither in deep nor in shallow periodontitis pockets of 6 PIDD subjects any significant changes in the subgingival flora during an 8–month follow–up. Overall, most studies indicate that the periopathogen micro–organisms in diabetics are no different from those seen in non–diabetic subjects. Further studies are needed to establish the true periodontal pathogenicity of oral spirochetes in poorly controlled diabetes.

2.5. **Histological studies.** The nature of most of the histological studies related to diabetes and periodontal disease is descriptive. There exist, however, acceptable morphometric methods to measure the cellular and volumetric composition of the subepithelial inflamed (ICT) or non–inflamed (NCT) connective tissue (Schroeder & Münzel–Pedrazzoli 1973, Liljenberg & Lindhe 1980, Berglundh et al. 1991). None of these numerous descriptive studies on diabetes have measured the volumetric or cellular composition of the ICT in PIDD, CIDD, or non-diabetic control subjects, although both cellular and humoral immune responses have been suggested to be defective in diabetic patients with periodontitis (Cutler 1991). An altered inflammatory cell response due to defective neutrophil and/or monocyte/macrophage functions in those with poorly controlled diabetes (McMullen et al. 1981, Oliver et al. 1991) suggests that it might be useful to evaluate the exact composition of the ICT beneath the junctional epithelium in metabolically poorly and well–controlled diabetic groups.

Prolonged exposure to hyperglycemia may result in vascular dysfunction and cellular changes (Feener & King 1997). Most histological studies have demonstrated that small blood vessels of the gingiva in long–term diabetic patients frequently shows microangiopathic changes with occlusion, and increased vascular thickness (Hove & Stallard 1970, von Heinrich 1980, Hiura 1991). These studies have failed to report metabolic control levels and correlations with other diabetic complications, such as retinopathy and nephropathy. In an electron microscopic study by Listgarten et al. (1974), a statistically significant increase in the width of the basement lamina of endothelial cells appeared in 10 IDDM and 10 control subjects, considering only the smallest thickness measurements per vessel.

Vlassara & Bucala (1995), Berg et al. (1997), and Chappey et al. (1997) have indicated that microvascular complications due to long–term hyperglycemia may occur due to modified proteins, the so–called advanced glycation end–products (AGEs). Potentially, AGEs
may also induce oxidant stress in the gingiva, resulting in accelerated periodontal tissue destruction (Schmidt et al. 1996). Vascular changes have not yet been studied by morphometric methods in poorly controlled versus controlled insulin–dependent diabetes, nor has the acellular or the cellular composition of the clinically healthy diabetic gingiva been compared to the gingiva of non–diabetic subjects.

2.6. **Biochemical studies.** In diabetes, the periodontium is evidently affected by pathologically elevated collagenase and matrix metalloproteinase (MMP) activities (Golub et al. 1992, 1997, 1998, Sorsa et al. 1992a), reduction of collagen and GAG synthesis (Willershausen–Zönnchen et al. 1991), and by other metabolic abnormalities in periodontal ligament fibroblasts (Sasaki et al. 1992). In this regard, Golub et al. (1983, 1992, 1998) have shown that in germ–free rats, diabetes increases collagenase levels in gingiva. Oxidants in concert with other human and potential periodontopathogenic bacterial proteases are capable of activating the tissue–destructive neutrophil, endothelial, epithelial, and bone–cell derived pro matrix metalloproteinases, such as MMP–8 and MMP–13 (Sorsa et al. 1990, 1992a,b, Westerlund et al. 1996, Sorsa et al. 1998). Poorly controlled diabetic patients with advanced periodontitis have been shown to express pathologically excessive active matrix metalloproteinase–8/collagenase–2 in their periodontitis gingiva and gingival crevicular fluid (GCF) (Sorsa et al. 1992a, 1996).

Identification of subjects at high risk for periodontal disease has significantly improved during the last decade (Grossi et al. 1996, Grossi & Genco 1998, Taylor et al. 1998). Understanding of the host’s pathological features has expanded, and new evidence demonstrates that the relationship between periodontal disease and diabetes with poor metabolic control is not accidental.
3. **AIMS OF THE INVESTIGATION**

- to evaluate the influence of metabolic control of diabetes mellitus on periodontal health of those with adult insulin–dependent diabetes mellitus (I).

- to evaluate in a two–year longitudinal study the healing and/or progression of periodontitis in those with poorly controlled and controlled insulin–dependent diabetes mellitus (II, III).

- to compare with dark field microscopy the proportional distribution of bacterial morphotypes, especially spirochetes, in adults with long–term poorly controlled and controlled diabetes mellitus (IV).

- to study histopathological features of clinically healthy gingival connective tissues from poorly controlled and controlled insulin–dependent diabetics and non–diabetic control subjects (V).
4. Material and methods

4.1. Subjects. Of 525 subjects, aged 17 to 65 years, with a medical history of long-term insulin-dependent diabetes mellitus (IDDM) most for ≥ 10 years (range 5 to 42 years), 317 were studied from 1984 to 1986 and 208 from 1990 to 1993. These patients were treated at the III Department of Medicine, University of Helsinki, and at two Helsinki health centers. Based upon their medical records, 183 subjects were included (inclusion criteria: age 17 to 65 years old, IDDM duration ≥ 10 years). The dental examination revealed 16 edentulous subjects; 14 subjects participated in another study, and 57 subjects were excluded due to various other reasons (e.g., changed address, severe illness, inability to participate, due to work). The remaining 96 subjects were allocated into two groups: 55 poorly controlled insulin-dependent diabetics (PIDD) and 41 controlled insulin-dependent diabetics (CIDD).

The medical history of the PIDD patients revealed problems involving their diabetes such as BGLs, GHBₐ₁ and/or GHBₐ₁c, the frequent hyper- or hypoglycemia, albuminuria, glycosuria, serum cholesterol, triglycerides, creatinine, C-peptide; they had recurrent infections, ketoacidosis, glycosuria, diabetic coma, frequent hospitalizations, and various stages of retinopathy, neuropathy, nephropathy or other vascular organ complications. Of those with cardiovascular complications, 70% were PIDD subjects.

The CIDD subjects presented with overall good metabolic control with acceptable blood glucose levels, lower glycosylated hemoglobin A₁ and/or A₁c levels, no ketosis, less hyper- or hypoglycemia, and few complications from their diabetes. The inter- and intraexaminer calibration were performed during two additional studies (Safkan & Knuuttila 1984, Rylander et al. 1987).

4.1.1. Study I. Groups were 44 PIDD and 27 CIDD subjects with a mean duration of 16.5 years. Of these 71 diabetic patients, 11 PIDD and 6 CIDD subjects revealed cardiovascular complications.

4.1.2. Studies II and III. Periodontal disease was assessed after one year from the baseline examination in 38 subjects and after two years in 22 subjects.

4.1.3. Study IV. Of 47 long-term IDDM subjects in this study, 26 (55%) were identified as PIDD and 21 (45%) as CIDD subjects.

4.1.4. Study V. Subjects included in the histological study were 29 long-term IDDM (19 PIDD and 10 CIDD) patients, 10 of them with
cardiovascular complications. Controls studied were 10 non–diabetic age– and sex–matched subjects.

4.2. Periodontal variables. Cross–sectional and longitudinal dental examinations were performed by B.S. The examiner was calibrated with an electronic periodontal probe at a standardized pressure of 20–25 g (Vine Valley Research, NY, USA), and was unaware during the periodontal examination of the subject’s group. Loss of marginal alveolar bone was measured on orthopantomograms taken at the Department of Oral Diagnosis, with Granex, OP3 (Studies I to III, V) or PM2002CC (Study IV) orthopantomographic equipment. Third molars were in all of the studies examined only when they replaced missing or extracted second molars. Periodontal treatment comprising scaling and root planing was given to all patients attending at baseline and at the one–year examination. Clinical recordings were made for the variables described in Studies I to V.

4.3. Sampling of the subgingival microflora. The composition of the subgingival microflora in 106 periodontal pockets of 47 subjects with insulin–dependent diabetes mellitus was examined by the method of Listgarten and Helldén (1978) (Study IV). Of the 106 pockets, 55 were healthy (probing depth < 4 mm) and 51 diseased (probing depth ≥ 4 mm).

4.4. Sampling of gingival biopsies and sample processing. Altogether 39 biopsy samples, one biopsy per subject, were taken from the buccal marginal gingiva. The gingival biopsy sample was chosen from a well–defined site, which made optimal and standardized biopsy technique possible (Study V). No evident signs of gingivitis or periodontitis were evident at the biopsy sites. Morphometric analysis was performed by two examiners (S. Münzel–Pedrazzoli & B.S.) according to the stereologic point–counting procedure described by Schroeder (1967), Weibel (1969), Schroeder & Münzel–Pedrazzoli (1973), Liljenberg & Lindhe (1980), and Berglundh et al. (1991). The multi–purpose test system, fitted into the viewing screen of the table projector unit, consisted of a square frame of defined size and 42 volumetric points (P42, Study V). With this test system, the relative volume and density of tissue components of ICT as well as of the area of blood vessels were calculated with the Zeiss Interactive Digital Analysis System (ZIDAS).
4.5. **Statistics.** Severity of diabetes was measured by means of nominal dichotomous variables (e.g., controlled/poorly controlled, presence or absence of retinopathy). Diabetic subjects were divided into groups based on numerical recordings of these variables.

Variables describing periodontitis (plaque index, gingival index, bleeding index, probing depth, gingival recession, loss of attachment and loss of alveolar bone) or the subgingival microflora (cocci, rods, filaments and fusiforms, motile rods, spirochetes) were continuous, and their distributions were compared between the various groups. In Studies II and III, attachment level and bone level were compared longitudinally between the PIDD and CIDD groups. The area of the lumen of the blood vessel was subtracted digitally from that of the total blood vessel area. When the assumptions of the $t$–test were satisfied, this test was used to compare the means of the variables describing periodontitis. If the assumptions of the $t$–test were not satisfied, the non–parametric $\chi^2$–test was used to compare the distribution of the variables among the different groups of subjects. The tests used are indicated in the tables of Studies I to V. The Systat (SYSTAT, Inc. 1990) and Cellanal (Nägli, 1984) programs served for statistical analysis and data processing with IBM PS/2 and PC Intel 486.
5. Results

5.1. Medical data. As the mean blood glucose and the GHbA\textsubscript{1} levels at baseline were higher in PIDD than CIDD subjects in all the five studies, except in Study V, for GHbA\textsubscript{1} \((p < 0.152, t\text{-test})\), this study design offers a good starting point for the evaluation of periodontal status in these two groups of subjects.

At the end of the two-year study, GHbA\textsubscript{1} levels had improved in PIDD subjects as compared to levels in CIDD subjects. This finding was, however, not significant \((p = 0.068, t\text{-test}, \text{II, III})\). During the two-year follow-up, the GHbA\textsubscript{1} level improved from 1.4 to 1.8\% in three of five PIDD subjects undergoing periodontal surgery.

5.2. Age. Mean age was similar in both groups, except in Studies II and III at baseline of the one-year study, with a slightly higher mean age of 4.4 years in the PIDD group \((p < 0.05, t\text{-test})\). No other significant differences occurred in age distribution during the study \((p > 0.05, t\text{-test})\).

5.3. Results of cross-sectional clinical studies (I to V).

5.3.1. Distribution of remaining teeth. The overall distribution of remaining teeth demonstrated that the PIDD subjects had lost more teeth than the CIDD subjects (Study I).

5.3.2. Oral hygiene and gingival conditions. Oral hygiene was in Studies I, II, III, and V similar in the two groups \((p > 0.05, t\text{-test} \text{ or } \chi^2\text{-test})\), but different in Study IV \((p < 0.01, t\text{-test})\). In Studies II and III the PIDD subjects also revealed higher mean gingival index scores than did CIDD subjects \((p < 0.001, t\text{-test})\).

5.3.3. Probing depth. No statistically significant differences regarding mean probing depths \((p > 0.05, t\text{-test})\) were observed between the PIDD and CIDD groups in Studies I to V.

5.3.4. Loss of attachment and alveolar bone. In Study I, the tooth-specific histogram of the mean attachment and bone level measurements clearly suggested that the PIDD subjects had lost more attachment and more alveolar bone than the CIDD subjects. With respect to tooth type, the mean proportions of attachment and bone loss were higher in some of the molars, premolars, canines, and incisors in the PIDD than in the CIDD subjects \((p < 0.05, t\text{-test})\). These differences seemed evident, but when all sites of the dentition were pooled for analysis, they did not reach statistical significance.
The main differences appeared in the patterns of loss of attachment and alveolar bone around molars and lower incisors \((p < 0.03, \ p < 0.01, \ t\text{-test})\). In Studies II and III, the long-term PIDD subjects had lost more tooth attachment and alveolar bone than the corresponding CIDD subjects \((p < 0.01, \ p < 0.001, \chi^2\text{-test})\). In Study IV, the PIDD subjects exhibited clearly more loss of alveolar bone than the CIDD subjects \((p < 0.05, \ t\text{-test})\). However, in Study IV, no differences existed between the PIDD and CIDD groups regarding mean attachment levels \((p = 0.113, \ t\text{-test})\). Furthermore, in Study V, the PIDD subjects exhibited more loss of attachment and alveolar bone than did the CIDD subjects \((p < 0.001, \ p < 0.01, \chi^2\text{-test})\).

5.4. Results of longitudinal clinical studies (II and III).

5.4.1. Loss of teeth. By the end of one year, PIDD subjects had lost 17 teeth, mainly incisors, 1st premolars, and molars, due to progressive periodontal disease, whereas the CIDD subjects had lost none. However, at the end of the second year, no further teeth were lost in either of the two groups.

5.4.2. Oral hygiene and gingival conditions. Both at one and at two years from baseline, the PIDD and CIDD subjects showed similar plaque index scores. At one and two years after baseline, the PIDD subjects exhibited higher mean gingival index scores \((p < 0.05, \ t\text{-test}, \text{II})\) than did CIDD subjects. This difference between groups in Study II was evident also regarding their bleeding index scores \((p < 0.05, \chi^2\text{-test})\). The poor gingival conditions improved after one and two years from baseline in PIDD subjects, but remained, at the two-year examination, still high as compared to those of the CIDD subjects \((21\% \ vs \ 3\%, \text{II})\). The PIDD subjects also exhibited higher percentages of sites with improved bleeding index scores than did CIDD subjects \((p < 0.01, \chi^2\text{-test})\).

5.4.3. Loss of attachment and alveolar bone. At the one-year and two-year examinations, the PIDD subjects had lost more attachment \((p < 0.01, \chi^2\text{-test}, \text{Study II})\) than did CIDD subjects. One and two years from baseline there appeared a difference of 0.1–0.3 mm of attachment loss between PIDD and CIDD subjects (Study II). A difference was also obvious in regard to the radiographically determined mean loss of alveolar bone \((p < 0.001, \chi^2\text{-test}, \text{Study II})\). The two-year longitudinal site-by-site examination of all teeth revealed more often a loss of \(\geq 2\) mm of alveolar bone in the PIDD
than in the CIDD subjects ($p < 0.05$, $\chi^2$–test, Study III). In addition, at both baseline and follow-up examinations the PIDD subjects exhibited more gingival recession than did CIDD subjects ($p < 0.05$, $\chi^2$–test, II).

The present longitudinal findings demonstrate that PIDD subjects, during their two-year follow-up, have experienced clearly more loss of attachment and alveolar bone, and also significantly more gingival recession than have CIDD subjects.

5.5. **Results of the microbiological study (IV).** The cross-sectional morphological study indicated no differences in relative numbers of cocci and filiform and fusiform bacteria in < 4 mm sulci of PIDD and CIDD subjects ($p > 0.05$, $t$–test, IV). In periodontitis sites with pockets $\geq$ 4mm the PIDD subjects revealed lower frequencies of cocci than did CIDD subjects ($p < 0.01$, $\chi^2$–test, IV). At baseline, the mean percentage of spirochetes and motile rods in deepened pockets $\geq$ 4mm were, however, clearly higher in PIDD than in CIDD subjects. These differences were statistically significant ($p < 0.01$, $p < 0.001$, $\chi^2$–test, IV).

5.6. **Results of the morphometric study (V).** The overall volumetric ($V_V$) and numeric ($N_V$) composition of the clinically healthy but histopathologically inflamed connective tissue (ICT) was similar in the PIDD and control subjects (Table 3, Study V). Light microscopic analysis of the inflamed gingiva revealed that the inflammatory cell infiltrate in the gingival connective tissue at the junctional epithelium was larger and extended more laterally and apically in PIDD than CIDD subjects. The volumetric ($V_V$) data on the connective tissue of PIDD subjects seemed also to indicate more chronic inflammation than was seen in CIDD subjects. Under similar plaque conditions, the composition of the ICT indicated elevated plasma cell levels in the diabetic subjects as compared to those of the control subjects (Table 3, Study V). PMNs were present in the gingiva of PIDD subjects and to a slightly lesser extent also in healthy control subjects ($31.3 \pm 5.3 \ [N_V \times 10^6]$ and $25.2 \pm 6.3 \ [N_V \times 10^6]$, respectively).

Moreover, fibroblasts ($V_{VF}$) occupied less volume in the ICT of diabetic subjects than of controls ($51.3 \pm 7.3 \ mm^3/100\ mm^3$ vs $80.5 \pm 42.3 \ mm^3/100\ mm^3$, Table 3, Study V). In addition, collagen occupied less of the volume of ICT lesions in diabetic than of control subjects ($12\%$ and $17\%$, V). The overall histological picture of the ICT in PIDD patients was thus characterized by a predominance
of plasma cells rather than of lymphocytes, associated with a clear reduction in fibroblasts and collagen fibers.

Swollen and proliferated endothelial cells, diminished or a total absence of pericytes, as well as enlarged Russel bodies were present in the inflamed connective tissue of the gingiva in insulin–dependent diabetics. Further, capillaries of the gingival ICT seemed more frequently to be obliterated by swollen endothelial cells, with a narrow capillary lumen, in long–term diabetics than in control subjects. The mean distance from the lumen to the outer border of the outmost basement membrane was greater in the non–infiltrated connective tissue (NCT) of the PIDD than in the non–diabetic control subjects ($p < 0.001$, $t$–test, V).
6. Discussion

The present studies (I to V) have focused on the relation between adult periodontal disease and poor metabolic control of type I diabetic subjects. When all sites of the dentition were pooled in the first cross-sectional study, no statistically significant differences in the mean periodontal measurements could be detected between the PIDD and CIDD subjects. In a recent Italian study, Sbordone et al. (1998) reported that IDDM patients and their healthy cohabiting siblings displayed no significant differences in these clinical parameters. Their study comprised only 16 patients and 16 controls, many patients were in relatively good metabolic state, and a subdivision into PIDD and CIDD would not have been possible without loss of statistical power. The present cross-sectional study (I), in contrast, comprised 44 PIDD and 27 CIDD subjects and provides more reliable data on the eventual association between periodontal disease and metabolic control of IDDM subjects. Because it can be assumed that poor metabolic control may accelerate the progression of periodontal disease, further evidence was required to specify the association in the subclasses of diabetic subjects and healthy control subjects at high risk for periodontal disease.

Despite the evidence that the pooled data from all sites (I) suggested an apparent lack of association between periodontal status and diabetic control, the tooth-specific data (I) demonstrated significantly more attachment loss and bone loss in PIDD than CIDD subjects ($p < 0.05$, $p < 0.01$, $t$-test), especially in some molars, premolars, canines, and incisors. Interestingly, Taylor & Becker (1998) have recently reported an increased efficiency of analysis using the cumulative logistic regression (CLR) instead of ordinary logistic regression (OLR) analysis in 21 NIDDM patients. In contrast to the OLR, the CLR model provided convincing evidence that the risk for more severe bone loss progression after two years is greater in subjects with poorly controlled than in subjects with better controlled NIDDM or in non-diabetic subjects (Taylor & Becker 1998).

The literature addressing studies of the periodontal status of IDDM subjects has been based on relatively small populations, probably due to increasing costs of manpower for large epidemiological populations, and therefore lack statistical power (Grossi & Genco 1998). However, large populations of Pima Indians have been studied in the United States and demonstrate convincingly that an increased risk for periodontal disease in diabetic subjects does exist (Shlossman et al. 1990, Emrich et al. 1991, Grossi et al. 1994, Taylor et al. 1996,
Taylor et al. 1998). Analysis of the literature in favor of the association between metabolic balance and periodontal status discloses that many of these previous papers dealt with type II diabetes mellitus (Shlossman et al. 1990, Emrich et al. 1991, Grossi et al. 1994, Taylor et al. 1996, Collin et al. 1998) or with mixed patient populations comprising both type I and II diabetes (Tervonen & Oliver 1993, Westfelt et al. 1996, Christgau et al. 1998). Metabolic control may be much more pertinent to periodontal status than is the type of diabetes, but selection of type I diabetes certainly avoids this possible bias. Furthermore, there are evident genetic (Groop & Tuomi 1997), etiologic (Åkerblom et al. 1997), and medical varieties (Tuomilehto & Reumanen 1997) of diabetics belonging to type II subgroups. Therefore, only type I or IDDM cases were selected for the present study.

The present study population was randomly chosen from among 525 subjects according to the following criteria: type of diabetes, age of subject, and duration of diabetes. Regarding the grouping of the type I patients, their metabolic control was not based upon a single measurement of glycosylated hemoglobin, but rather on continuous yearly evaluation of their medical status registered by the specialists/endocrinologists at the diabetes clinics (I). It is not possible in a cross-sectional study (I, IV, V) to evaluate how many of the "CIDD" subjects have periods of hyper- or hypoglycemia and actually represent PIDD, or conversely, how many of the "PIDD" subjects have normoglycemia and are misclassified as a result of sampling method, e.g. point estimate of blood glucose or GHbA\text{1c}. In this respect, longitudinal studies with yearly retro- and prospective evaluations give more reliable results than do cross-sectional studies. It is, naturally, possible that even patients classified as PIDD or CIDD as a result of longitudinal medical follow-up may shift category during the course of the study, but according to observations published by Tervonen & Oliver (1993), this is relatively rare.

GHbA\text{1c} reflects the BGL during the preceding 6 to 8 weeks. It has therefore been used in some earlier studies for the classification of those with IDDM into well, moderately, or poorly controlled subjects (Tervonen & Oliver 1993, Tervonen & Karjalainen 1997). In the present study, the classification of IDDM into CIDD and PIDD was based on a number of variables, such as BGLs, GHbA\text{1} and/or GHbA\text{1c}, frequency of hypo- or hyperglycemias, and presence or absence of various micro- and macroangiopathic complications, such as retino- and nephropathies as well as neuropathies, according to the evaluation of the specialists/endocrinologists at the diabetes clinics.
These data are supposed to provide a more reliable classification basis than the use of one or two criteria only.

It is important to observe that subjects with poor diabetic control may have other severe interfering systemic diseases, manual disabilities, or even blindness, resulting in poor compliance, unsatisfactory dental habits, and advanced periodontal disease (Thorstenson et al. 1989). For example, several potential pathomechanisms which may contribute to that end include insulin resistance associated with acute infections (Yki–Järvinen et al. 1989); advanced glycosylation end products (AGEs) affecting endothelial cells of the periodontal tissues (Lalla et al. 1998a); oxidant stress induced by vascular dysfunction (Schmidt et al. 1996, Lalla et al. 1998a); exaggerated inflammatory, such as prostaglandin E2 (PGE₂), interleukin–1 beta (IL–1β), interleukin–8 (IL–8), and tumor necrosis factor–alpha (TNF–α), responses in the human periodontium (Salvi et al. 1998); and identification of tissue–destructive MMPs in periodontal tissue and oral fluid of diabetic animals and humans (Ramamurthy & Genco 1983, Sorsa et al. 1992a, Sorsa et al. 1996, Lalla et al. 1998b).


The prevalence of periodontal disease is known to increase with age. Nevertheless, age could in none of these cross–sectional studies (I, IV, V), explain either greater attachment or bone loss in the PIDD subjects than in the CIDD subjects, despite of the small difference of 4.4 years recorded at baseline in the longitudinal studies. Overall, odd ratios indicated that diabetes, rather than age, increased risk for developing destructive periodontal disease, and increased it up to three-fold (Emrich et al. 1991, Grossi et al. 1994).

The loss of study subjects during the follow–up period was relatively substantial (II, III), probably mainly because patients with diabetes and its complications do not have the strength or interest,
or both, to be continuously engaged in dental examinations or to make regular dental visits (Thorstensson et al. 1989, Pohjamo et al. 1995, Rose et al. 1995, Kawamura et al. 1998). Nonetheless, the loss of subjects with periodontal disease was equally distributed between the PIDD and CIDD groups, indicating that these two groups were statistically comparable with each other also at the end of follow-up.

Patients from the CIDD group with the best periodontal status may have dropped out; such patients may not be motivated to attend regular dental follow-up visits because of their good periodontal status. In this case, the actual difference between PIDD and CIDD groups would have been even greater than that reported (II, III). Similarly, if the patients with the worst periodontal status from the PIDD group had dropped out of the study, then again this would be a bias, one which would decrease rather than increase the assumed difference between these two groups. Such patients might leave the study because they were, to start with, those least motivated to achieve good metabolic control, to take care of their oral hygiene, and to attend the dentist for follow-up examinations. In any case, it seems that the relatively high drop-out rate, which is usual in these kinds of studies (Cohen et al. 1970, Tervonen & Oliver 1993, Thorstensson 1995, Pohjamo 1996, Firatli 1997), does not invalidate the main clinical findings of the present study. On the contrary, it appears plausible that the actual difference is larger than smaller than that reported here.

Although our longitudinal study (II, Seppälä et al. 1993) was the first longitudinal study published on long-term poorly controlled and well-controlled IDDM subjects with a two-year follow-up, similar observations have been reported more recently (Tervonen & Karjalainen 1996, Westfelt et al. 1996, Firatli 1997). Firatli reported (1997) a statistically significant difference in the diabetic group in clinical attachment loss at baseline and five years later compared to that of healthy controls without any periodontal treatment. He did not, however, study the effect of metabolic control on the degree of attachment loss. Tervonen and Karjalainen (1996) reported that eight IDDM patients with severe diabetes, i.e., with poor long-term control and/or multiple complications, had a significantly higher extent of attachment loss (≥ 2 mm) at baseline and a rapid recurrence of probing pocket depth (≥ 4 mm) than did those with moderate or good control, which were comparable to the non-diabetic subjects. Westfelt et al. (1996) reported, based on their study of 20 moderately well-controlled diabetic and 20 non-diabetic subjects with moderate to advanced periodontitis, that their periodontal condition was–
after periodontal therapy—maintained during five years, as assessed every third month.

Only a few longitudinal studies analyzing all sites of each dentition site–by–site are available in periodontal research associated with diabetes mellitus (III), and none of those studies were based upon randomly chosen populations. Again, our longitudinal study (III, Seppälä & Ainamo 1994) was the first longitudinal site–specific study published on long–term poorly controlled and controlled IDDM subjects with a two–year follow–up. Technical difficulties regarding data processing were a challenge, and several cross–checkings and analytic runs of the data were needed to allow the data to be presented in a well–organized, comprehensible manner. In particular, the site–specific changes in PIDD and CIDD subjects have always been analyzed per subject in order to avoid statistical bias (Papapanou 1997). Instead of the use of means (I, II), the ability to evaluate distribution of periodontally active or stable sites was more informative, producing data demonstrating sites with both attachment and alveolar bone gains or losses among all sites (III). Importantly, the PIDD subjects had more rapidly progressing sites with more loss of attachment and alveolar bone than did the CIDD subjects (III). Another interesting longitudinal finding was that the PIDD subjects exhibited more gingival recession than did the CIDD subjects (II, III).

The tooth–specific analysis (I) demonstrated that PIDD subjects with elevated blood glucose and glycosylated hemoglobin levels had significantly more attachment loss than did CIDD subjects, especially in some of the molars, premolars, canines, and incisors. The distribution of teeth (I) was very much in accordance with that of Thorstensson (1995), although some of our subjects had fewer than 10 teeth remaining in each jaw. Local factors such as dental plaque and calculus, root anatomy and furcations, may at least partially be responsible for the increased loss of tooth attachment in PIDD subjects (I to III). Nonetheless, in our studies no statistically significant differences existed in the mean plaque and calculus levels between the two groups, and therefore plaque and calculus can only in part explain the differences in attachment and bone loss, which are indicators of true periodontal tissue destruction (Papapanou 1997, Oliver et al. 1998).

The exaggerated inflammatory response in diabetic subjects, especially involving chronic Gram–negative infections, has been suggested to induce insulin resistance (Yki–Järvinen et al. 1989), and thus contribute to the hyperglycemia complicating the metabolic control of diabetes. However, since the degree of insulin resistance
has not been quantified during acute infections, the real effect of impaired insulin action in diabetic subjects is unknown (Yki–Järvinen et al. 1989). Our results demonstrated that the progression of inflammatory lesions was more serious in PIDD than in CIDD subjects during a two–year follow–up (II, III). In particular, the increase in numbers of periodontal inflammatory cells seemed to result in more destructive loss of bone in poorly controlled than in well–controlled subjects.

According to the clinical studies, improved metabolic control of PIDD subjects seemed to be related to their improved periodontal condition ($p < 0.068$, $t$–test, II, III). This finding coincided with the findings of Miller et al. (1992) and Grossi et al. (1996), but differed from that of Smith et al. (1996). When severe periodontal or other inflammatory diseases such as acute periapical infections and infected cysts were eliminated either by non–surgical or surgical methods, a beneficial effect on the metabolic control of IDDM was achieved (II, III, results section, and unpublished data). On the other hand, in diabetic subjects with few deep periodontal pockets, no dramatic effect on their metabolic control could be observed (II, III).

Poor metabolic control involving diabetic complications was a risk factor affecting periodontal disease progression and severity, as evidenced in Study III ($p < 0.05$, $\chi^2$–test). However, prolonged exposure to hyperglycemia may be the primary factor responsible for the development of diabetic complications resulting in the formation of non–enzymatic advanced glycation end–products (AGEs); this has been demonstrated also in the gingiva of diabetic patients (Schmidt et al. 1996, Lalla et al. 1998a, Nishimura et al. 1998) and diabetic animals (Lalla et al. 1998b). AGEs can induce diabetic collagen cross–links and expansion of extracellular matrix, indicating that most of the macrovascular complications, such as hardening of arteries and narrowing of vascular lumina, may have been mediated by elevated glucose levels in serum and body fluids (Dennison 1998, Grossi & Genco 1998). Further, the AGE–modified proteins may bind to the macrophage receptor and possibly increase production of cytokines, such as IL–1 and TNF–$\alpha$ (Salvi et al. 1998). If synthesis of these cytokines is increased, as in hyperglycemia–induced excessive AGEs accumulation, a degradative cascade is triggered, resulting partly in MMP–mediated connective tissue degradation, endothelial proliferation, and focal thrombosis (Lalla et al. 1998a).

Thorstensson (1996) demonstrated that, in adult long–term IDDM subjects diabetic nephropathy and cardiovascular complications are
associated with severe periodontal disease. We found (II, III) that periodontal treatment does not directly seem to improve the metabolic control of these randomly chosen long–term IDDM subjects, perhaps partly due to the fact that the inflammatory process in the periodontium may be very local and discrete.

The clinical relevance of these studies (II, III) supports earlier findings indicating that even poorly controlled diabetic patients benefit directly or indirectly from the prevention and treatment of periodontal disease. Because PIDD patients have increased loss of attachment and bone, periodontal treatment is of utmost importance to prevent further deterioration and, eventually, loss of teeth. Because metabolic changes affect the development and progression of vascular complications of IDDM (Diabetes Control and Complications Trial Research Group, DCCT 1993), it is at least theoretically important to treat the periodontal diseases in IDDM to diminish the risk for development of cardiovascular complications (Mattila et al. 1989, DeStefano et al. 1993, Dennison 1998).

Results from microbiological studies on the prevalence of periodontal pathogens in diabetic subjects are somewhat variable (Sastrowijoto et al. 1990, Ueta et al. 1993, Grossi et al. 1994, Tervonen et al. 1994, Thorstensson 1995, Christgau et al. 1998, Collin et al. 1998, Sbordone et al. 1998). Grossi et al. and Thorstensson demonstrated that significantly more diabetic subjects harbored *Porphyromonas gingivalis* than did non–diabetic subjects, but Tervonen et al., Collin et al., and Sbordone et al. could not demonstrate in well or moderately controlled diabetic subjects more periodontopathogens, i.e., *Actinobacillus Actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia*, than in control subjects. These various results may originate from differences in study models (I, IV), and from methodological differences used to analyze partly non–cultivable putative periodontopathogens (Zappa et al. 1986, Ojima et al. 1998).

In our microbiological study (IV), when both periodontally healthy and diseased sites were analyzed, motile rods evidently representing spirochetes of the subgingival microflora were more frequent in periodontal pockets of PIDD than CIDD subjects. Our finding was thus in agreement with that of Mashimo et al. (1983), who found more spirochetes in diabetics than in control subjects (Mashimo et al. 1983, IV). However, also in healthy sites of PIDD subjects was an established microflora with spirochetes present more frequently than in the healthy sites of CIDD subjects ($p < 0.001$, $\chi^2$–test, IV), probably due to the high glucose levels of the GCF (Ficara et al.
Thus, elevated glucose levels in the GCF of PIDD subjects may have induced circumstances during an infection favorable for the growth of a periodontopathogen microflora with reduced oxygen generation in the periodontal tissues during an infection (Ueta et al. 1993). It is also well documented that diabetics are particularly susceptible to Candida albicans infections (Hostetter 1990).

Poorly controlled diabetes may, due to defectively functioning PMNs (Tater et al. 1987, Genco et al. 1990, Cutler et al. 1991, Marhoffer et al. 1992, De Toni et al. 1998), predispose PIDD subjects to a more virulent periodontopathogen microflora. Although our study could not document increased numbers of either PMNs or monocytes/macrophages in the ICT of PIDD subjects compared to numbers in non–diabetic control subjects, severe disruptions in the transmigration and/or function of these cells may occur due to abnormal exposure to glucose.

Increased cytokine (Iacopino 1995) and neutrophil–type matrix metalloprotei–nase–8 (MMP–8) or collagenase–2 and gelatinases act together with dental plaque–derived periodontopathogens and their virulence factors in diabetic subjects and in animal models of diabetes (Sorsa et al. 1992b, Ingman et al. 1996, Sorsa et al. 1996, Doxey et al. 1998, Lalla et al. 1998a). They may be responsible for an increase in the inflammatory tissue destruction of PIDD patients with advanced periodontitis (Sorsa et al. 1992a, 1996). It was of particular interest to observe, in the ICT of PIDD subjects, increased numbers of plasma cells (V) indicating a chronic, indolent local host–reaction to infective micro–organisms rather than clear signs of acute inflammation. Potentially, the pathogen microflora in hyperglycemic diabetic subjects leads, through exaggerated and/or prolonged host–responses, to periodontal breakdown. This finding was in agreement with findings of Grossi et al. (1994).

The histological study (V) included measurements of the shortest distance from the lumen to the outer border of each blood vessel finding this distance to be significantly greater in PIDD than in control or CIDD subjects (p < 0.001, t–test, V). Further, comparisons of the mean areas of circular cross–sections of several small blood vessels between the age– and sex–matched PIDD, CIDD, and control subjects showed CIDD subjects to have the smallest and PIDD subjects the largest mean areas of these three groups. This finding, although not statistically significant, suggests that well–controlled diabetic subjects are comparable to non–diabetic control subjects, but that vascular complications are present in the clinically healthy gingival tissues of long–term uncontrolled diabetic subjects.
The PIDD subjects had characteristics of chronic inflammation with a clear predominance of plasma cells and a reduction of fibroblasts as well as decreased collagen in their ICT (V). Based upon our histomorphometric study (V), the gingival tissues of PIDD subjects demonstrated not only microangiopathic changes but also an increased number of plasma cells, which characterize the chronic inflammatory process of the periodontal connective tissues and the local loss of attachment and alveolar bone in diabetic and non–diabetic subjects. Such a local activation of the inflammatory process may lack clinical relevance, and may not always lead to deepened probing depths, increased gingival indices, or increased bleeding after probing measurements, or to both. However, it does seem important to follow especially the attachment loss measurements, the radiological loss of alveolar bone, and those periodontal parameters related to gingival inflammation in diabetic subjects with moderate or severe periodontal disease. The significant differences between PIDD and CIDD in progression of attachment–loss site–specifically may indicate that poorly controlled diabetes accelerates destruction especially in those periodontitis sites affected by the disease in its active stage.

Based upon our histopathological results (V), the connective tissue changes may indicate increased degradation of the inflamed sites in gingiva, especially in PIDD subjects. Degenerated cell organs have also been observed in pericytes and endothelial cells of the mucosal and retinal capillaries, resulting in a narrowed capillary lumen (Serizawa 1988) as well as in impaired wound healing (Grant–Theule 1996). Hiura (1991) demonstrated that the percentage of capillaries with layered basement membranes and lysosome–like structures in their endothelial cells increases in the diabetic group and correlates with growing degrees of diabetic retinopathy. As PIDD diabetic patients seem to have more retinopathies than control subjects, Hiura’s findings agree well with the current study demonstrating thickening and obliteration of small arteries and postcapillary venules composed of endothelial cells and in many places surrounded by pericytes. Taken together, the vascular changes in the gingiva of PIDD subjects may be the result of a combination of metabolic and hormonal imbalances in conjunction with genetic factors.

It is generally accepted that the cellular composition of inflamed gingival tissue is characterized by a predominance of plasma cells (Chomette et al. 1987, Tew et al. 1989). However, characteristic of the inflammatory infiltrate of the diabetic gingiva was that it contained a greater number of plasma cells than seen in healthy controls.
under similar plaque conditions (V). This was the first attempt to quantify the cellular and volumetric composition of the sub-clinical inflammatory lesion of the gingival connective tissue in diabetic subjects (V). These studies provide some new basic information about the inflammatory process as well as the composition of the gingival tissues of randomly chosen long-term poorly controlled diabetic subjects.

This work confirms and extends earlier findings which suggest that good control of diabetes is associated with less severe and less progressive periodontal changes (Christgau et al. 1998, Collin et al. 1998, Oliver et al. 1998, Taylor et al. 1998). Although differences in mean cross-sectional periodontal measurements did not reach significance (I), such differences were statistically significant in the site-specific analysis of each subject's dentition (III). This represents one more reason why one should strive to obtain good metabolic control in diabetes, in particular in patients with poor periodontal status and other relevant risk factors. Because PIDD patients developed more diseased sites and displayed more rapid progression in already existing sites during the two-year follow-up than did CIDD subjects, we suggest that the poor control of metabolic status in insulin-dependent diabetes mellitus contributes both to the initiation and to the progression of attachment loss and bone loss. Further information could be obtained from long-term prospective clinical studies, which, however, for ethical reasons, cannot be performed in a totally randomized and controlled manner; it is impossible to allocate any patients to a "none or bad treatment" category. Secondly, it seems indicated and useful to treat gingivitis and periodontitis meticulously in diabetic patients with poor metabolic control, because they seem to respond favorably to periodontal treatment. In contrast, because PIDD was a marker and probably a risk factor for periodontitis, periodontal treatment and prophylaxis seem to be particularly important for the PIDD patients (II, III). Further, as demonstrated by these studies, the inflammatory response to plaque may in long-term and uncontrolled IDDM result in a more periopathogenic microflora (IV), more severe loss of collagen, and connective tissue degradation or degeneration, or both, as well as in plasma-cell-dominated infiltrates associated with reduced numbers of fibroblasts than occur in well-controlled IDDM subjects (V). Therefore, diabetic subjects with unsatisfactory metabolic control should be analyzed carefully during their annual medical and dental examinations in order to identify as early as possible subgroups of diabetic subjects at high risk for periodontal disease. This emerging
diagnostic, pharmacologic, and risk-based management will require a design for the benefit for these high-risk patients.
7. Summary and Conclusions

The purpose of the present study was to compare periodontal conditions in a randomly chosen population of poorly controlled insulin-dependent diabetic (PIDD) and controlled insulin-dependent diabetic subjects (CIDD) in a site-specific manner and over time. Classification of the insulin-dependent diabetics mellitus (IDDM) was based upon longitudinal assessment at annual follow-up visits. Fundamental questions were related to the influence of hyper- and hypoglycemia on the initiation, severity, and progression of periodontal disease in long-term insulin-dependent diabetic subjects. In addition, vice versa, the impact of periodontal treatment on metabolic control in IDDM underwent investigation. Further, the qualitative and quantitative differences were analyzed in the gingival inflammatory process and the composition of the subgingival microflora.

Under similar plaque conditions, adult subjects with long-term PIDD were found to have lost more attachment and alveolar bone than had subjects with CIDD. These differences were not equally obvious when the subjects were classified according to their history of medical complications, perhaps in part due to genetic variation in subjects. Both at the cross-sectional baseline and in longitudinal one-year and two-year examinations, the PIDD subjects had lost significantly more attachment than lost by the corresponding CIDD subjects. The influence of metabolic control on the site-specific progression of periodontal disease was also evident. Overall, this suggests that poor metabolic control of DM is prone to accelerate the progression of periodontitis, especially in the active phase of the periodontitis.

The benefit of periodontal treatment for on the metabolic control of the PIDD and CIDD subjects was estimated by levels of glycosylated hemoglobin, but this difference was not statistically significant. Results of the two-year longitudinal site-by-site examinations confirm that poorly controlled insulin-dependent diabetes mellitus is strongly related to amount of alveolar bone loss. At all three examinations, the PIDD subjects also exhibited more gingival recession than did CIDD subjects. Explanations for the increased prevalence and incidence of periodontal disease were increased risk for gingival inflammation, the specific pattern of the inflammatory response, the periopathogen microflora, the accelerated progression of vascular diseases, and the increased catabolism of the gingival connective tissue.
Dark field microscopy revealed that the percentage of spirochetes and motile rods in the periodontally diseased pockets was significantly higher in the PIDD than in the CIDD subjects. Moreover, the PIDD subjects had lower mean percentages of coccoid cells in their periodontally diseased sites than did CIDD subjects. This suggests that the metabolic state of IDDM patients affects their periodontal bacterial microflora. Further efforts should be made to analyze the subgingival microflora in long–term hyper– or hypoglycemic diabetic subjects to identify those periodontopathogens, if any, which act as mediators rather than markers for the severity and progression of periodontal disease.

The morphometric analysis of cellular changes in the gingival connective tissue samples revealed, under similar plaque conditions, that in the PIDD subjects, the inflammatory response was clearly influenced by hyperglycemia. In general, the ICT of CIDD subjects did not differ from the age– and sex–matched control subjects, but volumetric analysis of the healthy gingiva in PIDD subjects demonstrated elevated plasma cell levels compared to those of control subjects, with decreased numbers of fibroblasts and lymphocytes. In addition, the non–cellular components, such as collagen fibers, were also lower in the ICT of PIDD patients. Swollen and proliferated endothelial cells as well as diminished or totally lacking pericytes were frequent observations in PIDD subjects.

In conclusion, cellular, vascular, and connective tissue changes indicating increased catabolism in the gingiva are associated with poorly controlled long–term insulin–dependent diabetes mellitus.
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10. Studies I – V