PERIODONTAL DISEASES, DENTAL CARIES, AND SALIVA IN RELATION TO CLINICAL CHARACTERISTICS OF TYPE 1 DIABETES

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OU LU 2000
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To Jenni and Juuso
Abstract

Diabetes mellitus has been linked with an increased risk for oral diseases, especially periodontal diseases (Oliver & Tervonen 1994, Yalda et al. 1994). Further investigations have, however, shown that this risk is not equal in all patients with diabetes. These studies explored the relationship between the diabetic status and periodontal diseases, dental caries and salivary factors. In a group of diabetic adolescents aged 12 to 18 years, dental caries and gingivitis were shown to associate with poor metabolic control of diabetes. An increase of caries prevalence and the severity of gingivitis was evident in alarmingly poorly controlled patients with glycosylated haemoglobin (HbA₁) values of 13% or higher. The hyperglycaemia-associated increase of gingivitis was confirmed in a group of newly diagnosed diabetic children and adolescents, whose gingival inflammation decreased during a follow-up after the correction of hyperglycaemia by initiation of insulin treatment. Decreased salivary flow rates and elevated salivary glucose levels were observed during the hyperglycaemic state of children and adolescents with newly diagnosed diabetes. Higher salivary microbial counts, especially yeast counts, were related to the lower salivary flow rates and higher salivary glucose levels.

In adult patients with type 1 diabetes, the complex diabetic status was assessed by means of the level of metabolic control and/or the presence and severity of diabetic complications. Adult diabetic patients with poor metabolic control and/or complications exhibited more deepened pockets and clinical attachment loss, and after periodontal treatment, the recurrence of deepened pockets was faster in these patients compared to the other diabetic patients or the controls. The high-risk subjects among adults with type 1 diabetes were categorised as follows: subjects with long-term HbA₁ values over 10%, independently of whether the patient has diabetic complications or not; subjects with advanced diabetic complications, such as preproliferative or proliferative retinopathy, nephropathy, limb amputations or recurrent infections; and subjects with multiple diabetic complications, irrespective of the level of metabolic control.

In conclusion, dental professionals should be aware of the level of glycaemic control in their patients with type 1 diabetes, and the prevention and intensified treatment should be focused on those with a poor metabolic control (HbA₁c values around or over 10%). In the case of adult patients, more comprehensive knowledge about the diabetic status of the patients is needed in order to be able to identify the subjects at high risk for periodontitis and in need of regular maintenance care at least twice a year. The medical and nursing personnel should also be aware of periodontitis as a complication of diabetes, and especially in the case of adult diabetic patients, they should refer their patients to dental treatment when necessary.

Keywords: diabetes mellitus, gingivitis, periodontitis
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Oulu May, 2000

Kaisa Karjalainen
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGE</td>
<td>advanced glycosylation end product</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>analysis of co-variance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>CFU</td>
<td>colony-forming unit</td>
</tr>
<tr>
<td>DFS</td>
<td>decayed and/or filled tooth surfaces</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>DMFS</td>
<td>decayed, missing and/or filled tooth surfaces</td>
</tr>
<tr>
<td>DS</td>
<td>decayed tooth surfaces</td>
</tr>
<tr>
<td>FS</td>
<td>filled tooth surfaces</td>
</tr>
<tr>
<td>HbA$_1$</td>
<td>glycosylated haemoglobin, A$_1$</td>
</tr>
<tr>
<td>HbA$_{1c}$</td>
<td>glycosylated haemoglobin, A$_{1c}$</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>IDDM</td>
<td>insulin-dependent diabetes mellitus</td>
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<tr>
<td>IGT</td>
<td>impaired glucose tolerance</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IL-1$\beta$</td>
<td>interleukin-1$\beta$</td>
</tr>
<tr>
<td>IL-6</td>
<td>interleukin-6</td>
</tr>
<tr>
<td>LJM</td>
<td>limited joint mobility</td>
</tr>
<tr>
<td>MSR</td>
<td>macrophage scavenger receptor</td>
</tr>
<tr>
<td>NIDDM</td>
<td>non-insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>prostaglandin E$_2$</td>
</tr>
<tr>
<td>PMN</td>
<td>polymorphonuclear neutrophil</td>
</tr>
<tr>
<td>RAGE</td>
<td>receptor for advanced glycosylation end product</td>
</tr>
<tr>
<td>SPSS</td>
<td>statistical product and service solutions</td>
</tr>
<tr>
<td>TIA</td>
<td>transient ischaemic attack</td>
</tr>
<tr>
<td>TNF-$\alpha$</td>
<td>tumor necrosis factor-$\alpha$</td>
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List of original papers

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.


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1. Introduction

Diabetes mellitus consists of a group of diseases characterised by abnormally high blood glucose levels. The two main types of diabetes mellitus are type 1 or insulin-dependent diabetes mellitus (IDDM) and type 2 or non-insulin-dependent diabetes mellitus (NIDDM). Type 1 diabetes usually manifests in childhood or adolescence, and as the name (IDDM) implies, the patients require exogenous insulin because of the destruction of insulin-producing β cells in the pancreas by autoimmune reactions. The prevalence of type 2 diabetes begins to rise in early middle age and increases along with age. Exogenous insulin may not be a necessity for these patients, because insulin production is less or not at all decreased in type 2 compared to type 1 diabetes, and the basic reason for metabolic disturbance is insulin resistance. (Bennet 1990, Fajans 1990)

Diabetes is a major health problem in Finland, as the Finnish incidences of both type 1 and type 2 diabetes are among the highest in the world, and the incidence of childhood diabetes diagnosed under the age of 15 is actually the highest in the world (Karvonen et al. 1993). In the year 1996, the number of diagnosed diabetic patients in Finland was estimated to be about 170,000, out of whom 30,000 suffered from type 1 diabetes (Sarahimeo & Ilanne-Parikka 1999). The incidence rates have increased during the past decades and, unfortunately, the same trend seems to continue (Tuomilehto et al. 1995, Tuomilehto et al. 1999).

The oral health of patients with diabetes has been widely investigated. This research has mainly focused on periodontal diseases and dental caries, which can be considered as national diseases because of their high prevalence rates. Most of the studies have compared patients with diabetes to controls, but their results have been somewhat contradictory (Darwazeh 1990). These results, however, have indicated that diabetes can be seen as a risk factor for oral diseases, especially periodontal diseases (Oliver & Tervonen 1994, Yalda et al. 1994). The inconsistency of the results may relate to the fact that diabetes is a very complex multiform disease, and the study populations may therefore be very heterogeneous. Most evidently, not all patients with diabetes are at equal risk for oral complications, and some variation is apparently related to the differences in the diabetic status of the patients. Therefore, more attention has recently been given to the possible diabetes-related factors which might help to identify the
patients particularly prone to periodontal diseases or dental caries. The factors studied have included the duration of diabetes, age at diagnosis of diabetes, presence of diabetic organ complications and, since the beginning of the 1980s, the level of metabolic control.

Despite these efforts, there is not yet any agreement as to how the diabetic status should be evaluated with respect to the risk for oral diseases. An ability to identify the diabetic patients at a higher risk for oral complications would benefit the dental care of patients with diabetes, as the prophylaxis and treatment could then be targeted more efficiently to the risk subjects among the increasing number of diabetic patients. Moreover, there is evidence to suggest that treatment and prophylaxis of oral infections could benefit the maintenance of good metabolic control of diabetes (Miller et al. 1992, Grossi et al. 1996, 1997). The present studies were conducted to find out a suitable way to assess the diabetic status of patients, which could help both the dental and the medical professionals responsible for care of the treatment of patients with diabetes, to identify the patients most urgently in need for dental care.
2. Review of the literature

2.1. Diabetes mellitus

2.1.1. Common characteristics and epidemiology

Diabetes mellitus comprises a heterogeneous group of diseases, which cause the patients, if untreated, to have abnormally high blood glucose levels. Four main types of diabetes mellitus have been defined: type 1 or insulin-dependent diabetes mellitus (IDDM), type 2 or non-insulin dependent diabetes mellitus (NIDDM), gestational diabetes and diabetes related to other conditions, e.g. pancreatic diseases. The forms of diabetes mellitus other than type 1 or type 2 are comparatively rare. (Harris 1995, Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1998.) As the name indicates, at the time IDDM is diagnosed or soon after that, the patients are totally dependent on exogenous insulin therapy, because all the insulin-producing \( \beta \) cells in the Langerhans islets of the pancreas are ultimately destroyed by autoimmune reactions. The diagnosis of type 1 diabetes may be made at any age, but it usually manifests in childhood, adolescence or early adulthood, which means that a majority of the patients with type 1 diabetes are diagnosed before the age of twenty. Type 2 diabetes begins to manifest in middle age and its incidence increases over age. The prevalence of type 2 diabetes is much higher than that of type 1 diabetes. The treatment of type 2 diabetes does not necessarily require insulin, as its pathophysiology is primarily based on insulin resistance, and insulin secretion is not so badly disturbed as in type 1 diabetes. (Bennett 1990, Fajans 1990.) In Finland, the incidence of childhood diabetes is the highest in the world and it has been rising during the past few decades: in the year 1953, 12/100,000 new patients with type 1 diabetes aged under 15 were diagnosed compared to 36/100 000 during the years 1987–1990 (Tuomilehto et al. 1995). If the same trend continues, the predicted incidence in Finland in children aged 14 years or under will be approximately 50 new patients with type 1 diabetes per 100,000 per year in the year 2010 (Tuomilehto et al. 1999). The prevalence of type 2 diabetes in Finland is at least equal to the levels in the other developed countries, and the number of patients with type 2 diabetes has also been increasing. The number of diagnosed type 1 diabetes patients was about 30,000 and type
2 diabetes patients about 140,000 in Finland in 1996, but together with the undiagnosed cases, the estimated number of diabetic patients will rise up to 300,000 (Saraheimo & Ilanne-Parikka 1999).

Due to the high number of patients with diabetes and the exceptionally high incidence rates, diabetes research is active in Finland and especially focuses on the etiology of the disease. Genetic susceptibility to type 1 diabetes is related to the HLA gene region in chromosome 6, especially the HLA-DQ and HLA-DR regions. Finnish researchers have found haplotypes associated with a very high risk of type 1 diabetes, which have been found very rarely in populations other than Finns (Tuomilehto-Wolf et al. 1989). Family studies and twin studies have shown, however, that only about 30 percent of the risk for type 1 diabetes is genetically determined, while the rest may be related to environmental factors. At least certain viral infections, such as enterovirus and rotavirus infections, and nutritional factors, particularly cow’s milk proteins, have been suggested as possible causes of autoimmune reactions which destroy insulin-producing \( \beta \) cells in the Langerhans islets of the pancreas (for a review, see Knip & Åkerblom 1999). At the time of the clinical diagnosis, only a small number of \( \beta \) cells are capable of producing insulin, and the patients inevitably become dependent on exogenous insulin as a treatment of type 1 diabetes. (Palmer & Lernmark 1990, Rotter et al. 1990, Trevisan et al. 1998.)

Genetic background is much more strongly associated with type 2 than type 1 diabetes, but it is less clear and the risk gene or genes still remain unknown. The etiology of type 2 diabetes is heterogeneous, but the majority of patients with type 2 diabetes are believed to result from a combination of hyperinsulinemia / insulin resistance and \( \beta \) cell failure. The risk factors of type 2 diabetes are known to include older age, obesity and a family history of type 2 diabetes. Part of the patients with type 2 diabetes are treated with diet, physical activity and slimming of obese patients. Oral agents to induce insulin production can be used, and some patients with type 2 diabetes need insulin therapy, especially after a long disease duration. (Kahn & Porte Jr 1990, Rotter et al. 1990, Trevisan et al. 1998)

### 2.1.2. Diabetic organ complications

Vascular complications of diabetes occur in both micro- and macrovascular vessels. Microvascular complications include retinopathy, nephropathy and neuropathy. Macrovascular complications comprise peripheral vascular disease and cardiovascular complications, such as ischaemic heart disease and hypertension. These are all chronic illnesses which take 10–20 years to manifest. One of the most important factors in the pathogenesis of diabetic complications is the metabolic milieu of the diabetic patients, the main causative factor being hyperglycaemia (Diabetes Control and Complications Trial Research Group 1993). The severity of complications is modified by genetic factors, since many of the diabetic patients do not develop complications even when their glycaemic control is not optimal (Rosenstock & Raskin 1988).

Retinopathy is common in both type 1 and type 2 diabetes, and its prevalence is strongly related to the duration of diabetes. In patients with type 1 diabetes, the first changes in the small vessels of the retina may appear after 4–7 years of diabetes. About 20–30 percent of patients with type 2 diabetes already have retinopathic changes at the
time when their diabetes is diagnosed, presumably because type 2 diabetes may go undiagnosed much longer than type 1 diabetes. The incidence of retinopathy increases along with the duration of diabetes, and 90 percent of patients with type 1 and 60 percent of patients with type 2 diabetes have retinal changes after 20 years of the disease. The first signs of retinopathy are microaneurysms (minimal retinopathy), which are followed by haemorrhages and lipid exudates (background retinopathy), cotton-wool spots and venous reduplication (preproliferative retinopathy). When abnormal blood vessels and fibrous tissue, i.e. neovascularisation, occurs, the retinopathy is classified as proliferative. The etiology of retinopathy includes hyperglycaemia-associated biochemical, anatomical and functional changes. The main pathophysiological events have been linked with basement membrane pathology, i.e. thickening of retinal capillaries and small vessels. Non-enzymatic glycation and changes in growth factor profiles cause deposition of further extracellular matrix material along the basement membranes of small vessels. Also, an excess of glucose activates the polyol pathway, which causes accumulation of sorbitol in the lens and is accompanied by cataracts. (Kinoshita et al. 1990, L’Esperance et al. 1990, American Diabetes Association 1998a)

Nephropathy arises from glomerulosclerosis, which is characterised by glomerular basement membrane thickening and arteriosclerosis of small arterioles. The mechanisms proposed to induce glomerulosclerosis include hyperglycaemia, a hyperfiltration-related increase of glomerular pressure and increased blood viscosity. Clinically, nephropathy manifests as proteinuria, mostly albuminuria. The early phases of nephropathy are characterised by microalbuminuria, during which the urine albumin content (20–200 µg/min) can be recorded by sensitive laboratory measurements. This incipient diabetic nephropathy is evident in about 35 percent of patients with type 1 diabetes after 6–15 years of diabetes. Renal complications affect notably fewer patients with type 2 than type 1 diabetes. After 15–25 years, macroalbuminuria, i.e. clinically overt diabetic nephropathy, is estimated to develop in one third of diabetic patients. During macroalbuminuria, the protein content of urine is so high (> 200 µg/min) that it can be measured by simple stick tests. Along with macroalbuminuria the glomerular filtration rate falls consistently. Nephropathy may culminate in uraemia and, in fact, most of the hemodialysis patients and the patients receiving renal transplants, have diabetes. (Friedman 1990, Nelson et al. 1995, Mogensen 1997, American Diabetes Association 1998b)

High blood pressure manifests along with the progression of nephropathy: in patients with microalbuminuria, blood pressure gradually increases. Hypertension contributes notably to the progression of renal disease and retinopathy, and early antihypertensive treatment is crucial. Hypertension is not so clearly related to nephropathy in patients with type 2 diabetes as in patients with type 1 diabetes, but is more often part of the metabolic syndrome that includes glucose intolerance, insulin resistance, obesity, dyslipidemia and coronary heart disease. Due to arteriosclerosis, hypertension and dyslipidemias, diabetic patients are also at a higher risk for decreased peripheral blood flow, ischaemic heart disease and other cardiovascular problems compared to non-diabetic subjects. Abnormal fibrinolysis and altered platelet function also increase the risk of macrovascular complications among patients with diabetes. (Brunner & Chait 1990, Fein & Scheuer 1990, Steffes & Mauer 1990, Nelson et al. 1995, Mogensen 1997)
The third microangiopathic complication of diabetes is neuropathy, and the main histological finding is thickening of the basement membranes of nerve sheets and the capillaries that supply blood to the nerves. Reduced nerve perfusion is an important factor in the etiology of neuropathy, but many metabolic changes, such as an activated polyol pathway, non-enzymatic glycosylation and increased oxidative stress, for example, have recently been raised as possible reasons for defective nerve function. Neuropathy may be either focal or diffuse. It may affect both sensory and autonomic nerves, but distal symmetric polyneuropathy is probably the most common consequence which, together with peripheral vascular disease, is an important etiologic factor for foot ulcerations and lower limb amputations, which even nowadays are common complications of diabetes. Reliable estimates of the prevalence of neuropathy are not available, and the prevalence rates have varied within 20–100 percent, depending on the study population and the different way of assessing the presence of neuropathy. (Greene et al. 1990, Thomas 1997)

Limited joint mobility (LJM) and increased skin thickness in hands and feet have been documented as complications of diabetes, especially type 1 diabetes (Rosenbloom & Silverstein 1996). LJM seems to associate with the presence of microvascular complications, especially retinopathy (Arkkila et al. 1994, Balci et al. 1999, Duffin et al. 1999). Non-enzymatic glycosylation and changes in collagen structure are proposed to cause the development of LJM and skin changes (Seibold et al. 1985, Rosenbloom & Silverstein 1996).

2.1.3. Metabolic control of diabetes

Large follow-up studies have shown that good glucose control may prevent or delay the manifestation of complications despite the long duration of the disease (D’Antonio et al. 1989, Reichard et al. 1993, Diabetes Control and Complications Trial Research Group 1993, Wang et al. 1993). The evaluation of the level of metabolic control of diabetes is mainly based on the monitoring of blood glucose levels. Earlier, daily or occasional blood glucose determinations were the only way to get information on glucose control. These measurements still constitute the basis for day-to-day management of diabetes. Occasional blood glucose measurements can, however, produce quite variable results, depending, for instance, on the type and timing of insulin injections or the food intake and physical exercise.

Erythrocytes are freely permeable to glucose. In cells, glucose attaches to the free amino ends of haemoglobin molecules, and this process, called non-enzymatic glycosylation, causes glycosylated haemoglobin to be formed directly proportional to the blood glucose concentration. As the average erythrocyte life span is about 120 days, glycosylated haemoglobin levels give information on the mean average blood glucose levels over the past 2 to 3 months. Two main fractions of glycosylated haemoglobin, HbA1 or HbA1c, are commonly used in diabetes monitoring. The normal range for HbA1c is 4–6 percent, and the values for HbA1 are about two percentage points higher, as HbA1c is a smaller part of the HbA1 fraction. (Goldstein et al. 1995, American Diabetes Association 1998c.) According to generally accepted guidelines in the clinical monitoring of diabetic patients, diabetes is considered to be well controlled if the HbA1c levels are
below 7.5%, and moderately controlled if the HbA1c levels vary between 7.5–8.5%. Values from 8.6 to 10.0% indicate poor control of the disease, and values over 10% are considered alarmingly high (Suomen Diabetesliitto 1995).

Glycosylation of serum proteins, mainly albumin, has also been used in diabetes monitoring. The half-life of albumin is 2 to 3 weeks, and the degree of albumin glycosylation hence provides an index of glycaemia over a shorter period of time than glycosylation of haemoglobin. One of the most widely used measurements of glycosylated serum proteins is fructosamine assay. (Goldstein et al. 1995, American Diabetes Association 1998c.)

Although carbohydrate metabolism plays a central role in the pathophysiology of diabetes, the treatment aims at normalising blood glucose levels and treatment monitoring is mainly based on blood glucose levels, protein and lipid metabolisms are also affected (Kimball et al. 1990, Brunzell & Chait 1990). Other metabolic disorders are not, however, monitored so often and on such a routine basis as blood glucose or glycosylated haemoglobin levels. Different ways to evaluate the level of metabolic control can be used concomitantly to give complementary information. However, the availability of glycosylated haemoglobin measurements has made it possible to evaluate the role of long-term glucose control in developing diabetic complications, and has been adopted in dental studies as well.

2.1.4. Diabetes-related tissue alterations

Diabetes is known to lower the host’s resistance to infections and to impair wound healing. Insulin is necessary for glucose to enter cells and to provide a source of energy, for the uptake of amino acids to synthesise proteins, and for the inhibition of adipose tissue lipolysis. If insulin is not adequately supplied, basic cell functions in the body will consequently be disturbed. Signs of deterioration of the first-line host defence against microbes, i.e. impairment of PMN cell function with abnormalities of adherence, chemotaxis, phagocytosis and intracellular killing, are well known. Defects in PMN cell functions have been shown to be related to poor control of diabetes. (For reviews, see Pearl & Kanat 1988, Morain & Colen 1990, Rosenberg 1990, Terranova 1991.) Molenaar et al. (1976), however, found similar PMN cell defects in both diabetics and their first-degree of relatives, indicating a genetic background of the defect.

Changes in the turnover and structure of collagen, the main component of the extracellular matrix, such as decreased synthesis, increased degradation of newly synthesised collagen and decreased solubility of mature collagen, have been demonstrated in both human and animal diabetes studies. In diabetes, connective-tissue collagen is less soluble and more resistant to digestion, and the thermal rupture time and mechanical strength are also increased. (For reviews, see Sternberg et al. 1985, Reiser 1991.) Experimental animal studies have shown that these changes occur in gingival tissues as well (Ramamurthy et al. 1972, Schneir et al. 1984, Ramamurthy et al. 1985, Yu et al. 1993). Increased levels of collagenase activity have also been seen in gingival tissues (Golub et al. 1978, Golub et al. 1983, Ramamurthy & Golub 1983, Chang et al. 1988, Yu et al. 1993) and gingival crevicular fluid (McNamara et al. 1979, Kaplan et al. 1982).
The increased thickness of basement membranes, especially capillaries, in diabetes is well documented. Biochemical studies have shown that basement membranes in diabetes include excess amounts of type IV collagen, the main component of basement membranes, and decreased amounts of proteoglycans, both of which changes decrease the permeability of capillaries and disturb leukocyte diapedesis, oxygen diffusion, nutrition and metabolic waste removal. (For reviews, see Sternberg et al. 1985, Rosenberg 1990.) Tissue oxygenation is further impaired by the decreased ability of glycosylated haemoglobin to carry oxygen. Furthermore, hyperglycaemia increases blood viscosity, reduces erythrocyte deformability and increases platelet aggregation, which all cause blood flow abnormalities and, furthermore, platelet aggregation is followed by the release of serotonin and lysosomal enzymes (McMillan et al. 1978, Juhan et al. 1982, Mustard & Packham 1984, Kawamura et al. 1988).

The above mentioned alterations, proved to be related to hyperglycaemia, have been observed to cause changes in the function and/or structure of almost all cells and tissues explored. The extent of these alterations was found to be dependent on the level and duration of the abnormal metabolic state. Some of the changes are reversible, e.g. return to normal, when the glucose balance is corrected. Some, however, turn out to be irreversible and seem to accumulate, especially in tissues with a long half-life.

Non-enzymatic glycosylation has recently attracted increasing interest as a crucial pathophysiologic event behind all these hyperglycaemia-related alterations and in the pathophysiology of the development of diabetic complications. Proteins and lipids exposed to aldose sugars go through reactions which are not enzyme-dependent, and generation of reversible Schiff bases or Amadori products take place. Later, through further molecular rearrangements, irreversible advanced glycosylation end products (AGEs) are formed. This process also takes place during normal ageing, but in diabetes their formation is accelerated to an extent related to the level and duration of hyperglycaemia. (For reviews, see Reiser 1991, Vlassara et al. 1994, Vlassara 1997.) The potential pathophysiological significance of AGEs is associated with their accumulation in plasma, cells and tissues and their contribution to the formation of cross-links, generation of reactive oxygen intermediates and interactions with particular receptors on cellular surfaces (Schmidt et al. 1996a, Vlassara & Bucala 1996).

AGEs have direct effects on the host response by affecting tissue structures, e.g. by increasing collagen cross-links, which is followed by changes in collagen solubility and turnover (Monnier et al. 1996). Thickening of basement membranes is partly due to glycosylation of membrane proteins or entrapment of glycosylated serum proteins into basement membranes (Brownlee et al. 1988, Makita et al. 1991). Specific cell-surface receptors for the recognition of AGEs were first found on mononuclear phagocytes, and AGEs were observed to attract and retain mononuclear phagocytes (Schmidt et al. 1993). Later, these receptors have been identified on lymphocytes, endothelial cells and smooth muscle cells as well as on other cellular systems that participate in both normal tissue remodelling and tissue damage. AGEs are bound to the specific cell surface receptors for AGEs, within which family of receptors RAGE is the most well defined and resembles macrophage scavenger receptors (MSR). This interaction results in oxidant stress of the target cells, inducing production of different patterns of cytokines and growth factors, depending on the type of cells involved. Excess production of growth factors and cytokines plays an essential role in both micro- and macrovascular alterations. AGEs, by
themselves, appear to generate reactive oxygen intermediates and the interaction between AGE and RAGE further induces production of intra- and extracellular oxidants. Oxidative modifications of lipoproteins, in turn, accelerate atherogenesis (Witztum 1997). Free oxygen radicals cause tissue destruction directly and exaggerate the inflammation-related tissue destruction because activated monocytes produce proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α. Based on what has been said above, it is evident that AGEs can interact with cell functions, tissue remodelling and inflammatory reactions in several different ways. (For reviews, see Schmidt et al. 1994, Chappey et al. 1997.)

In conclusion, hyperglycaemia, either directly or through AGE formation, causes various structural and functional modifications of cells as well as quantitative and qualitative alterations of the extracellular matrix, which may all alter tissue homeostasis and modify the host response even in periodontal and other oral tissues.

2.2. Diabetes and oral health

2.2.1. Patients with diabetes vs. controls

The differences in oral health between patients with diabetes and non-diabetic subjects have been intensively studied. Widespread agreement exists about the increased risk for periodontal diseases among patients with diabetes, although this view has not been supported unanimously. Some authors have estimated that the risk for periodontal diseases is about 3-fold in patients with diabetes compared to non-diabetics, but these results have been obtained from type 2 study populations and may not be generalisable (Emrich et al. 1991). (For reviews, see Oliver & Tervonen 1994, Yalda et al. 1994.)

Comparisons of periodontal health between patients with diabetes and controls have been made both in children and in adolescent study populations as well as in adults in varying age ranges. The studies on young subjects have mostly concentrated on gingival inflammation, measured as bleeding on probing, since periodontal destruction is rare under the age of twenty. Cianciola et al. (1982) reported an exceptionally high prevalence of periodontitis in diabetic patients aged 11–18 years. Using their own classification of periodontal health or disease into six categories from normal to severe periodontitis, they reported that generalised periodontitis seemed to appear after the age of 12 and reached a prevalence of 9.8% in diabetic patients compared to 1.7% in controls in the age group of 13–18 years. Only a couple of studies (Leeper et al. 1985, Firatlı et al. 1996) have reported significantly deeper pockets and/or more attachment loss among adolescents with diabetes than in age-matched controls compared to Cianciola’s study. Absence of periodontitis (Barnett et al. 1984, de Pommereau et al. 1992) or no differences in the extent of periodontitis between adolescents with diabetes and controls have also been reported (Sandholm et al. 1989a, Novaes Jr et al. 1991, Pinson et al. 1995).

The results on gingival inflammation have been quite consistent: young diabetic patients have been reported to have significantly higher gingival inflammation scores than controls (Bernick et al. 1975, Ringelberg et al. 1977, Faulconbridge et al. 1981, Leeper et
Studies comparing periodontal health in diabetic and control adult populations are numerous. Periodontal disease has been found to be more common and more severe in diabetic patients than in controls (Belting et al. 1964, Finestone & Boorujy 1967, Cohen et al. 1970, Campbell 1972, Wolf 1977, Sznajder et al. 1978, Albrecht et al. 1988, Bacic et al. 1988, Bridges et al. 1996). The interpretation of the results is hampered somewhat by the variation in the indices used and the measurements made in the different studies; periodontal disease has been expressed as the prevalence or extent of gingival inflammation, deepened pockets or clinical attachment loss, or, in a few studies, as radiologically evident bone loss (Tervonen & Knuuttila 1986, Hugoson et al. 1989, Seppälä et al. 1993, Thorstensson & Hugoson 1993). Few studies have documented that the differences between diabetic and control subjects with respect to periodontal disease may not be evident until the age of 30 to 40 years (Glavind et al. 1968, Wolf 1977, Bacic et al. 1988). Thorstensson & Hugoson (1993), who compared diabetic patients and controls in ten-year age subgroups between 40 and 70 years, reported that periodontal disease began earlier in diabetics than in controls and that the differences were most obvious in the age group of 40 to 49 years. In the age groups of 50–59 and 60–69 years, no major differences were found.

A few studies have failed to indicate any differences in periodontal health status between diabetic patients and controls (Benveniste et al. 1967, Hove & Stallard 1970, Tervonen & Knuuttila 1986, Ben-Aryeh et al. 1993). Sbordone et al. (1998) followed up 16 patients with type 1 diabetes and their healthy siblings for three years. No differences in probing depth, attachment level, sulcus bleeding index or plaque index were found between the patients with type 1 diabetes and the controls. Another study (Firatli 1997) assessed periodontal conditions of 44 children and adolescents with type 1 diabetes and 20 healthy controls at baseline and five years later. The mean plaque index, gingival index and probing depth were comparable in the two groups both at baseline and at five years, and no significant changes in either group were observed in these parameters from baseline to five years. The attachment level at baseline was equal in the two study groups, but the loss of attachment from baseline to five years was significantly more marked in the diabetic group compared to the controls.

Despite the abundant evidence of more severe periodontal disease, which may start at a younger age among diabetic patients than controls, the response to treatment seems to be equal in the diabetic and control groups. No differences in the short-term (from a couple of weeks to a few months) response to non-surgical periodontal treatment were found between diabetic patients and controls by Bay et al. (1974), Tervonen et al. (1991), Smith et al. (1996) and Christgau et al. (1998). Westfelt et al. (1996) monitored for five years the periodontal conditions in a group of diabetic patients and controls with moderate to advanced periodontitis. Baseline recordings were made after initial periodontal treatment, and surgical treatment was done after 6 months if necessary. The data at baseline and at re-examinations at 12, 24 and 60 months did not reveal any differences between diabetic patients and controls. The changes in probing pocket depth and the loss of attachment from baseline to 60 months were also comparable between the
groups, and the authors concluded that "diabetics and non-diabetics alike, treated for moderately to advanced forms of adult periodontitis, during a subsequent 5-year period, were able to maintain healthy periodontal conditions."

The earliest suspicions, voiced in the 1920’s and 1930’s, that diabetes induces periodontal disease or causes specific changes in gingival tissues (Williams 1928, Hirschfeld 1934) have later been disproved, and periodontal disease has been observed to be histologically similar in diabetic and control animals (Glickman 1946). The role of diabetes as a predisposing or modifying factor with respect to the intensity of the host response initiated by a local etiology has been confirmed. However, the presence of local etiologic factors, i.e. plaque and subgingival calculus, has varied between the studies: mostly these factors have been found to be similar, but lower (Cohen et al. 1970) or even higher (Kjellman et al. 1970, Faulconbridge et al. 1981, Novaes Jr et al. 1991, Bridges et al. 1996) levels in diabetic study populations compared to non-diabetic ones have also been reported. This complicates the interpretation and comparison of the results of these studies and increases the confusion of what is the actual risk of diabetic patients compared to controls. In an experimental diabetes study, McNamara et al. (1982) found that the accumulation of plaque was faster and its microbiological composition different in diabetic rats compared to control animals. Earlier microbiological studies on humans also indicated possible differences in plaque composition between diabetic and control study populations. Mashimo et al. (1983) indicated that diabetic patients have more Capnocytophaga in their periodontal pockets, but no controls were studied and the previous literature on the microbiology of juvenile and adult periodontitis was used as a reference. Sandholm et al. (1989b) reported more gram-negative rods and a higher proportion of total gram-negative bacteria in plaque samples of adolescents with diabetes than in control samples. Later studies have not conclusively supported this, as they have revealed no significant differences in microbial species (Zambon et al. 1988, Sastrowijoto et al. 1989, Sbordone et al. 1995, Christgau et al. 1998, Sbordone et al. 1998) or antibody profiles (Sandholm et al. 1989b, Thorstensson et al. 1995). However, a depressed humoral immune response among diabetics was suggested by Smith et al. (1996), who detected lower IgG antibody titres against Porphyromonas gingivalis and Bacteroides forsythus in sera of diabetic patients compared to controls.

Dental caries or salivary factors have attracted less interest, and the results are divergent as to whether caries risk is different or salivary factors are affected in diabetic patients compared to controls. Equal caries rates in diabetic patients and controls have been reported in many studies (Kjellman et al. 1970, Wolf 1977, Faulconbridge et al. 1981, Sarnat et al. 1985, Goteiner et al. 1986, Tenovuo et al. 1986, Falk et al. 1989, Twetman et al. 1989, Swanljung et al. 1992). A higher caries risk among diabetic patients than healthy controls has also been demonstrated (Wegner 1971, Sarnat et al. 1979, Albrecht et al. 1988, Jones et al. 1992), but in contrast, some studies have found even less caries in diabetic patients than in controls (Sterky et al. 1971, Matsson & Koch 1975, Leeper et al. 1985, Kirk & Kinirons 1991). Bernick et al. (1975) got equal mean DMFS indices for both diabetic patients and controls, but if the DMFS index was categorised, the lowest DMFS class (0–5) was more common among diabetic patients (49%) than controls (25%). In short, 2- to 4-year follow-up studies have revealed either lower (Wegner 1975) or similar (Bernick et al. 1975) or slightly higher (Pohjamo et al. 1991) caries increments in diabetic children or adults compared to controls. Tavares et al. (1991) reported fewer
decayed and filled coronal and root surfaces in adults with diabetes compared to controls. Interestingly, Goteiner et al. (1986) reported less caries experience in diabetic patients with a family history of diabetes compared to the rest of the diabetic study population. He assumed this to be related to the better care and treatment of diabetes in these families.

Most studies on the possible differences in salivary factors between diabetic patients and controls have focused on the salivary flow rate. The results showing lower salivary flow rates in diabetic patients (Conner et al. 1970, Kjellman 1970a, Bánóczy et al. 1987, Ben-Aryeh et al. 1988, Thorstensson et al. 1989b, Sreebny et al. 1992) have been contradicted by studies which have failed to indicate any difference in salivary flow rates between diabetic patients and controls (Marder et al. 1975, Sharon et al. 1985, Tenovuo et al. 1986, Swanljung et al. 1992, Cherry-Peppers et al. 1992, Ben-Aryeh et al. 1993). One third of diabetic patients suffered from a feeling of a dry mouth, although their salivary flow rates were found to be normal (Thorstensson et al. 1989b). Higher salivary or gingival crevicular fluid glucose levels in diabetic patients than in controls reported by some studies (Englander et al. 1963, Campbell 1965, Kjellman 1970b, Faulgonbridge et al. 1981, Sharon et al. 1985, Harrison & Bowen 1987a, Ben-Aryeh et al. 1988, Thorstensson et al. 1989b, Darwazeh et al. 1991) have not been confirmed by some studies, either (Swanljung et al. 1992).

Most studies have not detected any differences in salivary lactobacilli counts or the counts of mutans streptococci or salivary pH and buffer capacity (Tenovuo et al. 1986, Thorstensson et al. 1989b, Swanljung et al. 1992). Swanljung et al. (1992), however, reported high counts of mutans streptococci (>10^6 CFU/ml) and lactobacilli (>10^5 CFU/ml) to be more common in diabetic patients than in controls. Only Kjellman (1970a) reported a higher buffer capacity, and one study has reported lower salivary pH values (Bánóczy et al. 1987) in diabetic patients vs. non-diabetic controls. Twetman et al. (1989) did not find any differences in the counts of mutans streptococci between diabetic patients and controls, but the lactobacilli counts were lower in patients with diabetes. Interest has also been focused on the colonisation of yeasts in the mouth, and the higher occurrence of yeasts in the mouth in diabetic patients is generally accepted (Bánóczy et al. 1987, Bartholomew et al. 1987, Lamey et al. 1988).

Miscellaneous other salivary factors, such as enzymes, other proteins and electrolytes, have been analysed in both human and animal studies, but the results do not agree on whether diabetic patients and controls do or do not differ with respect to these factors (Marder et al. 1975, Anderson & Johnson 1981, Muratsu & Morioika 1985, Sharon et al. 1985, Tenovuo et al. 1986, Fisher et al. 1991, Ben-Aryeh et al. 1993), probably because different salivary fractions and collection methods have been used.

The occurrence of periodontal diseases and dental caries is notably dependent on the subjects' home care procedures and use of professional dental treatment. This aspect has been only mentioned in a couple of studies: Thorstensson et al. (1989a) found more diabetic patients than controls who did not make regular dental visits, emergency dental treatment was more common among diabetic patients, and they were less willing to spend time and money on taking care of their teeth than controls. Swanljung et al. (1992) found the oral hygiene habits to be somewhat poorer among diabetic patients than controls.

The results of the above studies, which compare diabetic patients and controls, are not consistent. Based on them, it is difficult to evaluate the actual impact which diabetes might have on the risk for oral diseases, especially, as many studies fail to demonstrate
any differences between the diabetic and non-diabetic study populations. The differences in the results might relate to the heterogeneity of the study populations, as evidently not all diabetic patients are at equal risk for oral complications. Consequently, more attention has recently been paid to the possible diabetes-related risk factors to identify the subjects especially prone to periodontal diseases or dental caries. The factors studied have included the duration of diabetes, age at diagnosis of diabetes, the presence of diabetic organ complications, and since the early 1980s, the level of metabolic control.

### 2.2.2. Oral health in relation to age at diagnosis of diabetes, duration of diabetes and diabetic organ complications

Periodontal diseases in relation to various diabetic factors have been studied quite intensively, but less attention has been paid on the role of age at diagnosis of diabetes. Thorstensson & Hugoson (1993) analysed the periodontal disease experience in 40- to 70-year-old insulin-dependent diabetic patients and non-diabetic controls. They found significantly more periodontal disease in diabetic patients than in controls in the age group of 40 to 49 years. In the older age groups, however, no major differences were found. As the duration of diabetes in the age group of 40 to 49 years group was longer than in the older age groups, they concluded that age at diagnosis of disease has an impact on the extent of periodontal disease.

In many studies, the duration of diabetes in relation to periodontal diseases has been evaluated, mostly by correlation analyses. Only Hugoson et al. (1989) have so far reported the results of a study, which primarily focused on the impacts of the duration of diabetes on the severity of periodontal disease. Subjects aged from 20 to 70 years were divided into age groups of ten years, and in each of those groups age- and sex-matched pairs of subjects with the shortest and the longest duration of diabetes were selected. On the whole, diabetic patients had an equal extent of periodontal pocketing compared to the controls. When the study group was divided into those under and over the age of 45, moderate periodontal disease, i.e. 4–5 mm pockets, was found more often in patients with long-duration of diabetes than in controls, but only in the younger age group. Regardless of age, pockets 6 mm or deeper were found more often in patients with long diabetes duration than in diabetic patients with short duration or in controls. The differences in bone loss, for instance, were most obvious in the age group of 40 to 49 years. They concluded that a long duration of diabetes increases the risk for periodontal disease in diabetic patients.

Positive associations between diabetes duration and periodontal disease have been reported in other studies as well (Finestone & Boorujy 1967, Glavind et al. 1968, Thorstensson & Hugoson 1993, Firatli et al. 1996, Moore et al. 1999). Firatli (1997) examined the periodontal status of 44 children and adolescents at two examinations five years apart. The subjects lost attachment during the five-year period, and high correlation coefficients emerged between the change in attachment level and the duration of diabetes. The possible correlation between age and the duration of diabetes, however, was ignored.
In many studies, no attention has been paid to the role of diabetes duration or no relationship has been found (Nichols et al. 1978, Rylander et al. 1986, Bacic et al. 1988, de Pommereau et al. 1992, Tervonen & Oliver 1993).

The first attempts to relate diabetic organ complications to the severity of periodontal disease were made in the 1960s. Finestone & Boorujy (1967) reported that "periodontal index (PI) was related in positive fashion to age, duration of known diabetes, diabetic complications, and variation of blood sugar levels". They compared diabetic subjects with either no complications, one diabetic complication or different combinations of complications and found PI to be highest in the subjects with all the three most common diabetic complications, i.e. retinopathy, nephropathy and neuropathy. Retinopathy was found to be associated with attachment loss in the study of Glavind et al. (1968). Nichols et al. (1978) found significantly higher Ramfjord's periodontal disease index scores in two patients with limb amputations compared to other diabetic patients, but they did not want to generalise the result, as they did not find any overall differences between diabetic patients and controls or any other relationships between the diabetic status and periodontal disease. Diabetic patients with grade 2 retinopathy have been reported to have more deepened (6mm or over) pockets than subjects with grade 1 retinopathy (Bacic et al. 1988). Rylander et al. (1986), on the other hand, found more gingival inflammation in patients with both retinopathy and nephropathy than in diabetic patients without these complications. As early as 1970 (Kjellman 1970c), retinopathy was found to be related to the gingival index and alveolar bone loss. Galea et al. (1986) reported that 50 percent of their subjects with diabetic complications had deepened pockets compared to 11.5 percent of those without complications. Which complications were used in the comparisons, and how they were used was not mentioned. In another study (Wolf 1977), periodontal condition was found to be poorer in diabetic patients with nephropathy than in ones without. Ketoacidosis, retinopathy and neuropathy were more common in the periodontitis group than in the non-periodontitis group in the study of Rosenthal et al. (1988). In that study, the gingival index was significantly higher in diabetic patients with neurological complications or a history of chronic infections. In a recently published study report, Moore et al. (1999) stated that the only diabetic complication related to extensive periodontal disease was neuropathy.

Seppälä and co-workers (1992, 1993, 1994) classified their diabetic study subjects into two groups by assessing their diabetic status based on long-term medical records. Subjects with previous problems with their diabetes, such as ketosis, severe hyperglycaemia, recurrent infections, ketoacidosis, glycosurea, diabetic coma or different stages of retinopathies, neuropathies and nephropathies, were classified as having poorly controlled type 1 diabetes. Subjects with less severe diabetic complications were classified as well controlled ones. The subjects classified as poorly controlled diabetic patients experienced more periodontal disease and lost more alveolar bone and clinical attachment than the well controlled ones (Safkan-Seppälä & Ainamo 1992, Seppälä et al. 1993, Seppälä & Ainamo 1994). Complications alone did not associate with more severe periodontal disease, as comparisons of subjects with or without retinopathy, nephropathy or neuropathy did not reveal any differences in periodontal conditions (Safkan-Seppälä & Ainamo 1992). In an earlier study (Ervasti et al. 1985), gingival bleeding was not associated with diabetic complications.
During a five-year follow-up study including recall visits and treatment as needed, two subgroups of insulin-dependent adult diabetic patients based on the presence of diabetic complications were compared (Westfelt et al. 1996). From baseline to 5 years, the frequencies of sites with reduced inflammation, pocket depth and loss of attachment were equal in subjects with all the three common microvascular complications of diabetes, e.g. retinopathy and nephropathy and neuropathy, and in subjects with only beginning or no diabetic complications (Westfelt et al. 1996).

Thorstensson et al. (1996), in a prospective case-control study, found that the incidence of proteinuria, cardiovascular complications, such as stroke, TIA, angina, myocardial infarction and intermittent claudication, was higher in a group of subjects with periodontitis (cases) than in a group with only mild gingivitis (controls). Cases and controls were matched for sex, age and diabetes duration and all were insulin-dependent adult diabetic patients with a long disease duration. On the basis of their results, the authors concluded that there exists an association between periodontal disease and both renal disease and cardiovascular complications in patients with diabetes.

Aspects of oral health other than periodontal diseases in relation to the duration of diabetes or the presence of diabetic complications have been less intensively investigated. The duration of diabetes was found to have no effect on caries prevalence in a few studies (Sterky et al. 1971, Bernick et al. 1975, Faulgonbridge et al. 1981, Bacic et al. 1989, Falk et al. 1989). Galea et al. (1986), instead, found more caries in diabetic patients with a short duration of diabetes than in those who had suffered from diabetes for more than five years. This result was verified by Wegner (1975), who observed that caries activity was high in some diabetic children after the diagnosis of diabetes but, after stabilisation of metabolic disturbance, declined to the level found in non-diabetic children. Swanljung et al. (1992) obtained contradictory results: subjects with diabetes for more than six years tended to have higher caries indices than subjects with a shorter disease duration. The diagnosis of diabetes at an early age, i.e. before the age of 5–7 years, seems to associate with a lower caries risk than diagnosis after that age (Wegner 1971, Matsson & Koch 1975, Tenovuo et al. 1986, Kirk & Kinirons 1991).

Only a few studies on salivary factors include any references to the duration of diabetes. Tenovuo et al. (1986) did not find any correlation between salivary flow rate, salivary pH, buffer capacity or salivary microbial counts and the duration of diabetes, age or age at diagnosis of the disease. Salivary flow rate, pH and buffering capacity were not related to the duration of diabetes in the study of Swanljung et al. (1992). However, a tendency toward higher acidogenic bacterial counts in diabetic patients with diabetes duration of six years or more compared to less than six years was observed. They assumed that, along with the longer disease duration, the immune response might have been affected. Sreebny et al. (1992) reported that the occurrence of xerostomia was not related to the type of diabetes or its duration. No significant differences were found in salivary flow rate, pH, buffer capacity, glucose content or counts of salivary yeasts, lactobacilli or mutans streptococci between short- and long-duration diabetic patients by Thorstensson et al. (1989b). Oral yeast carriage and duration of diabetes were reported to be unrelated by Tapper-Jones et al. (1981).

Results on dental caries or salivary factors in relation to diabetic organ complications are rare; Bacic et al. (1989) mentioned that caries was not related to complications.
Many of the above mentioned studies share a common problem: each factor, i.e. disease duration and the different diabetic complications, have been separately compared with each other disregarding their interdependence. The duration of diabetes, and hence also the subjects’ age, determines to a certain extent whether complications develop. Metabolic control has, however, been shown to play a central role in the development and progression of diabetic complications (D’Antonio et al. 1989, Reichard et al. 1993, Diabetes Control and Complications Trial Research Group 1993, Wang et al. 1993), and it has therefore been emphasised in this work.

2.3. Oral health in relation to metabolic control of diabetes

2.3.1. Periodontal diseases

Quite early in the literature, some study reports appeared which postulated a role of the level of metabolic control in the severity of periodontal disease (Finestone & Boorujy 1967, Kjellman et al. 1970). Evaluation of metabolic control was, however, quite difficult until in the 1980s, when glycosylated haemoglobin values became available for monitoring metabolic control. One of the first comparisons of periodontal health between well, moderately and poorly controlled adult patients with diabetes was done and reported by Ervasti et al. (1985) and Tervonen & Knuuttila (1986). Gingival bleeding was observed to increase as the level of metabolic control deteriorated (Ervasti et al. 1985). With respects to pocketing and alveolar bone loss, the differences were not so obvious; however, well controlled subjects had better periodontal conditions than controls, and a trend towards severe periodontal disease in moderately and poorly controlled subjects compared to well controlled ones was obvious (Tervonen & Knuuttila 1986). Galea et al. (1986) reported that susceptibility to severe periodontal disease tended to increase with rising blood glucose levels in their study group, and all patients with pocketing had ”uncontrolled diabetes”.

Although the role of poor control of diabetes as a predisposing factor for periodontal disease has been reported in several studies even thereafter (Ainamo et al. 1990, Saikan-Seppälä & Ainamo 1992, Seppälä et al. 1993, Tervonen & Oliver 1993), contradictory results are also numerous (Wolf 1977, Rylander et al. 1986, Albrecht et al. 1988, Bacic et al. 1988, Rosenthal et al. 1988, Hayden & Buckley 1989, de Pommereau et al. 1992, Pinson et al. 1995, Bridges et al. 1996). In a case-control study, Thorstensson et al. (1996) compared diabetic patients with periodontitis to diabetic patients with only mild gingivitis. They did not find, in two examinations 6 years apart, any differences in any of the results of the laboratory tests performed (those used routinely to monitor the diabetic status, including HbA1c values) between the cases and controls.

In younger study populations, i.e. children and adolescents, the level of metabolic control has been mainly studied in relation to gingival inflammation. Gusberti et al. (1983) found more gingival inflammation in poorly controlled children than in well controlled ones before puberty. The increase of gingival inflammation during puberty and along with age was more obvious than the effect of poor control during and after puberty, however. In a couple of studies, more gingival inflammation in poorly controlled diabetic
children compared to healthy controls has been reported, but the differences between well and poorly controlled subgroups were less obvious (Gislen et al. 1980, Harrison & Bowen 1987b). Support for the role of metabolic control in relation to gingival inflammation was given by Firatli et al. (1994), who found a positive correlation between gingivitis and fructosamine, a short-term glycosylated protein, although the correlation between HbA1c and the gingival index was not statistically significant. The absence of an association between gingivitis and glycosylated haemoglobin values in young study populations has been reported in many surveys (Sandholm et al. 1989a, Sastrowijoto et al. 1989, de Pommereau et al. 1992, Pinson et al. 1995). Quite a few studies on children and adolescents have included pocket or attachment loss measurements in their protocols. Mostly, however, no differences in the extent of deepened pockets or attachment loss between well and poorly controlled young diabetic patients have been found (Sandholm et al. 1989a, Pinson et al. 1995).

Prospective studies on the relationship between glucose control and periodontal health are rare. Sastrowijoto et al. (1990) observed six diabetic patients who received intensified insulin treatment in order to improve their metabolic control. The observed decrease in HbA1c levels from a mean baseline value of 11.2% to 9.1% at 4 months and 9.6% at 8 months was followed by a decrease in gingival redness, but the other periodontal parameters remained unchanged. No periodontal treatment was given. Westfelt et al. (1996) observed a group of adult diabetic patients on regular recall visits for five years after treatment. The changes in bleeding on probing or pocket depth were not found to be related to HbA1c values. Christgau et al. (1998) monitored clinical, microbiological, medical and immunological effects of non-surgical periodontal treatment in 20 patients with either type 1 or type 2 diabetes and in healthy controls. No differences between the groups at baseline, 2 weeks or 4 months after treatment were observed, and the healing response was equal in the two groups. The median HbA1c value for the diabetic group was 6.5%, and only two subjects had moderate and one subject poor metabolic control of diabetes. The authors concluded that diabetic patients with good control of diabetes respond to therapy equally well as healthy controls.

Infections are generally known to disturb the metabolic control. A couple of studies have explored whether a reduction of periodontal infection has any effect on the level of glucose control. Miller et al. (1992) had 9 cases with gingivitis who underwent thorough periodontal therapy. Slight evidence to suggest that gingival inflammation might impair the glucose control was obtained, as a good response to treatment was accompanied by a decline of HbA1c levels, while no change in the HbA1c values was seen in those who did not respond favourably to the treatment. Grossi et al. (1996, 1997) had 113 poorly controlled type 2 diabetic patients with periodontitis who were randomised into five treatment groups. All underwent scaling and curettage combined with different combinations of antimicrobial treatment. Three months and six months after therapy, all groups showed a favourable response, i.e. a reduction of plaque scores, gingival scores and mean probing depth. From baseline to three months, a significant reduction of HbA1c values was observed, but only in the groups who received systemic doxycycline therapy. The change in fasting blood glucose levels, however, was not so obvious, and the ability of doxycycline to prevent non-enzymatic glycosylation might have reduced HbA1c values. At six months, HbA1c levels returned to baseline values, but no recurrence of periodontal infection was seen from three to six months. Based on these results, the
authors concluded that the control of periodontal infection has favourable effect on diabetes management. In another study (Christgau et al. 1998), no changes in HbA1c values were observed four months after non-surgical periodontal treatment. It is noteworthy, however, that the diabetic subjects of that study already had good metabolic control at baseline. Smith et al. (1996) failed to find any changes in HbA1c values at two months and Aldridge et al. (1995) at six weeks after periodontal treatment.

In most cases, local etiologic factors have not been different in the various subgroups based on the level of metabolic control, with a few exceptions: higher plaque or calculus scores in poorly controlled subjects compared to well controlled ones have been reported by Harrison & Bowen (1987b) and Tervonen & Oliver (1993). Seppälä & Ainamo (1996), in a dark field microscopy study, found higher percentages of spirochetes and motile rods and lower percentages of coccoid cells in periodontally diseased sites of poorly controlled than well controlled subjects with type 1 diabetes. No other microbiological studies (Tervonen et al. 1994, Sbordone et al. 1995, 1998) or antibody assessments (Morinushi et al. 1989) have revealed any significant associations between the microbiological composition of plaque and the level of glucose control. Mandell et al. (1992) had subjects with poorly controlled long-duration diabetes, and the high levels of organisms found in subgingival samples of diseased sites were similar to those associated with periodontal destruction in other patient populations. Sastrowijoto et al. (1989) reported a group of well controlled diabetic patients with HbA1c values equal to or lower than 7.7% and poorly controlled subjects with HbA1c values of 9.9% or higher. The periodontopathogens found were the same as those commonly seen in adult periodontitis, i.e. Actinobacillus actinomycetemcomitans and Bacteroides species. Capnocytophaga species, which have previously been suggested to be related to periodontitis of diabetic subjects (Mashimo et al. 1983), were found to be present in low numbers independently of the level of metabolic control.

2.3.2. Dental caries

There are only a few studies in which dental caries in relation to the level of metabolic control has been investigated. About half of them did not reveal any association between caries prevalence and the level of metabolic control (Sarnat et al. 1979, Sarnat et al. 1985, Harrison & Bowen 1987b, Bacic et al. 1989). Twetman et al. (1992) reported that young type 1 diabetic patients who developed caries had higher glycosylated haemoglobin values than patients without new decays. The subjects were followed up for two years after diagnosis of diabetes, and caries activity was higher during the first than the second year after the diagnosis of diabetes. Another follow-up study supported Twetman's results (Wegner 1975). The results of a cross-sectional study (Galea et al. 1986) mention that the diabetic patients with caries were mostly poorly controlled, i.e. had uncontrolled diabetes of short duration, and none of the subjects with high blood glucose levels were caries-free.
2.3.3. Salivary factors

Lower stimulated salivary flow rates in poorly controlled diabetic patients compared to well controlled diabetic patients and controls were reported by Harrison & Bowen (1987a). In another study on adult diabetic patients, inverse relationships were observed between salivary flow rates and HbA1c levels (Sreebny et al. 1992). Based on the equal flow rates between subjects with diabetes, those with impaired glucose tolerance and controls, Cherry-Peppers et al. (1992) concluded that salivary gland function is not significantly impaired in well controlled subjects with altered glucose metabolism. Reuterving et al. (1987) examined the salivary factors of 11 diabetic patients at baseline and after improvement of their level of metabolic control one to five months later. They reported that individual variations in salivary flow rates were conspicuous and were not affected by improved metabolic control. Salivary glucose levels, instead, were lower during better metabolic control. During poor metabolic control with high blood glucose values, high blood glucose levels could possibly be reflected as high salivary glucose levels. There is, however, little evidence of this since: some weak correlations between saliva glucose and blood glucose levels have been reported (Reuterving et al. 1987, Borg & Birkhed 1988, Darwazeh et al. 1991, Borg Anderson et al. 1998), but no such correlations have been found by others (Campbell 1965, Kjellman 1970b, Forbat et al. 1981, Sharon et al. 1985, Ben-Aryeh et al. 1988). Tenovuo et al. (1986) analysed glucose levels in more than one hundred simultaneously taken stimulated whole saliva samples and blood samples of seven patients. The variations in salivary glucose were found to be extensive, and the correlation between salivary and blood glucose was highly individual: some subjects showed high correlations, while some others showed no change in salivary glucose, even when their blood glucose levels were very high. Glucose in parotid saliva (Englander et al. 1963, Reuterving et al. 1987, Ben-Aryeh et al. 1988) or in gingival crevicular fluid (Kjellman 1970b, Ficara et al. 1975) is more strongly related to blood glucose levels than glucose in mixed saliva. Borg Andersson et al. (1998) reported that, after a standardised carbohydrate load, glucose levels in parotid saliva increased even in healthy subjects, but the levels were even higher in subjects with IGT or manifest diabetes. In an experimental animal study (Reuterving 1986), the salivary secretion rate decreased in diabetic rats and their flow rates correlated inversely with blood glucose levels. Insulin normalised the situation, and salivary secretion also improved in long-term diabetic rats compared to short-term diabetic rats. The author postulated that the change in salivary gland function is at least partly reversible, and that there might be a threshold value for blood glucose levels before which glucose is not excreted into saliva, comparable to the kidney threshold.

Salivary microbial counts in relation to glucose control have hardly been investigated. Reuterving et al. (1987) observed that the counts of mutans streptococci in mixed saliva decreased significantly as the metabolic control of 11 diabetic patients improved, but the counts of lactobacilli remained stable. Twetman et al. (1989) reported an increase of salivary lactobacilli counts along with a rise of salivary glucose levels. During a two-year follow-up of newly diagnosed patients with type 1 diabetes, salivary glucose levels tended to be lower during the second than the first year, and the counts of lactobacilli dropped significantly during the first six months, while the counts of mutans streptococci remained stable (Twetman et al. 1992). High salivary glucose levels might, however, be
connected with an increase of microbial colonisation, as also indicated by Darwazeh et al. (1991), who pointed out that subjects with yeasts in the mouth had higher salivary glucose levels than diabetic patients without yeasts. Yeast growth, however, has not been found to be associated with glycosylated haemoglobin values (Bartholomew et al. 1987, Fisher et al. 1987, Lamey et al. 1988) or serum glucose levels (Bartholomew et al. 1987, Lamey et al. 1988).

Other salivary factors, such as pH, buffer capacity or the contents of enzymes and other proteins or electrolytes, were the same in patients with type 1 diabetes on two occasions with poorer and better metabolic control of the disease (Reutervng et al. 1987).

In conclusion, the role of the level of metabolic control of diabetes with respect to oral health is still poorly defined. Presumably, poor metabolic control does have a role with respect to oral diseases, as it has with respect to diabetic organ complications, and this needs to be clarified further. This need is emphasised by recently published results, according to which average glycaemic control is poor in a majority of the diabetic patients in Finland (Valle et al. 1999). The mean HbA1c of the patients with type 1 diabetes was 8.8 percent, and about 25 percent of patients could be classified to have very poorly controlled diabetes with HbA1c values of 9.7 percent or more.
3. Aims

These studies aimed at identifying the diabetic patients at risk for periodontal diseases and dental caries.

In children and adolescents, the level of metabolic control is the only factor used to categorise the diabetic status without confounding factors, such as complications. The aim of the present studies was to investigate whether the level of metabolic control in young patients is related to gingival inflammation, dental caries and salivary factors.

In adults, the diabetic status is multifactorial and diabetes was therefore assessed to be an entity including the presence of diabetic organ complications and/or the level of metabolic control. The aim was to study whether the diabetic status, in terms of these criteria applied to adults, is related to the severity of periodontal disease or has any effect on the short-term response to periodontal therapy and the maintenance of the healing results during a follow-up period.
4. Material and methods

4.1. Subjects and study protocol

This work relates to four separate study groups. All data were not available for all of the subjects, and the number of subjects therefore varies between the Papers (Table 1). All subjects gave informed consent. The study protocol has been approved by the Ethical Committee of the Faculty of Medicine, University of Oulu, Oulu, Finland.

Table 1. Characteristics of the study populations.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of subjects</th>
<th>Sex (female/male)</th>
<th>Age (years)</th>
<th>Duration of diabetes (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Papers I, II)</td>
<td>80</td>
<td>36/44</td>
<td>14.5 ± 1.6 (11–18)</td>
<td>6.0 ± 3.9 (3 mo–15 yrs)</td>
</tr>
<tr>
<td>Subgroup A (Paper III)</td>
<td>50</td>
<td>20/30</td>
<td>14.4 ± 1.7 (11–18)</td>
<td>6.2 ± 4.1 (3 mo–15 yrs)</td>
</tr>
<tr>
<td>Group 2 (Paper III)</td>
<td>14</td>
<td>7/7</td>
<td>11.0 ± 2.4 (6–14)</td>
<td></td>
</tr>
<tr>
<td>Subgroup B (Paper I)</td>
<td>12</td>
<td>7/5</td>
<td>10.6 ± 2.4 (6–14)</td>
<td></td>
</tr>
<tr>
<td>Group 3 (Paper IV)</td>
<td>26</td>
<td>17/9</td>
<td>30.0 ± 2.4&lt;sup&gt;a&lt;/sup&gt; (25–34)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.1 ± 5.5 (11–31)</td>
</tr>
<tr>
<td>Group 4 (Paper V)</td>
<td>36</td>
<td>23/13</td>
<td>29.4 ± 3.7 (24–36)</td>
<td>16.9 ± 6.7 (3–29)</td>
</tr>
<tr>
<td>Controls (Paper V)</td>
<td>10</td>
<td>8/2</td>
<td>30.1 ± 3.8 (25–35)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> standard deviation; <sup>b</sup> range.

The first study group included a random sample of 80 adolescents with type 1 diabetes who visited the paediatric diabetes outpatient clinic, Oulu University Hospital, Oulu, Finland, for routine check-ups (Papers I and II, Group 1, Table 1). The subjects were randomly selected from among those aged 12–18 years (approximately 150 patients). In the Papers, this group is referred to as the long-term IDDM cases, as their diabetes had
lasted for 3 months to 15 years (mean 6 years). After the diagnosis of diabetes, the subjects had received several injections of insulin daily, and had visited the University Hospital approximately every three months for follow-up and adjustment of diabetes treatment. They were otherwise healthy and not on regular medication. Two subjects exhibited minimal retinopathic changes. Two examinations were made on consecutive visits approximately three months apart. Three subjects did not participate in the second examination, and the HbA1 values of three subjects were not determined on Examination 2. The results of salivary tests were available for 50 of these subjects (Paper III, Subgroup A, Table 1).

Papers I and III report on the results of a group of children with newly diagnosed type 1 diabetes (Group 2 and Subgroup B, Table 1). In the Papers, this group is referred to as the newly diagnosed IDDM cases. All new diabetes patients over six years of age referred to the Department of Paediatrics, Oulu University Hospital, Oulu, Finland, during the six-month study period for whom dental examinations could be arranged (e.g. by excluding weekends), were included in the study. They were examined three times. The first examination was carried out as soon as possible after the diagnosis of type 1 diabetes, on the third day (3.1 ± 1 day) of hospitalisation (Day 1). Insulin treatment had been started immediately after the diagnosis. The second examination was performed on the day of discharge from the hospital (12.4 ± 1.6 days later) (Day 2). The third examination was made on the first outpatient visit to the hospital approximately one month later. Saliva samples and tests were available for 14 subjects (Paper III, Group 2, Table 1) and a periodontal examination was carried out on twelve subjects (Paper I, Subgroup B, Table 1), one of whom did not attend the third examination.

The third study group (Paper IV) included young adult patients with type 1 diabetes from the diabetes outpatient clinic of the Oulu Health Center, Oulu, Finland. The age range of the subjects was restricted to 25–35 years, the selection being based on the year of birth, and a minimum duration of diabetes of ten years was required. All the patients fulfilling the inclusion criteria who visited regularly the diabetes outpatient clinic of the Oulu Health Center were invited. Twenty-six patients out of 40 patients volunteered to participate (Group 3, Table 1). All subjects were on multiple daily insulin injections and made regular visits to the diabetes outpatient clinic to monitor their diabetes treatment. Other diseases apart from diabetes were rare, but two subjects received thyroxin substitution. Diabetic organ complications (Table 2), which will be discussed later, were common, and the only medications in regular use were those for hypertension.

The subjects of the fourth study group (Paper V, Group 4, Table 1) were selected from the diabetes outpatient clinics of the Oulu Health Center and the Oulu University Hospital, Oulu, Finland. All the patients with type 1 diabetes aged 25–35 (the selection being based on the year of birth) attending the outpatient clinic of the Oulu Health Center were invited (n=65). An additional random sample of patients of the same age was invited from among the patients attending the diabetes outpatient clinic of the Oulu University Hospital, to recruit more subjects. Forty-three patients volunteered to participate, approximately one fourth of them were patients from the University Hospital. Paper V relates to the results of 36 subjects for whom information on the long-term glucose control was available (Group 4, Table 1). Diseases other than diabetes and diabetes-related complications (Table 2) were rare. Three subjects were on thyroxin substitution,
one subject was receiving medication for epilepsy, and one for a psychological disorder. Ten randomly selected non-diabetic subjects of similar ages from one of the Oulu Dental Health Care Center clinics served as controls (Table 1).

Table 2. Prevalence of diabetic organ complications in the subjects of Group 3 (Paper IV) and Group 4 (Paper V).

<table>
<thead>
<tr>
<th>Number of subjects with</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No retinopathy</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Minimal retinopathy (Grade 1)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Background retinopathy (Grade 2)</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Preproliferative retinopathy (Grade 3)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Proliferative retinopathy (Grade 4)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Albuminuria/Nephropathy</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

4.2. Periodontal assessments

The periodontal examinations described in Papers I, IV and V were performed by the first author, who was unaware of the general health status of the subjects at the time of the clinical examination. All periodontal variables were recorded on four sites (mesial, buccal, distal and lingual) of all teeth, excluding wisdom teeth in adults and the exfoliating primary teeth or erupting permanent teeth in children and adolescents. A ball-pointed periodontal probe with a tip diameter of 0.5 mm and a 2mm graduation (LM-Instruments Oy, Finland) was used in the periodontal examinations. The following variables were measured in all the studies: presence of visible plaque corresponding to scores 2 and 3 of the plaque index (Silness & Löe 1964) and bleeding on probing. In Papers IV and V, the presence of subgingival calculus corresponding to scores 2 and 3 of the Björby and Löe retention index (1967), probing depth and clinical attachment level measured from the cemento-enamel junction to the base of the crevice/pocket were also recorded. The percentages of sites with visible plaque, subgingival calculus, bleeding on probing, probing depths equal to or deeper than 4 mm (Papers IV and V) and 6 mm (Paper IV), and clinical attachment loss equal to or greater than 2 mm (Papers IV and V) and 3 mm (Paper IV) were calculated for each subject.

4.3. Dental caries registration

The presence of dentine caries (Paper II) was clinically diagnosed on all tooth surfaces by visual examination and probing, following the guidelines of the World Health Organisation (1987). No x-rays were taken. Decayed (DS), filled (FS) or decayed and/or filled (DFS) surfaces were calculated and the means of the indices were used in analyses.
Missing teeth were not included into any indices, because extractions were rare and done for orthodontic reasons (twelve premolars in five subjects) in the study group of 80 children and adolescents. Thirteen subjects had few teeth totally or partially non-erupted (second molars and/or premolars). Altogether 30 primary teeth existed in 14 subjects. When the teeth were clinically free from both caries and fillings, dentition was defined as intact, and the presence or absence of intact dentition was used as a parameter in the analyses.

4.4. Saliva sampling and salivary tests

Paraffin-stimulated whole saliva samples were collected over five-minute periods at least half an hour after the last meal between the hours 10 a.m. and 2 p.m. (Papers II and III). The amounts of saliva were recorded (ml/5 minutes) and pH was measured electrometrically (Orion Research, model SA 210) immediately after saliva collection. Buffering capacity (final pH) was determined by using the Dentobuff® colorimetric strip test (Orion Diagnostica, Espoo, Finland) and coded in three categories according to the model chart (low, intermediate, high). Dentocult®LB and Oriicult®N dipslides and Dentocult® SM Strip mutans test strips were prepared and incubated at 37 °C, following the manufacturer’s instructions (Orion Diagnostica, Espoo, Finland). After incubation, salivary lactobacilli counts (Dentocult® LB) and counts of mutans streptococci (Dentocult® SM) were expressed as colony-forming units (CFU) per millilitre of saliva according to the manufacturer’s model density chart. Lactobacilli densities were categorised as 0, 10³–10⁴ and 10⁵–10⁶ in Paper III. In paper II, lactobacilli counts were grouped as low (CFU ≤ 10⁵), intermediate (CFU 10⁵) and high (CFU 10⁶–10⁷). The corresponding limits for counts of mutans streptococci were <10⁵, 10⁵–10⁶ and >10⁶. The density of yeast growth on Oriicult® N dipslides was grouped as 0, 10³–10⁴ and 10⁵–10⁶ in Paper III. The presence or absence of yeasts was used in the analyses in Paper II.

After the above mentioned tests, the rest of the saliva was centrifuged and frozen and stored at −20 °C until glucose determination with a D-Glucose UV™ test kit (Mannheim Boehringer, Germany) (Paper III).

4.5. Diabetes assessment

The duration of diabetes, age at diagnosis of diabetes and glycosylated haemoglobin values (HbA₁ or HbA₁c) were retrieved from the medical records after the clinical periodontal examinations. Either actual or mean HbA₁ or HbA₁c values over various lengths of time were used, depending on which study population was concerned, and detailed information is given in the results. HbA₁ values were available for children and adolescents, and the limits were set at <10%, 10–12.9% and ≥13% in Papers I and II, and at <10%, 10–13% and >13% in Paper III, respectively. The subjects in Paper IV were divided into two groups: well to moderately (HbA₁ < 10%) and poorly controlled (HbA₁ ≥ 10%). In Paper V, the subjects were classified as well controlled (HbA₁c ≤ 8.5%), moderately controlled (HbA₁c 8.6–9.9%) or poorly controlled (HbA₁c ≥ 10%).
For the patients with newly diagnosed type 1 diabetes, glucose control was also evaluated by calculating the mean of several blood glucose measurements done on the day of examinations during the inpatient episode. The blood glucose variable and, in part of the analyses, the glycosylated haemoglobin values were kept as continuous. HbA\(_1\), HbA\(_{1c}\) and blood glucose determinations were part of the routine monitoring of diabetic patients, and the tests were run in the hospital laboratory or the laboratory of the health center.

The presence of diabetic complications was derived from the medical records (Papers IV and V). Albuminuria was defined as macroalbuminuria. In addition, the subjects of Group 3 (Paper IV) were referred to the Department of Ophthalmology, Oulu University Hospital, Oulu, Finland, where direct and indirect ophthalmoscopy was performed by an ophthalmologist (KD). Fundus photographs were also taken, and retinopathy was assessed into four grades according to the clinical evaluation and fundus photography. The subjects of Group 3 (Paper IV) were divided into three groups as follows: patients without organ complications; patients with minimal or background retinopathy (incipient retinopathy); and patients with advanced organ complications. Most subjects in the last group had many concomitant complications (Table 3). In Paper V, three diabetic subgroups based on long-term glucose control and the presence of diabetic complications were formed as follows: subjects without diabetic complications and a good long-term glucose control with HbA\(_{1c}\) levels constantly ≤ 8.5%; subjects with moderate metabolic control with/without retinopathy; and subjects with poor long-term metabolic control (HbA\(_{1c}\) values constantly ≥ 10%) and/or multiple diabetic complications (Table 4). Retinopathy was assessed as present or absent, as no exact classification of retinopathy was possible on the basis of the medical records.

Table 3. Distribution of diabetes-associated organ complications in the group of subjects with advanced complications (Paper IV).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Retinopathy Grades 0–2</th>
<th>Retinopathy Grades 3–4</th>
<th>Albuminuria</th>
<th>Neuropathy</th>
<th>Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>10</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The subjects were interviewed about their home care habits (frequency of tooth brushing and the use of toothpicks or dental floss) and about their visits to a dentist (frequency and the time elapsed since the last visit to a dentist).
Table 4. Frequency of poor metabolic control and various diabetic complications in subjects in Group D3 (Paper V).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Poor metabolic control 3 years</th>
<th>4–5 years</th>
<th>Retinopathy</th>
<th>Nephropathy</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>neuropathy</td>
</tr>
<tr>
<td>B</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>skin infections (abscess)</td>
</tr>
<tr>
<td>C</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>neuropathy, skin infections</td>
</tr>
<tr>
<td>D</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>neuropathy, skin infections with necrosis (amputation of toes)</td>
</tr>
<tr>
<td>E</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>hypertension</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>hypertension</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>neuropathy, hypertension, skin infections (abscesses)</td>
</tr>
<tr>
<td>H</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>neuropathy, hypertension, skin infections (abscesses)</td>
</tr>
</tbody>
</table>

4.6. Hygienic phase of periodontal therapy and follow-up examinations

All the diabetic subjects (Paper V, Group 4) and the controls (Paper V) were subjected to the hygienic phase of periodontal therapy given by one of the authors (KK). Home care instructions were given at baseline and repeated, if necessary, during the follow-up examinations. Periodontal therapy included the removal of plaque-retentive overhangs of fillings and scaling and root planing. According to individual needs, prophylaxis, restorative caries treatment and occlusal adjustment were also performed. Re-examinations were done four weeks, six months and twelve months after the periodontal treatment. One control subject missed the 6-month examination and another control, and one poorly controlled diabetic subject did not participate in the 12-month examination.

4.7. Statistical analyses

These studies aimed to explore the diabetic status in relation to oral health. In Papers IV and V, the diabetic status was graded mainly based on the presence of diabetic complications, and the three subgroups were compared. In Papers I, II and III, the diabetic patients were divided into three subgroups according to the level of metabolic control. The statistical significance of the differences between the three groups of subjects was tested by analysis of variance (ANOVA) or by means of the Kruskal-Wallis test, depending on the normality of the variables concerned. If any two groups were compared, as in Paper IV, the statistical testing was done by using the Student’s t-test or the Mann-Whitney U-test. Age, which is as a common confounding factor, was controlled by restricting the age range of the study subjects. Adjustments for other covariates was done by using analysis of covariance (ANCOVA). In Paper IV, stepwise multiple regression analysis was used to confirm the main result of the study, as many confounding factors were found to be present in the study population. The correlation
coefficients quoted are Pearson’s or Spearman’s, the latter for variables with skewed distributions. The significance of the differences in the distribution of categorical variables between groups was tested using the chi-square test or Fisher’s exact test. In Papers I and III, the possible changes in salivary factors or gingival inflammation between two occasions were compared. Student’s paired $t$-test for normally distributed variables or Wilcoxon’s signed rank test for skewed distributions was used to determine the significance of the differences between paired comparisons. The analyses in Paper IV were done using a Pato statistical package. All the other analyses were performed using the SPSS Version 6.0 or 7.0 statistical package for Windows.
5. Results

5.1. Gingival bleeding in relation to metabolic control in children and adolescents with type 1 diabetes

Gingival bleeding in the patients with newly diagnosed type 1 diabetes decreased during the approximately two weeks of follow-up, as the abnormally high blood sugar levels normalised after the initiation of insulin treatment (Table 5). The extent of gingival bleeding was the same on the one-month follow-up examination as on the day of discharge from the hospital. On the one-month examination, glucose control was found to be good. Similar amounts of plaque were seen on all examinations.

Table 5. Changes in gingival bleeding, plaque and glucose control after the initiation of insulin treatment in children and adolescents (n = 12) with newly diagnosed type 1 diabetes (Subgroup B, Paper I).

<table>
<thead>
<tr>
<th></th>
<th>Day 1 (3th day in hospital)</th>
<th>Day 2 (12th day in hospital)</th>
<th>One month after discharge from hospital</th>
<th>p (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingival bleeding (%)</td>
<td>37.8 ± 15.3(^c)</td>
<td>19.0 ± 7.3</td>
<td>23.1 ± 13.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Plaque (%)</td>
<td>50.0 ± 22.4</td>
<td>44.3 ± 18.2</td>
<td>43.8 ± 19.9</td>
<td>0.5</td>
</tr>
<tr>
<td>HbA(_1) (%)</td>
<td>14.9 ± 3.8</td>
<td>13.1 ± 2.9</td>
<td>8.4 ± 1.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Mean blood glucose level (mmol/l)</td>
<td>13.4 ± 3.3</td>
<td>6.1 ± 2.2</td>
<td>na(^d)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^a\) n = 11, one subject was not available for the one-month examination; \(^b\) paired \(t\)-test: p-values show significant differences between day 1 and day 2, and between day 1 and one month, the differences between day 2 and one month were not significant (except HbA\(_1\), \(p < 0.001\)); \(^c\) ± standard deviation; \(^d\) na = not available.

In children and adolescents with a longer disease duration, gingival bleeding was significantly more abundant in the poorly controlled individuals (actual HbA\(_1\) ≥ 13\%) compared to the moderately (actual HbA\(_1\) 10.0–12.9\%) and well controlled ones (actual HbA\(_1\) < 10\%) on two examinations three months apart (Table 6, \(p = 0.03\) on Examination 1, \(p = 0.04\) on Examination 2, ANOVA). The amounts of plaque were comparable between the groups, and the differences in the extent of gingival bleeding were significant after adjustments for age, age at diagnosis of diabetes, duration of diabetes and pubertal
stage (p < 0.05, ANCOVA). The mean HbA1 values of the poorly controlled groups were alarmingly high (16.0% on Examination 1, 15.3% on Examination 2). The same amounts of plaque induced more gingival bleeding in the poorly controlled cases compared to the well controlled diabetic subjects (Fig. 1).

Table 6. Comparison of gingival bleeding and plaque in children and adolescents with well, moderately and poorly controlled long-term type 1 diabetes on two examinations 3 months apart (Group 1, Paper I).

<table>
<thead>
<tr>
<th></th>
<th>Well controlled (HbA1 &lt;10%)</th>
<th>Moderately controlled (HbA1 10–12.9%)</th>
<th>Poorly controlled (HbA1 ≥13%)</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>examination 1 (n = 80)</td>
<td>18</td>
<td>27</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>examination 2 (n = 74)</td>
<td>12</td>
<td>31</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Gingival bleeding (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>examination 1</td>
<td>35.2 ± 14.5c</td>
<td>35.6 ± 16.7</td>
<td>46.3 ± 19.3</td>
<td>0.027</td>
</tr>
<tr>
<td>examination 2</td>
<td>26.9 ± 16.0</td>
<td>33.4 ± 17.3</td>
<td>41.7 ± 18.8</td>
<td>0.036</td>
</tr>
<tr>
<td>Plaque (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>examination 1</td>
<td>45.5 ± 19.6</td>
<td>42.3 ± 22.9</td>
<td>50.3 ± 26.5</td>
<td>0.4</td>
</tr>
<tr>
<td>examination 2</td>
<td>43.0 ± 21.8</td>
<td>45.1 ± 26.2</td>
<td>45.8 ± 25.0</td>
<td>0.9</td>
</tr>
<tr>
<td>HbA1 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>examination 1</td>
<td>8.5 ± 1.1</td>
<td>11.7 ± 0.7</td>
<td>16.0 ± 1.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>examination 2</td>
<td>8.3 ± 1.3</td>
<td>11.3 ± 0.8</td>
<td>15.3 ± 1.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

a ANOVA; b three subjects did not participate in examination 2, and the HbA1 values of three subjects were not determined on examination 2; c ± standard deviation.

Fig. 1. Scattergrams of plaque and gingival bleeding values in well (HbA1 < 10%) and poorly (HbA1 ≥ 13%) controlled adolescents with long-term type 1 diabetes at two examinations 3 months apart.
5.2. Dental caries in relation to metabolic control in children and adolescents with type 1 diabetes

The mean value of DFS was significantly higher in the group of poorly controlled (mean HbA₁ over the past six months ≥ 13%) children and adolescents with type 1 diabetes than in any other group (Table 7). After controlling for differences in age, age at diagnosis of diabetes and the duration of diabetes between the groups, the statistical significance disappeared (p = 0.1, ANCOVA). The prevalence of intact dentition was much lower in the poorly controlled group compared to the moderately (mean HbA₁ 10.0–12.9%) and well (mean HbA₁ < 10%) controlled groups (Table 7), which difference remained significant after controlling for the co-variates (p = 0.008, ANCOVA).

Table 7. Comparison of well, moderately and poorly controlled children and adolescents with type 1 diabetes according to the mean HbA₁ during the past six months (Group 1, Paper II).

<table>
<thead>
<tr>
<th></th>
<th>Well controlled (n = 21)</th>
<th>Moderately controlled (n = 25)</th>
<th>Poorly controlled (n = 34)</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.8 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.2 ± 1.4</td>
<td>15.3 ± 1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>9/12</td>
<td>8/17</td>
<td>19/15</td>
<td>0.2</td>
</tr>
<tr>
<td>Age at diagnosis of diabetes (years)</td>
<td>11.4 ± 3.0</td>
<td>7.2 ± 3.9</td>
<td>7.7 ± 3.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>2.3 ± 2.5</td>
<td>6.9 ± 3.8</td>
<td>7.6 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean HbA₁ (%), 6 months</td>
<td>8.9 ± 0.9</td>
<td>11.5 ± 1.0</td>
<td>15.3 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subjects with intact dentition</td>
<td>10/21</td>
<td>12/25</td>
<td>5/34</td>
<td>0.008</td>
</tr>
<tr>
<td>Decayed and filled surfaces (DFS)</td>
<td>4.1 ± 6.9</td>
<td>2.3 ± 3.3</td>
<td>7.5 ± 9.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Positive Oricult® N test</td>
<td>11/21</td>
<td>10/25</td>
<td>26/34</td>
<td>0.015</td>
</tr>
</tbody>
</table>

<sup>a</sup> ANOVA or chi-square test; <sup>b</sup> standard deviation.

The subjects with non-intact dentition had significantly poorer short-term (mean six-months HbA₁ 14.1%) and long-term (mean twelve months HbA₁ 13.2%) metabolic control than the subjects with intact dentition (mean HbA₁ values 11.7% and 11.5%, respectively) (Table 8). Age, age at diagnosis of diabetes and the duration of diabetes as co-variates did not affect this finding. A positive Oricult® N test result was related to both poor control (Table 7) and non-intact dentition (Table 8). The results of the Dentobuff<sup>®</sup>, Denticut<sup>®</sup> LB and Denticut<sup>®</sup> SM tests and the salivary flow rates were comparable in the well, moderately and poorly controlled groups and also between the groups of subjects with intact and non-intact teeth.
Table 8. Comparison of subjects with clinically intact dentition and subjects with non-intact dentition among children and adolescents with diabetes duration of at least 2 years (Paper II).

<table>
<thead>
<tr>
<th></th>
<th>Subjects with intact dentition (n = 21)</th>
<th>Subjects with non-intact dentition (n = 41)</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14.3 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.0 ± 1.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>9/12</td>
<td>21/20</td>
<td>0.5</td>
</tr>
<tr>
<td>Age at diagnosis of diabetes (years)</td>
<td>6.3 ± 3.1</td>
<td>7.7 ± 3.4</td>
<td>0.09</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>8.0 ± 3.0</td>
<td>7.3 ± 3.2</td>
<td>0.4</td>
</tr>
<tr>
<td>HbA&lt;sub&gt;1c&lt;/sub&gt; (%) (6 months)</td>
<td>11.7 ± 2.0</td>
<td>14.1 ± 2.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean HbA&lt;sub&gt;1c&lt;/sub&gt; (%) (24 months)</td>
<td>11.5 ± 1.8</td>
<td>13.2 ± 2.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Positive Oriculos&lt;sup&gt;®&lt;/sup&gt; N test</td>
<td>8/21</td>
<td>28/41</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>a</sup> t-test or chi-square test; <sup>b</sup> standard deviation.

5.3. Salivary factors in relation to metabolic control in children and adolescents with type 1 diabetes

Fig. 2 shows the increase of the salivary flow rate (from 5.4 ± 3.3 ml/5 min to 7.3 ± 2.6 ml/5 min, p < 0.01, paired t-test) of the patients with newly diagnosed type 1 diabetes after two weeks of insulin treatment along with the elimination of the hyperglycaemia that existed at the time of the diagnosis of diabetes. A positive correlation between the blood glucose and salivary glucose levels was only noted in the case of severe hyperglycaemia upon the diagnosis of diabetes (Fig. 3). After the initiation of insulin treatment, the blood glucose levels of the patients with newly diagnosed diabetes decreased, and there was no correlation between the blood glucose and salivary glucose levels (Fig. 3).

![Fig. 2. Subjects with newly diagnosed type 1 diabetes: salivary flow rates in the hyperglycaemic state (Exam. 1) and after the initiation of insulin treatment (Exam. 2). Each line represents one case, two lines (marked by †) represent two cases each.](image-url)
In the patients with long-term type 1 diabetes, the salivary flow rates and HbA1 values were determined twice, three months apart. No differences were observed between the well (HbA1 <10%), moderately (HbA1 10–13%) and poorly controlled (HbA1 > 13%) groups with respect to the salivary flow rate or the salivary glucose level on either examination (Figs 1 and 2 in Paper III).

In the patients with long-term type 1 diabetes, a tendency toward higher salivary glucose levels and HbA1 values and lower salivary flow rates was observed along with increasing lactobacilli counts (Dentocult® LB test) and especially increasing yeast counts (Oricult® N test) (Figs 3 and 4 in Paper III).

5.4. Periodontal diseases in relation to the diabetic status in young adults with type 1 diabetes

A comparison of subgroups according by the existence of complications (Paper IV) revealed that the severity of periodontal disease increased as the complications progressed (Table 9). The subjects in Group C with advanced complications had the highest percentages of deepened pockets (≥ 4 mm, ≥ 6 mm), bleeding on probing and attachment loss (≥ 2 mm, ≥ 3 mm). The difference in the amount of plaque was statistically significant between the Groups A and C; most subgingival calculus was found in Group C, but the differences between the groups were not statistically significant. The mean percentage of deepened pockets at sites with subgingival calculus was 2.7% in Group A, 12.5% in Group B and 28.6% in Group C (p = 0.05, Kruskal-
Wallis test). In multiple stepwise regression analysis, the following factors were significantly related to pockets equal to or deeper than 4 mm: organ complications (p = 0.004), subgingival calculus (p = 0.006), sex (p = 0.014) and smoking (p = 0.067) (Table 4 in Paper IV). The following variables were not included in the final model: age, number of teeth, plaque, duration of diabetes and long-term metabolic control.

Table 9. Characteristics of diabetic subjects without complications (Group A), incipient retinopathy (Group B), and advanced complications (Group C) (Paper IV).

<table>
<thead>
<tr>
<th></th>
<th>Group A n = 4</th>
<th>Group B n = 11</th>
<th>Group C n = 11</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.7 ± 2.1^b</td>
<td>29.3 ± 2.5</td>
<td>31.2 ± 2.0</td>
<td>0.06</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>4/0</td>
<td>8/3</td>
<td>5/6</td>
<td>0.1</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>12.3 ± 2.5</td>
<td>18.8 ± 5.0^c</td>
<td>22.0 ± 4.6^d</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean HbA1 5-year (%)</td>
<td>8.8 ± 0.5</td>
<td>9.9 ± 1.1</td>
<td>12.0 ± 1.3^de</td>
<td>0.001</td>
</tr>
<tr>
<td>Plaque (%)</td>
<td>31.9 ± 8.7</td>
<td>33.5 ± 14.0</td>
<td>61.3 ± 23.4^de</td>
<td>0.02</td>
</tr>
<tr>
<td>Subgingival calculus (%)</td>
<td>7.0 ± 11.6</td>
<td>20.7 ± 24.7</td>
<td>26.8 ± 16.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Gingival bleeding (%)</td>
<td>29.1 ± 16.6</td>
<td>29.4 ± 20.8</td>
<td>52.1 ± 25.5^e</td>
<td>0.045</td>
</tr>
<tr>
<td>Pockets ≥ 4 mm deep (%)</td>
<td>1.6 ± 1.3</td>
<td>5.1 ± 6.9</td>
<td>13.3 ± 11.9^de</td>
<td>0.01</td>
</tr>
<tr>
<td>Pockets ≥ 6 mm deep (%)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>2.42 ± 5.2^f</td>
<td>0.02</td>
</tr>
<tr>
<td>Attachment loss ≥ 2 mm (%)</td>
<td>0.22 ± 0.45</td>
<td>0.26 ± 0.64</td>
<td>5.36 ± 9.26^e</td>
<td>0.046</td>
</tr>
<tr>
<td>Attachment loss ≥ 3 mm (%)</td>
<td>0</td>
<td>0</td>
<td>3.2 ± 6.3^f</td>
<td>0.046</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>0/4</td>
<td>5/6</td>
<td>4/7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

^a Kruskal-Wallis test or chi-square test; ^b the values are means ± standard deviations, paired comparisons using Mann-Whitney U-test, p ≤ 0.05; ^c between Groups A and B; ^d between Groups A and C; ^e between Groups B and C; ^f difference between Groups B and C, p = 0.07; ^g difference between Groups B and C, p = 0.1.

The subjects with poor long-term glucose control (mean 5-year HbA1 ≥ 10%) had significantly more plaque, subgingival calculus and gingival bleeding than the well to moderately controlled subjects (HbA1 < 10%) (p < 0.05, Mann-Whitney U-test). The difference in the mean percentages of pockets ≥ 4 mm (5.1% vs. 10.2%) did not reach statistical significance, but all subjects with severe attachment loss (≥ 3 mm) or deep pockets (≥ 6 mm) were poorly controlled. Poor long-term glucose control and the duration of diabetes were closely related to complications, as can be seen from the values of these variables in the Groups A, B and C (Table 9).

5.5. Response to periodontal treatment in relation to the diabetic status in young adults with type 1 diabetes

At baseline before periodontal treatment, the diabetic group as a whole and the non-diabetic control group did not differ with respect to the amounts of plaque (Table 3 in Paper V) and subgingival calculus (Table 4 in Paper V) or the extent of gingival bleeding,
deepened pockets or attachment loss (Table 5 in Paper V). Age and the number of teeth were the same in the diabetic patients and controls as well as in the diabetic subgroups (Table 1 in Paper V).

When the diabetic subgroups were compared, subjects with poor control and/or complications (Group D3) had significantly higher percentages of sites with clinical attachment loss equal to or greater than 2 mm (24.4 ± 19.5 %) at baseline than subjects with moderate control and with/without retinopathy (Group D2, 6.6 ± 7.8%) or subjects with good long-term control and without complications (Group D1, 8.9 ± 7.6%) (p = 0.003, ANOVA). The difference in the mean percentages of deepened pockets (≥ 4 mm) did not reach statistical significance at baseline (Fig. 4), and a favourable short-term response to periodontal therapy was seen in all groups after four weeks (Fig. 4). However, faster recurrence of deepened pockets was seen in group D3, and the subjects in that group had significantly more deepened pockets ≥ 4 mm in the 12-month examination than the subjects in the D2 or D1 groups.

![Fig. 4. Percentages of sites with pocket depth ≥ 4 mm in the subjects with type 1 diabetes as a whole group (D), in the non-diabetic controls (C) and in the diabetic subgroups (D1, D2, D3) in the 4-week and 6- and 12-month examinations. The p-values of ANOVA between the diabetic subgroups are indicated: in the 12-month examination, the Group D3 subjects have a significantly higher percentage of pocket depth ≥ 4 mm when compared to the Group D1 and D2 subjects (p = 0.04).](image)

At baseline, the amount of subgingival calculus was not significantly different between the groups, but within six and twelve months, significantly more calculus was formed in the subjects of group D3 than in the groups D2 or D1 (Table 4 in Paper V). The plaque percentages of all the groups first decreased, but then increased, almost reaching the baseline values by the 12-month examination (Table 3 in Paper V). No statistically significant differences in the mean plaque percentages were seen between the diabetic subgroups (Table 3 in Paper V).
6. Discussion

6.1. Study design

As already pointed out, comparisons between patients with diabetes and controls have not resulted in consensus as to whether diabetes is a risk factor with respect to periodontal diseases or dental caries. Nor did the present study on adult patients with type 1 diabetes reveal any differences in periodontal disease severity or healing response between the diabetic group as a whole and the controls (Paper V). Oral diseases, such as periodontal diseases and dental caries, but also the diabetic status are multifactorial. Most evidently, not all diabetic patients are at equal risk for oral diseases, and more attention has recently been paid to possible diabetes-related risk factors to identify subjects who are more prone to periodontal diseases or dental caries. The factors studied have included the duration of diabetes, age at diagnosis of diabetes, the presence of diabetic organ complications, and since the early 1980s, the level of metabolic control. The results of these studies, however, have still been inconsistent, possibly because of methodological differences and differences in the characteristics of the study populations, such as the type of diabetes, the level of metabolic control, the duration of diabetes and the age range of the subjects. The present work was carried out to elucidate the relationship between characteristics of type 1 diabetes and periodontal diseases and dental caries. These studies aimed at obtaining more precise information by selecting the subjects carefully and controlling for possible confounders in the data analysis. Also, three different study populations were used: children and adolescents with recent-onset or previously diagnosed type 1 diabetes and young adult subjects with type 1 diabetes. Moreover, both cross-sectional and follow-up data were obtained.

A goal was to use homogenic study populations to be able to control for various confounding factors. As implicated in the literature review, type 1 and type 2 diabetes have differences in their genetic background, etiology, treatment strategies and the presence of complications. Moreover, the difference in age at diagnosis causes inevitable differences in the duration of diabetes and/or age between subjects with type 1 and those with type 2 diabetes. Therefore, only subjects with type 1 diabetes were included in this work. Along with increasing age, other factors apart from the level of metabolic control, such as the duration of diabetes, diabetic complications and the interrelationships between
these factors, complicate the interpretation of the diabetic status. To avoid this, the age range of the subjects was restricted to be quite narrow, which has usually not been the case in previous studies.

Most attention was paid to the level of metabolic control, as poor metabolic control has been shown to be related to diabetes-associated alterations in tissues and is a key factor in the development of diabetic organ complications (D’Antonio et al. 1989, Reichard et al. 1993, Diabetes Control and Complications Trial Research Group 1993, Wang et al. 1993). Therefore, it was assumed that poor glucose control would affect the occurrence of oral complications as well. The role of metabolic control in relation to oral health was considered a feasible target of investigation in children and adolescents, in whom metabolic control is the best, and often the only, indicator of the diabetic status, because complications are rare in these age groups, as the long-term effects of the disease are not yet obvious. Accordingly, variation in the duration of diabetes is also more limited than in adult populations, and variations in the history of previous dental treatment as a confounding factor are relatively small. In order to increase the understanding of the role of metabolic control in relation to oral health, many aspects, such as gingivitis, dental caries and salivary factors, were concomitantly evaluated in the same study population. This is the first study to include subjects with newly diagnosed diabetes and observed from diagnosis. This group of patients was included because it represents the most extreme cases of hyperglycaemia and, as such, could give further information on the effects of glucose control on oral health. In previous studies, the level of metabolic control has been evaluated over various lengths of time, ranging from actual HbA1 or HbA1c values at the time of oral examinations to mean values over a few years. In this work, different evaluation times were used, depending on whether salivary factors, gingivitis, dental caries or periodontitis were studied. Part of the biological side-effects of poor control appear within a short time and may be reversible, e.g. changes in PMN cell function. Some other changes, such as alterations in collagen structure, however, require a long time to develop and are usually irreversible. Accordingly, the short-term level of glucose control was thought to serve as a better reference for changes in salivary factors or gingivitis, while information on long-term control will be needed in the case of chronic and slowly progressing oral diseases, such as dental caries and periodontitis.

In adults, both the diabetic status and factors affecting oral health are more complicated. Diabetes-associated structural and functional changes in tissues and cells result in the development of diabetic organ complications, such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. The duration of diabetes, and hence also the subject’s age, determines to a certain extent whether complications develop, but the most important causative factor for complications has been shown to be poor metabolic control (D’Antonio et al. 1989, Reichard et al. 1993, Diabetes Control and Complications Trial Research Group 1993, Wang et al. 1993). Despite that, there are both patients who develop complications after a short duration of diabetes and with a reasonable level of metabolic control and patients who never get complications even with long-standing poorly controlled disease. This individual susceptibility has been assumed to be related to differences in genetic background (Rosenstock & Raskin 1988, Deckert et al. 1989). In this work, the diabetic status of adult patients was defined according to the level of long-term metabolic control and the presence of diabetic complications or their combination, when the diabetes-related risk factors for periodontal disease were studied.
Complications were thought to indicate individual susceptibility to biological alterations, which are inevitable even in the oral environment and could thus affect oral health. On the other hand, the age range of the subjects was set to be 10 years, and the subjects selected were young adults, in order to control for the confounding effect of age on periodontal disease experience. In cross-sectional studies, dental treatment history is a confounding factor with respect to the prevalence of caries or the severity of periodontal disease. In order to adjust the subjects with respect to the unknown previous dental treatment, one of the present studies included initial intervention with a hygienic phase of periodontal therapy followed by observation.

6.2. Periodontal diseases in relation to the diabetic status

6.2.1. Periodontal diseases in relation to metabolic control of diabetes

The present results confirmed that poor metabolic control increases gingival inflammation in children and adolescents with type 1 diabetes (Paper I). This was evident in the patients with newly diagnosed diabetes, as gingival bleeding decreased after the initiation of insulin treatment, i.e. after the hyperglycaemic phase was reversed. Cross-sectional data on adolescent patients with varying duration of diabetes supported this: the bleeding on probing scores were significantly higher in poorly controlled subjects compared to moderately or well controlled ones (Paper I). However, according to the present findings, it seems that the effect of metabolic control on gingival bleeding is not very strong, as the increase of gingival bleeding was not obvious until the metabolic control was really poor. The mean glycosylated haemoglobin value was 14.9% in the group of newly diagnosed patients and 15.3% (Examination 1) and 16.0% (Examination 2) in the group of poorly controlled adolescents, respectively. These mean values indicate alarmingly poor metabolic control in these patients. In the previous studies which have not revealed an association between HbA1c and gingival inflammation in young study populations, the mean HbA1c values of poorly controlled subjects were lower than in the present study: 10.9% in the study of Sandholm et al. (1989a), 11.5% in the study of Sastrowijoto et al. (1989), and 8.8% in the study of Firatli et al. (1994), or the means were not given (de Pommereau et al. 1992, Pinson et al. 1995). Firatli et al. (1994) reported a significant correlation between the gingival index and fructosamine values, but not between HbA1c and the gingival index. First, this may relate to the relatively low mean HbA1c value of 8.8% of their diabetic study group. Secondly, fructosamine is a glycosylated protein with a shorter half-life than glycosylated haemoglobin, and the gingival index is also likely to change quite quickly, as was observed in the patients with newly diagnosed diabetes. Presumably, the better correlation between fructosamine and the gingival index than between HbA1c and the gingival index in the study of Firatli et al. (1994) relates to these factors. In addition to Firatli’s study, support for the result on the association between gingival inflammation and the level of metabolic control has previously been reported in adolescents by Gislen et al. (1980) and in adult patients with diabetes by Ervasti et al. (1985).
Some studies have measured the extent of periodontal destruction (e.g. deepened pockets or attachment loss) in relation to metabolic control in age groups comparable to these studies, but have not found any associations (Sandholm et al. 1989a, Pinson et al. 1995, Firatli et al. 1996). In the present study of adolescent patients, deepened pockets and clinical attachment loss were registered and explored in relation to the diabetic status, but no statistically significant associations were found (data not shown), partly because periodontitis is so rare in such young age groups as those included in the present study. Cianciola et al. (1982) reported an exceptionally high prevalence of periodontitis in diabetic adolescents of comparable ages as in the present study, but the relationship between periodontal disease and the level of metabolic control was not explored, and any evaluation of their results in relation to the diabetic status is impossible.

In the study concerning young adults (Paper IV), the level of long-term metabolic control in relation to periodontal disease severity, measured by the prevalences of deepened pockets or clinical attachment loss, was explored, but the differences between well to moderately and poorly controlled groups did not reach statistical significance. The extent of gingival bleeding, however, was greater in the poorly controlled patients. The strong relation between gingivitis and the level of metabolic control and the weaker relation between periodontitis and the level of metabolic control in adults most probably relates to the more complex nature of periodontitis compared to gingivitis, as well as to the complexity of the diabetic status in adults. The observed association between gingival inflammation and poor glucose control also supports the role of poor metabolic control as a phenomenon behind periodontitis. In conclusion, intensified prevention and effective treatment of gingivitis in young diabetic patients with poor metabolic control is important, although not all gingivitis proceeds to periodontitis.

When the association between gingival inflammation and the level of metabolic control is evaluated, the contribution of local etiology, i.e. plaque and calculus, needs to be controlled for. In this study, the amount of local etiology did not explain the differences in gingival bleeding, as the subgroups of children and adolescents categorised by the level of metabolic control had comparable plaque indices and the amount of plaque remained unchanged during the follow-up of the subjects with newly diagnosed diabetes. Nor have microbiological studies shown significant differences in the composition of plaque between patients with diabetes or control subjects (Zambon et al. 1988, Sbordone et al. 1995, Christgau et al. 1998) or between subgroups based on the level of metabolic control (Sastrowijoto et al. 1989, Mandell et al. 1992, Tervonen et al. 1994, Sbordone et al. 1995). In a follow-up study (Sastrowijoto et al. 1990), the improvement of metabolic control by intensified insulin treatment did not alter the prevalences of known periodontopathogens. Only the percentage of streptococci was significantly higher in diseased periodontal pockets during improved metabolic control. These results, however, do not exclude the possibility that microbial changes may occur in alarmingly poorly controlled patients, as seen in the present study. In fact, Tervonen et al. (1994) speculated that their results might have been affected by the fact that the "vast majority of the subjects (73%) were under good or moderate diabetic control". The above referred results, presuming that microbiological changes are not likely, together with the information given in Fig. 1 supports the assumption that inflammatory reactions are intensified during poor metabolic control, as the same amounts of plaque induced more gingival bleeding in the poorly controlled subjects compared to the well controlled ones.
Infections are known to deteriorate the metabolic state of diabetes (Rayfield et al. 1982). Unfortunately, the present studies were not designed to explore this aspect, and the data hence did not allow to assess whether periodontal treatment was followed by any changes in the level of metabolic control. Miller et al. (1992) and Grossi et al. (1996, 1997) reported a favourable effect on HbA1c values after periodontal treatment. The study of Miller et al. (1992) covered only nine subjects, five of whom showed a significant decrease of their HbA1c values. Part of that drop might have been related to the Hawthorn effect, i.e. the subjects might have improved their diabetes self-care when involved in the study. In the studies of Grossi et al. (1996, 1997), improvement of metabolic control was only evident in the groups receiving doxycycline. The ability of doxycycline to prevent non-enzymatic glycosylation (Ryan et al. 1998) cannot be ignored: although HbA1c levels dropped, fasting blood glucose levels remained unchanged. Other studies (Aldridge et al. 1995, Smith et al. 1996) were not able to detect any changes in HbA1c levels after scaling and root planing. It is noteworthy, however, that the mean HbA1c levels were lowest in that study (around 8%) compared to about 10.5% in the studies of Grossi et al. (1996, 1997) and 9.5% in the study of Miller et al. (1992). Therefore, the possible favourable effect of the treatment of periodontal infections on the metabolic control of diabetes has not yet been proven conclusively. However, a two-way relationship between diabetes and periodontal disease has been suggested (Grossi & Genco 1998), and it is reasonable to assume that the effect of periodontal treatment on the level of metabolic control will be clarified in populations with more severe periodontal disease and/or including subjects with higher glycosylated haemoglobin values than those studied so far.

6.2.2. Periodontal diseases in relation to the overall diabetic status

In adult study populations, periodontal disease severity was not clearly associated with the level of metabolic control alone (Papers IV and V). In study IV, however, it became evident that a stronger association between periodontal disease severity and the level of metabolic control was found when long-term mean HbA1 values rather than the most recent single HbA1 value was used as an indicator of metabolic control. Also, diabetic organ complications related more strongly to the long-term level of glucose control (Table 6 in Paper IV). The association between periodontitis and the level of metabolic control did not seem to be straightforward, as the differences between the subgroups did not reach statistical significance with respect to deepened pockets (Paper IV). This may relate to the fact that, apart from metabolic control, other factors, such as the duration of diabetes and individual susceptibility, are also involved, as in the development of complications, especially if the risk factors for periodontitis and organ complications are assumed to be similar. The clinical picture and progress of diabetes are complex and vary between individuals, and periodontitis is also multifactorial. However, the presence of organ complications can be seen to reflect the whole entity of the diabetic status, i.e. it is a kind of end-point of the consequences of diabetes in each individual.

The subgroups of diabetic patients with severe diabetes (Tables 3 and 4), e.g. advanced complications and poor long-term metabolic control, had more attachment loss, which reflects the history of periodontal disease, in both of the present studies. In study IV, these
subjects also had significantly more deepened pockets. In study V, the extent of deepened pockets was not statistically significantly greater at baseline, but faster recurrence of deepened pockets during the 12-month follow-up after the periodontal treatment was obvious in the subjects with severe diabetes compared to the other diabetic patients or the controls. This variation in results is most likely, because active disease with deepened pockets was not so common at baseline of study V, as the subjects of that study had received more regular dental treatment than the subjects in study IV. However, if the diabetic status was not assessed and the whole patient group was compared with the controls, the response to treatment was similar, and the rates of recurrence of pockets and gingival inflammation in the patients and the controls were comparable (Paper V), as has been reported in many studies previously (Bay et al. 1974, Tervonen et al. 1991, Smith et al. 1996, Westfelt et al. 1996, Christgau et al. 1998). The present results clearly showed that, in adults, periodontal disease was more strongly associated with the diabetic status when it was evaluated as an entity. Accordingly, the progression of periodontitis can be seen as a complication of diabetes – dependent on the same risk factors as diabetic organ complications. In fact, Löe (1993) reviewed a few studies on periodontal disease in subjects with diabetes and suggested that periodontal disease should be considered "the sixth complication of diabetes mellitus".

Periodontal disease will not develop and progress without a local etiology, e.g. plaque and calculus, and in that respect it differs from the other diabetic complications. The diabetic status remained, however, associated with periodontitis (assessed by deepened pockets), when the differences between the patient subgroups in the amounts of plaque and subgingival calculus were controlled for (Table 4 in Paper IV). Moreover, the presence of deepened pockets on sites with subgingival calculus increased significantly along with the severity of complications (Paper IV). Similar observations have been previously reported in relation to poor long-term metabolic control (Tervonen & Oliver 1993).

The approach to assess the diabetic status by means of the presence of complications has been used in earlier studies as well. Earlier reports provide support for the present finding on an association between periodontal disease and diabetic complications (Finestone & Boorujy 1967, Glavind et al. 1968, Bacic et al. 1988, Rylander et al. 1986). Retinopathy and its grading constituted the basis for forming the groups (Paper IV), as retinopathy is the first microvascular complication to appear, and it can be easily classified into incipient and more severe forms, and most importantly, retinopathy is the diabetic organ complication most clearly related to the level of metabolic control (Wang et al. 1993). In concordance, Kjellman (1970c), Glavind et al. (1968) and Bacic et al. (1988) also reported an association between the severity of periodontal disease and retinopathy. There are, however, also studies which have not found any associations between periodontal disease and diabetic complications (Ervasti et al. 1985, Safkan-Seppälä & Ainamo 1992, Westfelt et al. 1996). Moreover, as evident from the literature review, the complication with which periodontal disease has been observed to be associated, vary considerably between the studies. One reason may lie in the fact that many of them have used each complication separately in their comparisons, although many subjects in their study groups presumably had several complications concomitantly. This means that one-to-one comparisons between subjects with complications may not have included representative controls. Only the studies of Finestone & Boorujy (1967)
and Westfelt et al. (1996) accounted for the concomitant occurrence and severity of diabetic complications. Only the results of Finestone & Boorujy (1967) are consistent with the present results, as they found more severe periodontitis in subjects with many concomitant complications compared to those with either one or no complications.

These observations suggest that the differences with respect to the diabetic status and the way to assess diabetes explain a lot of the controversies of previous results. Periodontal disease in relation to the diabetic status should be assessed in study populations with enough variation with respect to both the level of metabolic control and the presence and severity of diabetic complications. Such study populations, however, may easily turn out to be very heterogeneous with respect to diabetes duration and the subjects’ age and periodontal disease experience, and the confounding effect of the interdependence of these factors should be considered. Also, the means to assess diabetes depends on what variable is under survey; in children and adolescents, gingivitis was clearly associated with short-term metabolic control, but the extent of periodontitis had to be evaluated in relation to long-term metabolic control or, even more appropriate, in combination with the presence of complications.

Irregular dental visits, poor dental hygiene and smoking tended to be more common among the subjects with poor control and/or advanced complications (Paper IV). Thorstensson et al. (1989a) reported that irregular dental visits were more common among diabetic patients than controls. Missed dental appointments without cancellation were more common among subjects with diabetes than controls in the study of Pohjamo et al. (1995). The level of metabolic control is highly dependent on the subject’s ability and motivation to carry out selfcare procedures, especially if all efforts have been made to optimise the subject’s treatment regimens, by having e.g. multiple daily insulin injections (Drash & Becker 1990). Individual differences certainly exist and in some patients the ability to maintain good blood metabolic control may be affected by genetic factors. The association between adherence to diabetes self-care regimens and the level of metabolic control is interesting, however, as self-care is also closely related to oral diseases, such as periodontal diseases and dental caries. This aspect has recently received support from the results of Kneckt et al. (1999) and Syrjälä et al. (1999). Kjellman (1970c) was the first to report that the degree of gingivitis and the amounts of supragingival calculus correlated with "DM-co-operation". This indicates that when the overall diabetic status as a risk factor for oral diseases is evaluated, other risk factors, such as the subject’s health behaviour and adherence with both diabetes and dental treatment, should also be considered. They all contribute to the entity of diabetes and will ultimately be reflected as biological alterations, which explain the consequences of diabetes, presumably in the oral cavity as well.

### 6.2.3. Diabetes-related biological alterations in relation to periodontal diseases

Diabetes research has yielded a lot of information on the susceptibility to infections (Rayfield et al. 1982) and compromised wound healing (Pearl & Kanat 1988, Morain & Colen 1990, Rosenberg 1990, Terranova 1991) among patients with diabetes. As high-
lighted by the literature review, a variety of biological consequences are known to be related to diabetes, mainly with hyperglycaemia, which could also induce impaired infection resistance, altered host response and modifications of tissue destruction in periodontal tissues. These aspects could hence explain the present results.

These biological phenomena, however, have mainly been studied in tissues other than periodontal tissues and in experimental diabetes or in vitro conditions. The earliest studies addressed diabetes-associated PMN cell dysfunctions in relation to periodontal disease. In experimental studies in rats, the number and chemotactic response of leukocytes in gingival sulcus were found to decrease in diabetic animals compared to control rats (Ramamurthy et al. 1979, Golub et al. 1982). Later, in human, PMN cell defects were linked to both periodontitis and diabetes, as the chemotactic response of PMN cells was mostly compromised in diabetic subjects with periodontitis compared to diabetic patients with mild periodontitis or healthy subjects with either mild or severe periodontitis (Manouchehr-Pour et al. 1981a, 1981b, Bissada et al. 1982). Abnormalities in the basement membranes of gingival capillaries have been documented in many studies (Keene Jr 1969, Campbell 1971, Frantzis et al. 1971, Seppälä et al. 1997), but the association of these alterations with the metabolic control of diabetes or the severity of periodontal disease has not been proven yet (Listgarten et al. 1974, Seppälä et al. 1997).

In experimental studies, hyperglycaemic culture conditions have been shown to decrease the synthesis of collagen and the glycosaminoglycans of gingival fibroblasts (Willershausen-Zönnchen et al. 1991) and to cause abnormal proliferation of both gingival fibroblasts and periodontal ligament cells (Ohgi & Johnson 1996). Also, the chemotactic response of periodontal ligament cells is impaired (Nishimura et al. 1996), which might compromise wound healing. Under hypoglycaemic conditions, periodontal ligament fibroblasts were found to dissociate and undergo apoptosis (Nishimura et al. 1996). Both hyperglycaemia and hypoglycaemia can thus directly impair the biological functions of periodontal connective tissues, at least in experimental diabetes or in vitro study conditions. In experimental animal studies, changes in the turnover and structure of collagen, the main connective tissue component of periodontal tissues, have been shown in gingiva (Ramamurthy et al. 1972, Schneir et al. 1984, Ramamurthy et al. 1985, Yu et al. 1993). According to some more recent studies on collagen glycosylation (for a review, see Reiser 1991) in skin, for example, it can be assumed that these changes are also connected with the glycosylation of collagen in gingival tissues. These collagen alterations most probably modify both periodontal disease progression and healing after therapy, as tissue turnover and remodelling are affected.

Accelerated immune responses, assumed to be related to AGE formation, have been supported indirectly by the observations on significantly higher levels of PGE$_2$ and IL-1β in gingival crevicular fluid in patients with diabetes than in controls matched for periodontal disease severity (Salvi et al. 1997). AGE-upregulated immune responses are in oral tissues indirectly supported by the observations that diabetes induces pathologically excessive collagenase activity in gingival tissues (Golub et al. 1978, Golub et al. 1983, Ramamurthy & Golub 1983, Chang et al. 1988) and gingival crevicular fluid (McNamara et al. 1979, Kaplan et al. 1982). This may relate directly to the immunogenity of glycosylated collagen or the indirect activation of collagenase-productive cells by AGE formation. Schmidt et al. (1996b) demonstrated that
immunoreactivity to AGEs is increased in the gingiva of diabetic animals and human, and a marker of enhanced oxidative stress was detected in diabetic gingiva compared to control tissue.

The known biological consequences of diabetes have so far been used only partly in the attempt to explain the increased susceptibility to periodontal diseases in a proportion of patients with diabetes, especially in those with poor metabolic control and severe diabetes – a phenomenon also seen in the present studies. Theoretically, many diabetes-related biological changes could explain the altered host response, but these changes have mostly been studied only in tissues other than periodontal tissues or in experimental or in vitro conditions. No conclusions can therefore be drawn directly from these results. Moreover, inflammation, which is always present to some extent in gingival tissues, most probably modifies the association between diabetes-related alterations and the host response in the periodontium.

6.3. Dental caries and salivary factors in relation to metabolic control of diabetes

6.3.1. Dental caries in relation to metabolic control of diabetes

Dental caries, either compared to healthy controls or in relation to the diabetic status, is a much less investigated topic than periodontal disease in subjects with diabetes, and the results have been notably inconsistent, as is evident from the literature review. The present finding that the mean DFS value was significantly higher in poorly (HbA1 ≥ 13%) than moderately (HbA1 10.0–12.9%) or well (HbA1 < 10%) controlled adolescents with type 1 diabetes was not statistically significant, when the differences in age, age at diagnosis of diabetes or the duration of diabetes between the groups were controlled for (Paper II). The DFS and DFMS values are age-dependent, and ignorance of this fact, especially in studies with wide age variations, may partly explain the controversies in the results. Apart from using DFS, the data were analysed by using the presence of intact dentition as a dependent variable, because it is not confounded by age as strongly as DFS values. Moreover, the precise timing of the placement of fillings in relation to the diagnosis of diabetes and of the dental treatment history of the subjects were unknown, both of which confound the use of DFS or DFMS indices in a cross-sectional study design. The present results clearly showed that non-intact dentition was significantly more common among poorly than moderately or well controlled adolescents with diabetes. Support for the role of metabolic control in relation to caries experience has been previously obtained from studies which either have been prospective (Wegner 1975, Twetman et al. 1992) or have used a very narrow age range of the subjects included (Galea et al. 1986). Noteworthingly, in the present study, subjects with poor control had alarmingly high HbA1 values. In conclusion, the need for prophylaxis and frequent dental examinations among children and adolescents with type 1 diabetes is greater for the patients with poor metabolic control (HbA1c higher 13% or HbA1c higher 10%) than for those with good or moderate control of the disease.
6.3.2. Salivary factors in relation to metabolic control of diabetes

Most attention has been paid to the possible changes in salivary flow rate, but no agreement has been reached, either in comparisons between diabetic patients and controls or about the relationship between salivary flow rate and the level of metabolic control (see literature review). In the cross-sectional part of the present study on diabetic adolescents who had been on insulin treatment for various lengths of times, no differences in stimulated whole salivary flow rates or salivary glucose levels were obvious between the well, moderately and poorly controlled subjects (Paper III). Occasional blood glucose measurements and salivary glucose levels did not correlate in patients with long-term type 1 diabetes (data not shown), and HbA1 values and salivary glucose levels were not related either. However, salivary flow rates increased and salivary glucose levels decreased during the follow-up of children with newly diagnosed diabetes after the correction of severe hyperglycaemia. Salivary glucose levels and blood glucose levels correlated significantly during the hyperglycaemic phase in patients with newly diagnosed diabetes, but not at the end of the follow-up, when the blood glucose levels were significantly lower. It was concluded that both the salivary flow rate and the salivary glucose levels are affected, but only in the presence of reasonably high blood glucose levels. In fact, Reutervin et al. (1987) have suggested that there is a threshold at blood glucose concentrations of about 10–15 mmol/l, and blood glucose levels higher than that increase salivary glucose levels. Hyperglycaemia-related increases in the salivary glucose levels have previously been shown by Reutervin et al. (1987) and Twetman et al. (1992). Inter- and intraindividual variations in salivary glucose levels were observed in the present study and have been reported by others as well (Tenovuo et al. 1986, Borg & Birkhed 1988, Borg Anderson et al. 1998). Tenovuo et al. (1986) found that blood glucose levels were reflected in the salivary glucose levels in some subjects, whereas in others high blood glucose did not result in any notable elevation of salivary glucose. A few studies have shown that blood glucose levels are reflected in parotid saliva to a certain extent (Englander et al. 1963, Reutervin et al. 1987, Ben-Aryeh et al. 1988, Borg & Birkhed 1988, Borg Andersson et al. 1998). In addition to parotid saliva, gingival crevicular fluid is another probable source of glucose in mixed saliva, as blood glucose levels have been shown to correlate with gingival crevicular fluid glucose levels (Kjellman 1970b, Ficara et al. 1975). High HbA1 values indicate that the patient has had hyperglycaemic periods during the past 6 to 8 weeks, but these values do not give information about the actual situation at the time when the salivary test was performed, which explains why no direct relationship between salivary factors and HbA1 values was observed. In support of this, Darwazeh et al. (1991) found that glucose levels in unstimulated mixed saliva correlated with actual blood glucose levels but not with HbA1 values.

A slight effect of glucose control on salivary lactobacilli counts (determined by Dentocult® LB test) and yeast counts (determined by Oricult® N test) was observed: in the patients with long-term type 1 diabetes, the HbA1 values of the subjects with the highest lactobacilli and particularly yeast counts tended to be higher than the values in those with the lowest microbial counts. Furthermore, the lactobacilli counts dropped during the initial follow-up period in half of the children with newly diagnosed type 1 diabetes. The same was also observed in the study of Twetman et al. (1992), where the
lactobacilli counts dropped significantly during the 6-month period after diagnosis of diabetes. They have previously reported that the lactobacilli counts correlated with the salivary glucose levels (Twetman et al. 1989). The present results are in agreement with this, because the salivary glucose levels showed a tendency to increase along with the increasing lactobacilli and yeast counts (Figs 3 and 4 in Paper III).

The associations of salivary yeast counts with both the level of metabolic control and the caries experience are interesting. It is well known that yeast growth is more common among patients with diabetes than in controls (Bánóczy et al. 1987, Bartholomew et al. 1987, Lamey et al. 1988). Salivary glucose levels have been shown to be higher in diabetic subjects with than without yeast growth (Darwazeh et al. 1991). Wilson & Reeves (1986) observed that in vitro intracellular killing of yeasts by neutrophils is impaired under conditions of hyperglycaemia and ketosis, and glucose in saliva has been reported to promote the adhesion of Candida albicans to buccal epithelial cells (Samaranayake & MacFarlane 1982, Darwazeh et al. 1990). Thus, glucose can indirectly increase the amounts of yeasts in the oral cavity. Yeasts do have properties, such as proteolytic capacity, which are enhanced if the glucose content of saliva increases (Samaranayake et al. 1984), and yeasts could thus be a factor in caries etiology. As yeast growth is connected with low saliva secretion and high salivary glucose levels, high levels of yeasts in saliva can be considered a reasonable indicator of a cariogenic environment in the mouths of diabetic subjects.

Saliva pH and buffer capacity were among the other salivary factors studied, and they were not observed to be related to the level of metabolic control, which is in accordance with the results of Reuterving et al. (1987).

In conclusion, among the salivary factors studied, only the salivary flow rate, salivary glucose level and salivary yeast count were found to be related to the level of glucose control. All changes were only evident at the time of severely impaired metabolic control, most clearly during the hyperglycaemic phase at the time of the diagnosis of type 1 diabetes.

6.3.3. Diabetes-related aspects in relation to dental caries and salivary factors

Dietary factors are important in caries etiology, and the key factors are whether sucrose or free sugars are consumed and the frequency of their consumption (Gustafsson et al. 1954, Scheinin et al. 1974, Shannon 1977). The diabetic diet has previously included less carbohydrates and more fat (Sterky 1962) and less refined carbohydrates (Sterky et al. 1971) than usually. These factors might have suggested lower caries activity in patients with diabetes than in healthy controls, and in support of this, Sterky et al. (1971) reported less caries in subjects with diabetes than in controls. Nowadays, the proportion of carbohydrates in the diet has increased to the same level as that recommended for a healthy diet composition, but restricted use of raffinated carbohydrates is still recommended (American Diabetes Association 1998d). However, in order to keep the blood glucose levels balanced, patients with diabetes usually have a higher frequency of meals and snacks than non-diabetic subjects. Also, in case of frequent hypoglycaemia
episodes, free sugars might be consumed in an uncontrolled fashion. Sarnat et al. (1985) reported equal caries rates in patients with diabetes and in healthy subjects, and both caries risk and protective factors were found in the diet of diabetic patients: carbohydrate consumption was the same in both groups, but the carbohydrates were mainly derived from starch in the diabetic diet. The frequency of consumption, however, was higher and the meals were longer in patients with diabetes than in controls. When the role of poor metabolic control as a caries risk factor is evaluated, it should not be forgotten that impaired metabolic control may be a consequence of poor dietary compliance, which usually is reflected by too many meals and snacks and too much carbohydrates.

Apart from the dietary composition, salivary factors, such as secretion rate, buffer capacity, saliva pH, antimicrobial defence mechanisms and microbial counts are important in caries etiology. As a central defence system in the oral cavity, saliva and its constituents are important for all aspects of oral health. The possible explanations for diabetes-related changes in the salivary flow rate or glucose levels may in the short term include the effect of absolute or relative insulin deficiency, which impairs the function of salivary gland cells. This has been supported by experimental animal data, since the initiation of insulin treatment has been shown to normalise salivary gland function, i.e. salivary flow rates increased to the level seen in control animals (Reuterving 1986, Anderson 1987). Hyperglycaemia-related overall dehydration should not be forgotten as a reason for a reduced salivary flow rate. In the present study, the salivary flow rate and the salivary glucose level turned out to be negatively correlated, which partly explains the higher salivary glucose levels, when the salivary flow rate is decreased. Ignorance of this may explain the previous contradictory results on whether salivary glucose levels are altered, or whether there is a relationship between salivary and blood glucose levels. Along with the longer duration of diabetes, long-term alterations in salivary glands, such as histologically evident lipid accumulation and degenerative changes (Hand & Weiss 1984, High et al. 1985, Anderson & Garrett 1986), may relate to salivary alterations. Neuropathic changes, evident as altered reactivity to stimulation, and histologically evident neuroaxonal abnormalities (Anderson et al. 1989), have also been demonstrated. According to Newrick et al. (1991), the lower salivary flow rates in subjects with diabetes compared to controls were more obvious in patients with than without neuropathy. Basement membrane alterations could also contribute to salivary changes. Some authors have hypothesised that basement membrane alterations in the salivary glands are the reason for higher salivary glucose levels, because leakage of glucose through salivary gland ductal cells increases due to a basement membrane damage (Sharon et al. 1985, Harrison & Bowen 1987a).

As discussed in connection with periodontal diseases, a lack of metabolic control in diabetes induces a series of various biological changes. In the case of dental caries, the biological effects of diabetes are mainly mediated through alterations in salivary glands and saliva. However, aspects of health behaviour, such as adherence to the dietary recommendations, should not be forgotten, either. These factors have direct effects on caries experience, but if poorly monitored, also indirect effects through the development of biological alterations.
7. Conclusions

Diabetic children and adolescents with poor metabolic control were found to be predisposed to gingivitis and dental caries. It is noteworthy that the glycaemic control of the poorly controlled group in the present studies was alarmingly poor, as their HbA1c values were 13% or higher. In view of the currently used HbA1c values, this would mean a value in excess of 10%. Hyperglycaemia was also associated with a decreased salivary flow rate, increased salivary glucose levels and a tendency towards higher salivary lactobacilli, and especially yeast, counts. It can be proposed that dental professionals should be aware of the level of glycaemic control of their child and adolescent patients with type 1 diabetes, and prevention and intensified treatment should be focused on those with poor glycaemic control.

In adults, on the other hand, the overall diabetic status assessed by the level of long-term glycaemic control and the presence and severity of diabetic complications was related to more severe periodontal disease and recurrence of periodontitis after periodontal treatment. Glycaemic control alone was not directly associated with periodontitis. The risk subjects for periodontitis among adult patients with diabetes can be categorised as follows: subjects with long-term HbA1c values higher 10% alone or combined with the presence of diabetic complications; subjects with advanced diabetic complications, such as preproliferative or proliferative retinopathy, nephropathy, limb amputations, recurrent infections or subjects with multiple diabetic complications, even with good or moderate metabolic control. The risk subjects will need regular recall visits at shorter intervals, at least twice a year, than the other patients with diabetes or controls. However, the short-term response to periodontal treatment was equally favourable in all the subjects.

It would be emphasised that although the long-term level of metabolic control should be assessed in the case of dental caries and periodontitis, even shorter hyperglycaemic periods may induce alterations in salivary factors and may aggravate inflammation. Accordingly, unexpected changes in oral health may indicate an impairment of glucose control or even undiagnosed diabetes in a dental patient.

In conclusion, dental professionals need to have comprehensive knowledge of their patients’ diabetes: knowledge that the patient has diabetes is not sufficient to assess the effects of diabetes with respect to oral diseases and dental treatment. This need is
emphasised by the high and ever increasing number of patients with diabetes in Finland. On the other hand, the members of the team responsible for diabetes treatment should pay attention to dental care and guidance to dental treatment, especially in the case of adult patients with diabetes. Periodontal disease can be seen as a complication of diabetes and the importance of its treatment is comparable to the treatment of other diabetic complications. Finally, co-operation and consultation between all the members of the team responsible for the treatment of patients with diabetes is highly recommended.
8. References


Original papers