Some salivary factors in insulin-dependent diabetics

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The aim of the study was to compare salivary flow rate, salivary pH, buffer capacity of the saliva, salivary glucose content, and number of *Candida albicans*, lactobacilli, and *Streptococcus mutans* in the saliva in age- and sex-matched adult long- and short-duration insulin-dependent diabetics and non-diabetics. Ninety-four long-duration and 86 short-duration diabetics and 86 non-diabetics, aged 20–70 years, participated in the study. Paraffin-stimulated whole saliva was collected. Both long- and short-duration diabetics had a decreased salivary flow rate and an increased salivary glucose content compared with non-diabetics. However, the differences were small. There were no significant differences between the groups in salivary pH, buffer capacity, or bacterial counts. □ *Diabetes mellitus; salivary glucose; salivary microorganisms; salivary secretion*

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The importance of well-functioning salivary glands for oral health is well known. Impaired salivary flow rate, altered composition of the saliva, and increased oral microbial counts may increase the susceptibility to caries, periodontal disease, and oral mucosal lesions.

Diabetes mellitus has been associated with altered salivary secretion and mouth dryness. Some investigators have reported reduced salivary flow rate in diabetics (1–4). In other studies, no change in the salivary flow rate has been found either in resting or in stimulated whole saliva (5–7). In a study of diabetic boys, Faulconbridge et al. (8) found a lower salivary pH than in non-diabetics, whereas Tenovuo et al. (7) could not find any difference in salivary pH or in buffer pH between adult diabetics and non-diabetics. Kjellman (4), on the other hand, found a higher buffer pH in diabetics than in non-diabetics.

There are few studies on the composition of the saliva in diabetics. Englander et al. (9), Campbell (10), Mehrotra & Chawla (11), Kjellman (12), Knight & Fletcher (13), Faulconbridge et al. (8), and Harrison & Bowen (2) demonstrated increased salivary glucose content in diabetics compared with non-diabetics. Sharon et al. (6) could not find a corresponding increase of glucose in whole saliva. However, the glucose content in parotid saliva was increased in the diabetics. In other studies, increased salivary concentrations of calcium, potassium, IgG, IgA, and peroxidase have been demonstrated in diabetics (2, 5, 6, 14).

With regard to the presence of microorganisms in saliva, Tenovuo et al. (7) found no difference between diabetics and non-diabetics in colony-forming units of mutans streptococci and lactobacilli per milliliter of saliva. However, the proportion of mutans streptococci from the total cultivable aerobic flora was higher in the diabetics. Furthermore, infection by *Candida albicans* has been reported to be commoner in diabetics than in non-diabetics (13, 15–20).

Reported changes of the salivary glands in diabetics include asymptomatic parotid gland enlargement (21–23) and parotid gland basement membrane abnormalities (24).

The results of the few published clinical investigations of salivary factors in diabetics are in many respects contradictory, and comparison of the results is difficult, mainly due to vague or differing classification of the diabetes disease.

The aim of this investigation was to study some salivary factors in three well-defined

groups of adults, namely age- and sex-matched long- and short-duration diabetics and non-diabetics. The salivary factors studied were flow rate, salivary pH, buffer capacity of the saliva, salivary glucose content, and number of *C. albicans*, lactobacilli, and mutans streptococci in the saliva.

Materials and methods

The studied population comprised the 741 insulin-dependent diabetics, 20 to 70 years old, examined at the Department of Medicine at the Central Hospital in Jönköping, Sweden. They constituted all insulin-dependent diabetics living in the borough of Jönköping. A sample from this population was selected for a detailed examination of dental health. From each age class, comprising individuals born the same year, the man and the woman with the longest and shortest diabetes duration were selected. The group thus selected consisted of 194 diabetics. Of these 194 individuals, 180 took part in the examination, 94 (48 women and 46 men) with long and 86 (44 women and 42 men) with short diabetes duration. The average duration of the disease in the two groups was 28.9 ± 10.19 years and 5.2 ± 1.92 years, respectively (Table 1). In conjunction with the medical examination, the selected patients were invited by the physician to attend a dental examination.

The control group was selected from the County Council's register of persons residing in the borough of Jönköping and consisted of an age- and sex-matched random sample of 102 non-diabetics. All selected individuals received a written invitation, containing the same information as that given to the diabetics, to attend a dental examination. Of the 102 non-diabetics, 86 (49 women and 37 men) took part in the study (Table 1).

Fourteen diabetics (7.2%) and 16 non-diabetics (15.7%) did not participate in the study. A detailed description of the material and non-respondents has been given in a previous report (25).

All participants were instructed not to eat or smoke for at least 1 h before the salivary sampling. The sampling was carried out between 0800 h and 1130 h. After they had chewed paraffin for 1 min, the produced saliva was swallowed and the sampling started. Stimulated saliva was collected in a graduated glass cup, placed on ice, during 5 min or until at least 3 ml of saliva was obtained. After collection the glass cup was sealed with wax. The secretion rate was read immediately (in ml/min). Then 1 ml of the sample was transferred to VMGII transport medium (26) for culture of *C.* albicans, lactobacilli, and mutans streptococci. The same day, 0.1 ml of the VMG solution was transferred to Sabouraud's agar. After dilution of the VMG solution (1/10, 1/100, 1/1000), 0.1 ml of each dilution was transferred to Rogosa agar and Mitis Salivarius agar. The Sabouraud plates were incubated for 48 h at 37°C in a moist chamber. The Rogosa plates were incubated with 5% CO_2 at 37°C for 48 h. The Mitis Salivarius plates with bacitracin added were incubated anaerobically at 37°C for 48 h

Table 1. Number of individuals examined (20–70 years old) and diabetes duration. Means ($\bar{x}$)

	Dentate			Edentulous
	Total	Women	Men	Total
Diabetics, *n*				
Long duration ($\bar{x}$ = 28.9 years)	82	42	40	12 (12.8%)
Short duration ($\bar{x}$ = 5.2 years)	72	34	38	14 (16.3%)
Non-diabetics, *n*	77	43	34	9 (10.5%)

and then for an additional 24 h at room temperature. After that the number of colony-forming units (CFU) of lactobacilli and mutans streptococci was calculated. *C. albicans* was identified with a serum test, and the number of CFU was calculated. Salivary pH was determined electrometrically and buffer capacity by the method of Ericsson (27) but with exclusion of the air stream step.

Salivary glucose content was determined with an enzymatic colorimetric method (Sigma 510). Glucose is oxidized in the presence of the enzyme glucose oxidase and oxygen to gluconic acid and hydrogen peroxide. The hydrogen peroxide and *o*-dianisidine are then oxidized in the presence of the enzyme peroxidase to a brown end product (oxidized *o*-dianisidine). The intensity of the brown color measured at 450 nm is proportional to the original glucose concentration. Then 2.5 ml of glucose oxidase reagent was added to blank solution 1 (0.25 ml distilled water), to the standard solution (0.25 ml of 1.0 mg/100 ml glucose solution), and to the test solution (0.25 ml saliva), and 2.5 ml of 0.9% sodium chloride was added to blank solution 2 (0.25 ml saliva). The solutions were stored in the dark for 45 min and then centrifuged at 2000 *g* for 10 min, after which they were read in a spectrophotometer (Hitatchi) at 450 nm. The glucose content (mg/100 ml) was calculated as (absorption of salivary sample divided by absorption of standard solution minus absorption of blank solution 1) × standard concentration.

The absorption of the salivary sample was calculated as absorption of the test solution minus absorption of blank solution 2 minus absorption of blank solution 1.

Statistical methods

Student's *t* test for unpaired data was used to determine the significance of differences between two independent groups. Comparisons between more than two groups were made using analysis of variance (ANOVA). If the ANOVA rejected the multi-sample null hypothesis, a multiple comparison procedure (Newman–Keuls test) was used to detect where the differences were located. When frequencies in discrete categories constituted the research data, the chi-square test was used to determine the significance of differences between independent groups. Bacterial data were logarithmically transformed before statistical analysis to normalize the populations and equalize the variances. All statistical tests were two-tailed and at the 5% significance level. The data are presented as mean values and standard errors. Since the number of edentulous individuals was small, only dentate individuals have been included in the analysis.

Table 2. Salivary flow rate (ml/min). Means ($\bar{x}$), standard errors (SE), and Newman–Keuls test (NK)

	Total	Women	Men
Diabetics			
Long duration (A)			
$\bar{x}$	1.21	1.11	1.32
SE	0.07	0.08	0.11
Short duration (B)			
$\bar{x}$	1.18	1.05	1.29
SE	0.10	0.11	0.15
Non-diabetics (C)			
$\bar{x}$	1.71	1.52 —— * ——	1.95
SE	0.11	0.12	0.20
NK	A,B$\overset{*}{\neq}$C	A,B$\overset{*}{\neq}$C	A,B$\overset{*}{\neq}$C

* $p < 0.05$.

Results

Salivary flow rate

Both long- and short-duration diabetics had a significantly lower salivary flow rate than non-diabetics ($p < 0.05$) (Table 2). Of the non-diabetics women had a lower flow rate than men ($p < 0.05$) (Table 2). A flow rate lower than 0.7 ml/min was found in 17.2% of the long-duration and 16.2% of the short-duration diabetics and in 11.7% of the non-diabetics (Fig. 1).

pH

No significant difference was found between long- and short-duration diabetics and non-diabetics with regard to salivary pH ($\bar{x} = 7.22 \pm 0.17$, $\bar{x} = 7.31 \pm 0.16$, and $\bar{x} = 7.23 \pm 0.14$, respectively).

Buffer capacity

No significant difference in salivary buffer capacity was found between long- and short-duration diabetics and non-diabetics ($\bar{x} = 4.67 \pm 0.13$, $\bar{x} = 4.71 \pm 0.13$, and $\bar{x} = 4.79 \pm 0.13$, respectively).

Glucose content

Most individuals had a very low salivary glucose content (Fig. 2). Both long- and short-duration diabetics had an increased salivary glucose content compared with non-diabetics ($p < 0.05$) (Table 3). This increased glucose content in diabetics was found mainly in men.

Candida albicans, *lactobacilli and mutans streptococci*

No significant differences were found between long- and short-duration diabetics and non-diabetics with regard to the mean number of CFU of *C. albicans*, lactobacilli, and mutans streptococci (Table 4). The distribution of the subjects by CFU of these microorganisms is shown in Figs. 3, 4, and 5.

Discussion

The participants in this study constituted a well-defined group of diabetics divided in accordance with disease duration, age, and sex. The control group consisted of an age- and sex-matched random sample of non-dia-

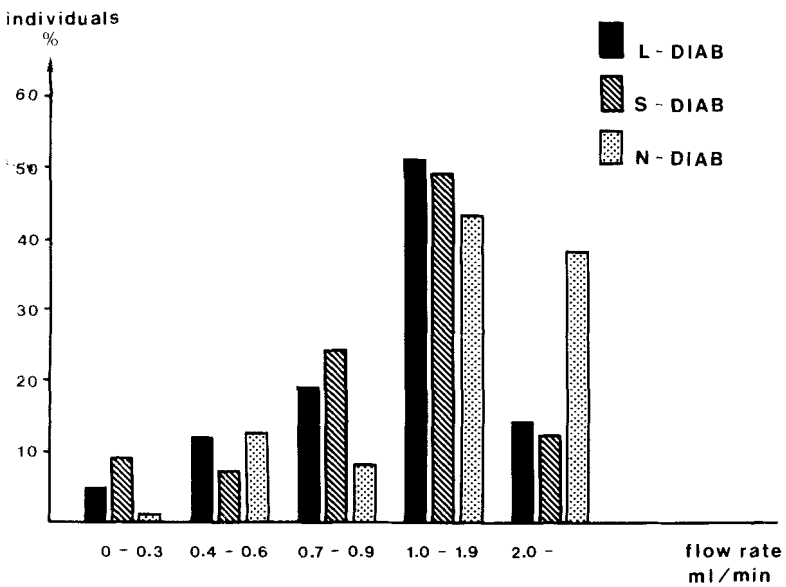

Fig. 1. Percentage distribution of the subjects in accordance with salivary flow rate (ml/min).

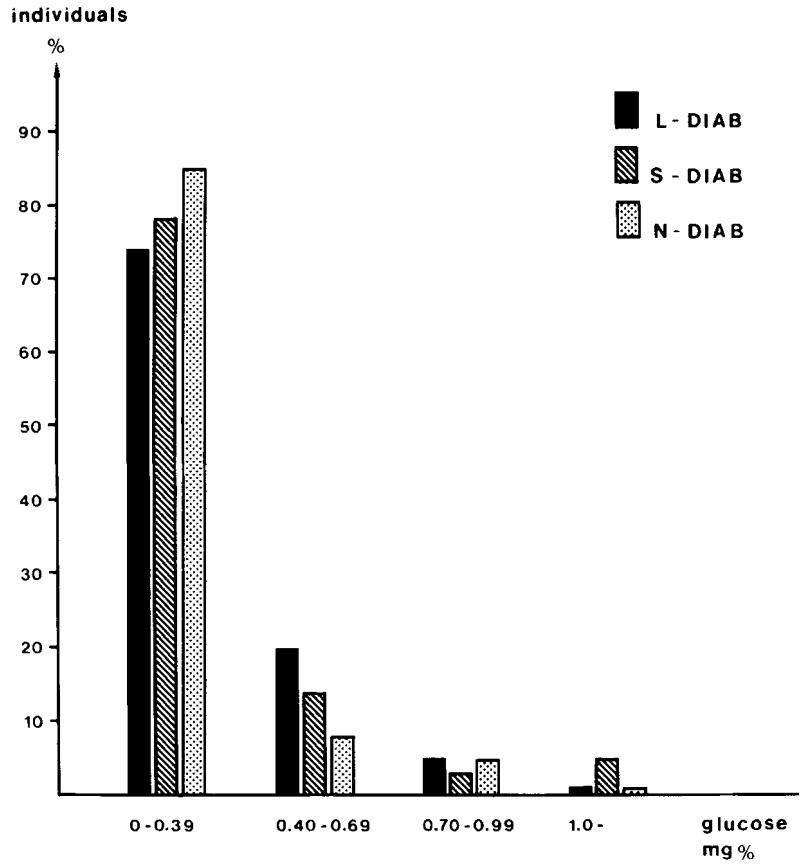

Fig. 2. Percentage distribution of the subjects in accordance with salivary glucose content (mg/100 ml).

betics from the same district. The non-response rate of 7.2% for diabetics and 15.7% for non-diabetics has been discussed in a previous report and was considered not to have any significant effects on the results (25). In previous studies the periodontal conditions (25), the number of teeth, the prevalence of caries and periapical lesions (28), and the dental care habits and knowledge of oral health (29) of these long- and short-duration insulin-dependent diabetics have been accounted for.

Long- and short-duration diabetics had a significantly lower stimulated salivary secretion rate than non-diabetics. However, the reduction in the flow rate was moderate, and all group mean values were within the limits of normal secretion rate. This is in accordance with the findings of Connor et al. (3), Kjellman (4), and Bánóczy et al. (1). Most diabetics with low salivary flow rate were found within the limits of 0.7 to 0.9 ml/min (Fig. 1). It is doubtful whether such a small reduction in salivary secretion has any major influence on the development of dental diseases. Among both diabetics and non-diabetics there were individuals with a markedly low salivary flow rate (< 0.7 ml/min), but these individuals were few.

Xerostomia among diabetics, particularly in cases of uncontrolled disease, has in the literature been explained by general dehydration (30). Asymptomatic parotid gland enlargement has been reported among diabetics (21–23), and this might indicate a direct influence of the diabetes disease on the salivary glands. Biopsy specimens from enlarged parotid glands have shown acinar hypertrophy, fatty infiltration, fibrosis, and glucogenic degeneration (22). On the other

Table 3. Glucose content (mg/100 ml). Means ($\bar{x}$), standard errors (SE), and Neuman–Keuls test (NK)

	Total	Women	Men
Diabetics			
Long duration (A)			
$\bar{x}$	0.33	0.32	0.34
SE	0.03	0.04	0.04
Short duration (B)			
$\bar{x}$	0.33	0.29	0.36
SE	0.04	0.05	0.06
Non-diabetic (C)			
$\bar{x}$	0.21	0.21	0.20
SE	0.03	0.04	0.03
NK	$A,B \overset{*}{\neq} C$		$A,B \overset{*}{\neq} C$

* $p < 0.05$.

hand, no reduced salivary secretion has been demonstrated in diabetics with asymptomatic parotid gland enlargement, which might indicate that parotid enlargement is a compensatory mechanism to combat reduced salivary flow rate. The above findings have been associated with newly discovered or uncontrolled diabetes, and they are therefore probably not applicable to the diabetics in this study. The diabetes disease is accompanied in time, even in adequately treated cases, by various complications such as micro- and macro-angiopathies, leading to circulatory disturbances and hypertension. As a consequence, a considerable proportion of diabetics are treated with antihypertensive drugs (31). Some of these drugs have a negative influence on salivary secretion (32) and therefore constitute one probable cause of the reduced salivary flow rate. About 30% of the diabetics actually reported a feeling of mouth dryness (29). This feeling of mouth dryness might be explained by reduced resting saliva or reduced secretion from the minor salivary glands as a consequence of these antihypertensive drugs.

Table 4. Number of colony-forming units of *Candida albicans*, lactobacilli, and mutans streptococci per 1 ml saliva (logarithms). Means ($\bar{x}$) and standard errors (SE)

	Candida albicans	Lacto-bacilli	Mutans streptococci
Diabetics			
Long duration			
$\bar{x}$	1.64	4.26	5.01
SE	0.15	0.15	0.13
Short duration			
$\bar{x}$	1.73	4.53	5.03
SE	0.18	0.12	0.13
Non-diabetics			
$\bar{x}$	1.48	4.05	4.86
SE	0.16	0.18	0.13

For determination of buffer capacity, a modification of Ericsson's method (27) was used, which might explain the somewhat lower values compared to those found in some other studies. Kjellman (4) reported a higher buffer capacity in diabetics than in non-diabetics and suggested that the diet recommended for diabetics may have an effect on the buffering capacity. These results could not be verified in the present study, in which no such differences were found. The result of the present study is also in agreement with results from Tenovuo et al. (7).

The method used for the assay of glucose in saliva is well established for studies of glucose in biologic fluids, such as blood, urine, and cerebrospinal fluid (33–36). The results showed that long- and short-duration diabetics had a higher salivary glucose content than non-diabetics. This finding is in

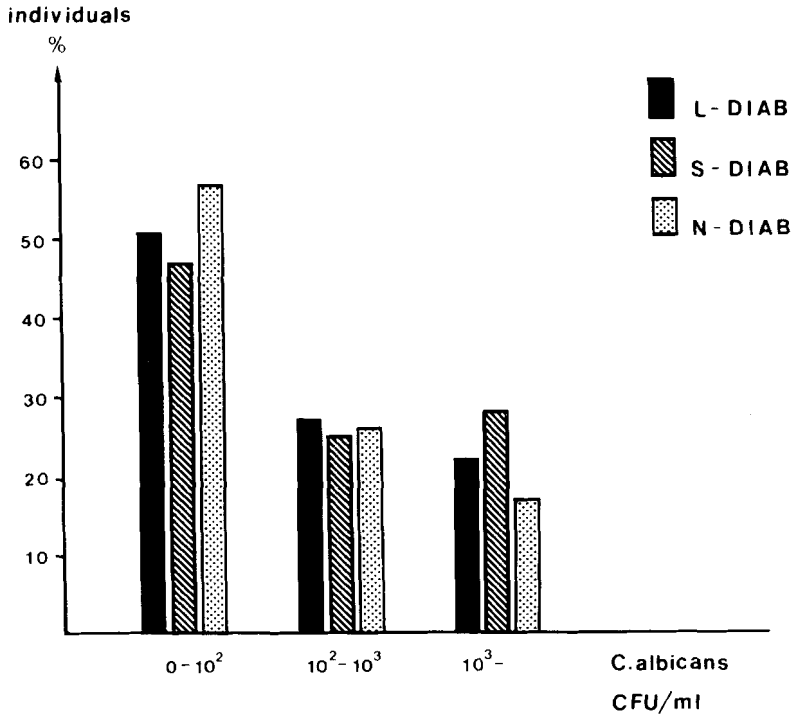

Fig. 3. Percentage distribution of the subjects in accordance with CFU of *Candida albicans* (CFU/ml saliva).

agreement with the results of Englander et al. (9), Campbell (10), Mehrotra & Chawla (11), Kjellman (12), Knight & Fletcher (13), Faulconbridge et al. (8), and Harrison & Bowen (2). The differences between the groups were small, however. Although membrane abnormalities of the parotid gland have been reported in diabetics (24),

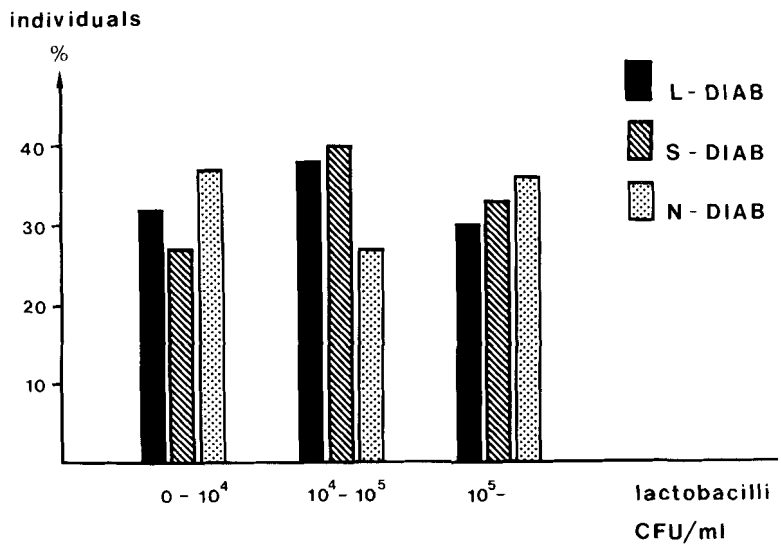

Fig. 4. Percentage distribution of the subjects in accordance with CFU of lactobacilli (CFU/ml saliva).

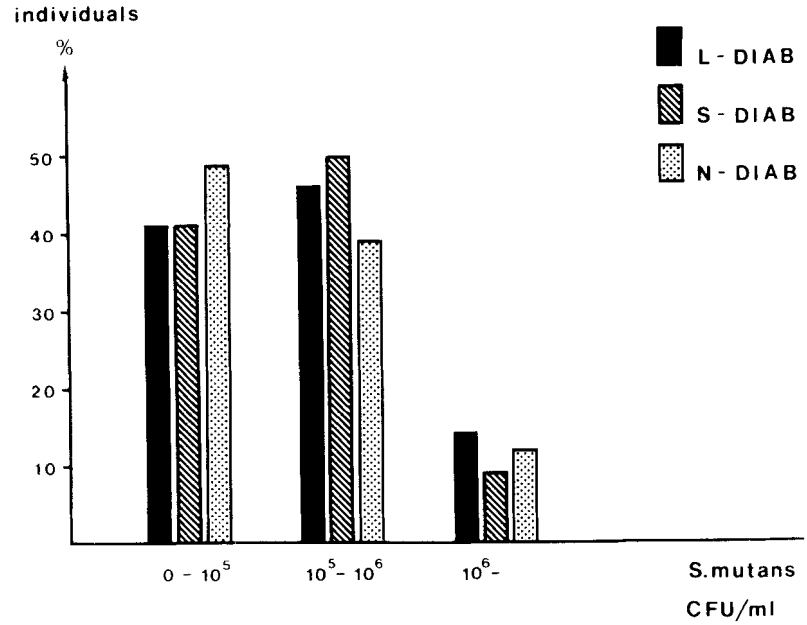

Fig. 5. Percentage distribution of the subjects in accordance with CFU of mutans streptococci (CFU/ml saliva).

there might also be other reasons for the increased salivary glucose content than changes in the glandular tissues. Increased glucose content in the gingival fluid has also been reported in diabetics (12, 37). It is therefore possible that part of the registered salivary glucose content originates from the gingival fluid. This hypothesis is supported by the finding that the present diabetics had more inflamed gingival units than non-diabetics (25) and hence larger amounts of gingival fluid. In addition, an increased salivary glucose content (Table 3) and more gingival inflammation were found in male diabetics compared with female diabetics (25).

There were no statistically significant differences between long- and short-duration diabetics in the mean number of CFU of *C. albicans*, lactobacilli, and mutans streptococci. Nor could any differences be demonstrated in the total number of decayed and filled tooth surfaces between the diabetics and the non-diabetics (28). It has also been shown that the compliance with dietary advice was poor among the diabetics; for example, they had frequent food intake between principal meals and did not always consistently omit sucrose (29). This finding, combined with the somewhat increased salivary glucose content, might explain the similarity in bacterial counts among the diabetics and non-diabetics. Many authors have reported an increased disposition for candidal infections among diabetics (13, 15–20). Access to salivary glucose has then been considered to promote candidal growth (13). It is possible, however, that the glucose content found in this study is not sufficient to promote growth of *C. albicans*.

In this study small but statistically significant differences were found in salivary flow rate and glucose content between long- and short-duration diabetics and non-diabetics. However, since the differences were small, their clinical importance may be questioned. However, both among the diabetics and among the non-diabetics there was a small group of individuals with such salivary qualities that they must be considered at risk of developing dental diseases.

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