

Flow rate and organic constituents of whole saliva in insulin-dependent diabetic children and adolescents

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Abstract

The purpose of this investigation was to examine the association between control of diabetes and composition of whole saliva in insulin-dependent diabetic children. Thirty diabetic children and adolescents ages 4-19 years were matched by age, sex, and race to 30 healthy children. Metabolic control was determined for each diabetic child by percentage of glycosylated hemoglobin, HbA_{1c}. Fourteen children were judged to be well controlled (HbA_{1c} ≤ 10%); 16 were poorly controlled (HbA_{1c} ≥ 10%). Whole stimulated saliva was collected from each subject and flow rate was evaluated. Amount of glucose, total protein, IgG, IgA, lysozyme, peroxidase, and lactoferrin were analyzed for each subject. Analyses of covariance were performed to determine the relationship between subject group and factors in saliva.

Well controlled and poorly controlled diabetic children demonstrated increased output of glucose to saliva. Poorly controlled diabetics had decreased flow of saliva and a significantly increased concentration of lactoferrin in whole saliva. The observed changes in composition of saliva in diabetic subjects suggested that poor control of diabetes may affect functioning of salivary glands.

The complications of insulin-dependent diabetes affect most tissues of the body, but relatively few investigators have examined the complications of diabetes that may be manifested in salivary glands. Investigators have reported an altered flow and composition of saliva in adult diabetics¹ and several studies have shown that experimental diabetes in laboratory animals causes structural and functional changes in salivary glands.² These documented effects of diabetes on salivary glands in adults and laboratory animals demonstrate a need for investigation of alterations in salivary glands associated with diabetes in children. Variation in concentration of constituents of saliva like glucose and proteins will

indicate an effect of diabetes on functioning of salivary glands.

Diabetic children offer a unique opportunity to study the association between diabetes and salivary gland function because children are less likely to suffer from other systemic disorders and are free of the degenerative processes associated with aging. The difficulty in maintaining metabolic control in diabetic children and adolescents, in addition to the severity of the disease, reinforces the need for examination of salivary gland function in diabetic children.

This investigation was undertaken to study composition of whole saliva and functioning of salivary glands in insulin-dependent diabetic children.

Materials and Methods

Subject Population

Details concerning the group of insulin-dependent diabetic children and adolescents and healthy control subjects were outlined in a previous article (Harrison and Bowen 1987, see preceding article).

Collection of Saliva

Whole saliva was collected from each subject at least one hour after their having eaten between the hours of 9 a.m. and noon. Each subject chewed a clean, 2 x 2-inch square of Parafilm[®] and then expectorated into a tared, sterile 50-ml beaker which was supported on crushed ice in a Styrofoam[™] collection vessel. After the time required for collection was recorded, beaker and saliva were weighed to the nearest 0.1 mg and flow rate was calculated. Saliva was centrifuged at 12,500 xg for 20 min at 5° C. Supernatant was dispensed and stored at -5° C until required for laboratory assays.

Glucose

Glucose in saliva was assayed on the day of collection using the glucose oxidase method (Keston 1956).

¹ Conner et al. 1970; Kjellman 1970a; Mandel and Baurmash 1978; Marder et al. 1975.

² Anderson and Johnson 1981; Anderson and Shapiro 1980; Muratsu

[®] American Can Co; Neenah, WI.

The assay was linear in the range of glucose concentrations in saliva.

Total Protein

A modification of the Lowry method (Lowry et al. 1951) was used to measure total protein in saliva (Ohnishi and Barr 1978).

IgG and IgA

Single radial immunodiffusion was used to quantify IgG and IgA (Mancini et al. 1965). Standards used were human colostrum IgA and human IgG.^b Antisera were α -chain specific rabbit antiserum to human IgA and γ -chain specific rabbit antiserum to human IgG.^c

Lysozyme (E.C.3.2.1.17)

Lysozyme activity was assayed on the day of collection using an agar plate (lyso-plate) technique (Osserman and Lawlor 1966). Standard dilutions in 0.0066 mol/L phosphate buffer of lysozyme from human milk (100,000 units per mg desiccated lysozyme^e), were included with each plate of test samples.

Peroxidase (E.C.1.11.1.7)

A spectrophotometric technique which measured the rate of oxidation of iodide to tri-iodide was used to assay peroxidase activity (Hosoya and Morrison 1967)

Lactoferrin

An ELISA assay using a double antibody sandwich technique was used to determine amount of lactoferrin in saliva (DiPaola and Mandel 1980).

Lactoferrin standards from human milk^c were used to establish a standard curve. IgG fraction rabbit antihuman lactoferrin was the source of the antibody to lactoferrin.^d

Data Management

Coefficient of variation (CV) was determined for each assay. The CV was less than 11% for each of the assays.

Least squares means, adjusted for differences in age and sex among the groups of subjects, were computed for salivary factors for each group. Subject groups were poorly controlled diabetics, well-controlled diabetics, and healthy control subjects. Analysis of covariance was performed to determine the relationship between subject group and factors in saliva. Newman-Keuls nonsignificant groupings were determined if the subject group was found to be significantly ($P \leq 0.05$) associated with a constituent of saliva.

^b Sigma Chemical Co; St. Louis, MO.

^c Calbiochem-Behring Corp; San Diego, CA.

^d Cappel Scientific Division - Cooper Biomedical; Malvern, PA.

Results

Flow of Saliva

Diabetics in poor control had a significantly lower flow of whole saliva than well-controlled diabetics and healthy subjects (Tables 1, facing page, and 2, below).

Organic Constituents of Saliva

Values for salivary immunoglobulins, innate defense proteins, total protein, and glucose were expressed in three ways: (a) amount (mg, μ g) per ml of saliva, or activity per unit volume (mean values of all proteins assayed for three subject groups are given in Table 1); (b) amount produced per min (output) which indicated availability to the mouth of each salivary constituent and adjusted for the concentrating or diluting effect of differing flow rates (units/min); (c) percentage or proportion of total protein in saliva.

Glucose concentration in saliva was significantly higher in poorly controlled diabetics compared with well-controlled diabetics and healthy subjects (Tables 1, 2). Output of glucose in both groups of diabetics was significantly higher than that of the healthy children (Table 2).

The mean IgA concentration in saliva of poorly controlled diabetics was significantly higher than that

TABLE 2. Differences Associated With Subject Group in Salivary Values

Salivary Values	P Value	Newman-Keuls Non-significant Groupings [†]
Rate of flow	0.02	[1, 2][0]
Glucose concentration	0.003***	[0][1, 2]
Output	0.0002****	[0, 1][2]
Total protein concentration	0.15	[0, 2, 1]
Output	0.67	[1, 2, 0]
IgA concentration	0.04*	[0, 1][1, 2]
Per cent total protein	0.54	[2, 1, 0]
Output	0.67	[1, 2, 0]
IgG concentration	0.21	[0, 2, 1]
Per cent total protein	0.24	[2, 0, 1]
Output	0.52	[2, 0, 1]
Lysozyme activity	0.70	[0, 2, 1]
Ratio of activity to total protein	0.91	[0, 1, 2]
Output	0.77	[1, 0, 2]
Peroxidase activity	0.71	[0, 1, 2]
Ratio of activity to total protein	0.44	[1, 2, 0]
Output	0.03*	[1, 2][2, 0]
Lactoferrin concentration	0.009**	[0][2, 1]
Per cent total protein	0.03*	[0][2, 1]
Output	0.40	[0, 2, 1]

Statistical analysis: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.005$.

[†] Newman-Keuls groupings ranked beginning with subject group with highest mean value; groups in separate brackets are significantly different ($P \leq 0.05$), groups placed together in brackets not significantly different from each other. Group 0 = poorly controlled diabetics; group 1 = well-controlled diabetics; group 2 = healthy subjects.

of healthy subjects; the IgA concentration of the well controlled diabetic group was not significantly different from either of the other groups (Table 2). IgG concentration was highest in the poorly controlled diabetic group, but was not significantly different from the other two groups. Output of IgA or IgG and percentage of total protein contributed by either IgA or IgG were independent of subject group.

Lysozyme activity, output and ratio of lysozyme activity to total protein, was unrelated to presence of diabetes (Table 2).

Salivary peroxidase activity and ratio of peroxidase activity to total protein were independent of subject group. However, output of salivary peroxidase was significantly different among the three groups (Table 2). The peroxidase output in the poorly controlled diabetic group was significantly lower than the well controlled diabetic group; peroxidase output was not significantly different in the healthy subjects compared to either diabetic group.

Lactoferrin concentration in saliva and percentage of total protein contributed by lactoferrin were highest in diabetics in poor control (Table 2). Output of lactoferrin also was increased in poorly controlled diabetics, but was not significantly different from the other groups.

Concentration and output of total salivary protein were independent of the presence of diabetes.

Discussion

The significantly reduced flow of whole saliva observed in the poorly controlled subjects in the present study confirmed the results of previous investigators (Kjellman 1970a; Conner et al. 1970). Several factors may have caused decreased flow of saliva in diabetics in poor control. Glucosuria caused by even mild hyperglycemia leads to elevated fluid loss and dehydration which may diminish salivary gland output. In addition, decreased salivary flow may have resulted from a direct effect of diabetes on salivary gland structures. If structural changes in human salivary glands are similar to those reported in salivary glands of diabetic animals (Hand and Weiss 1984), then poorly controlled diabetes may lead to a diminished flow of saliva.

The present study confirmed the results of previous investigators who reported elevated glucose concentrations in whole and duct saliva of diabetics.³ A significantly increased availability of glucose to the mouth was observed for both well controlled and poorly con-

TABLE 1. Salivary Values for all Subjects*

Salivary Value	Units	Diabetics				Healthy Subjects	
		Poor Control		Good Control			
Rate of flow	ml/min	0.7 ± 0.2		1.2 ± 0.2		1.1 ± 0.1	
Glucose	μg/ml	22.0	7.0	11.0	2.0	5.0	1.0
Total protein	mg/100 ml	140.5	11.5	117.5	11.3	118.0	6.0
IgA	μg/ml	189.3	24.3	152.9	16.1	131.3	10.0
IgG	μg/ml	48.3	6.5	32.4	6.0	41.5	4.3
Lysozyme	units/ml	3063.0	520.0	2404.0	581.0	2788.0	401.0
Peroxidase	μmoles/min/ml	6.2	0.8	5.7	0.6	5.5	0.4
Lactoferrin	μg/ml	3.4 ± 0.8		1.2 ± 0.3		1.5 ± 0.3	

* Least square mean ± SD.

trolled diabetic children. Glucose is a small molecule which easily diffuses through semipermeable membranes (Kjellman 1970b). Thus, large amounts of glucose become available to saliva when blood glucose levels are elevated, as they are in diabetes.

Factors other than elevated blood glucose may have led to elevated salivary glucose. Increased glucose in parotid saliva also has been reported in parotitis as a result of breakdown of normal salivary-blood barriers (Mandel and Baurmash 1980). Alterations in permeability occurring as a result of basement membrane changes in diabetes may be an additional explanation for increased concentrations of glucose in saliva.

Concentration of IgA was significantly increased in saliva of poorly controlled diabetics. This increase was probably a result of unchanged production of IgA by plasma cells, but significantly reduced output of saliva (i.e., a concentrating effect). The amount of IgA available to the mouth in a fixed unit of time, the output of IgA, was similar for all subject groups regardless of flow of saliva, confirming the authors' hypothesis that increased IgA concentration resulted from a decreased flow of saliva in poorly controlled diabetic subjects. Because of no significant reduction in any IgA values, transport of IgA by secretory component into saliva was also apparently not impaired in diabetic subjects.

The amount of peroxidase, an enzyme primarily produced by acinar cells, may be diminished if decreased salivary flow in poorly controlled diabetic children resulted from malfunctioning acinar cells. In this study, activity of salivary peroxidase was independent of subject group. However, output of salivary peroxidase was significantly lower in the poorly controlled diabetic group than the well-controlled group, an observation consistent with decreased flow of saliva in the poorly controlled diabetic subjects. This association between poorly controlled diabetes and decreased output of saliva suggested that synthesis of peroxidase by acinar cells also may be impaired.

Lysozyme and lactoferrin are produced primarily by intercalated duct cells of salivary glands (Korsrud and Brandtzaeg 1982), but lysozyme values, in contrast to lactoferrin, were independent of subject group. Sali-

³ Forbat et al. 1981; Kjellman 1970b; Tenovuot et al. 1985.

vary mucins in whole saliva form a complex with lysozyme, thereby reducing lysozyme activity (Virella and Goodswaard 1978). The absence of a relationship between diabetes and lysozyme in saliva partially may be explained by the authors' assay technique which did not dissociate lysozyme-mucin complexes. Another explanation of the diverse result observed with lysozyme compared with lactoferrin is that diabetes can have selective effects on secretion of different proteins originating from the same type of cells. Synthesis or transport of lysozyme by intercalated duct cells may be influenced differently from lactoferrin by poorly controlled diabetes.

Results of the assay of lactoferrin in saliva provided good evidence that poorly controlled diabetes affected salivary gland cells. Concentration of lactoferrin was significantly increased to such a high degree that mechanisms in addition to the concentrating effect of decreased flow may have led to this increase. Results of a previous study involving diabetic hamsters suggested that increased ratio of lactoferrin to total protein in saliva was a direct result of diabetes (Muratsu and Morioka 1985).

Increased lactoferrin in whole saliva of the poorly controlled diabetics, the group which had increased periodontal disease, also may have arisen from infiltrating neutrophils (PMNs). Lactoferrin from PMN granules accounts for about 10% of the lactoferrin in mastitis (Tabak et al. 1978). A similar situation probably prevails in salivary glands, suggesting a minimal contribution to saliva by lactoferrin from PMNs.

Diabetes may be poorly controlled for reasons such as poor dietary habits, stress, inadequate home blood glucose monitoring, or failure to administer necessary insulin. One or all of these reasons may have contributed to poor control in the diabetics in the present study which make it difficult to attribute a definite explanation to the observed changes in functioning of salivary glands. Altered functioning of salivary glands may reflect a response by particular cells to fluctuating levels of circulating insulin or a response to the effect of diabetes on neural control of salivary secretion. Changes in salivary flow and composition may, in part, result from leakage of macromolecules through damaged basement membranes (Hand and Weiss 1984) which has been reported in epithelial, vascular, and neural components of parotid glands of diabetic rats.

Further study is warranted to clarify the relationship between diabetes and protein synthesis by salivary glands. A longitudinal study monitoring salivary proteins in diabetic children from time of diagnosis will permit assessment of salivary gland function as diabetes is brought under control.

Conclusions

1. Poorly controlled diabetics had a decreased flow of saliva.
2. Well controlled and poorly controlled diabetic children demonstrated increased output of glucose to saliva.
3. Diabetic children in poor control displayed a significantly increased concentration of lactoferrin in whole saliva. The increase in concentration of lactoferrin and percentage of total protein contributed by lactoferrin and the observed decrease in flow of saliva suggested that poorly controlled diabetes may affect functioning of salivary glands.

The authors thank Mrs. Norma Strathearn for typing the manuscript and Dr. Dennis Mangan, Kurt Schilling, Dr. Ed Thibodeau, and Dorothy Young for advice on technical procedures. They also acknowledge the cooperation of Dr. Ken McCormick and the staff and patients of Strong Memorial Hospital Pediatric Diabetic Outpatients Clinic, Rochester, New York, and Mark Espeland who completed the statistical analysis of the data.

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Smokeless tobacco linked to gum disease

Periodontal ligament cells, responsible for the attachment of teeth to bone, have been found to be severely limited or even destroyed when they are exposed to high concentrations of smokeless tobacco extract.

Investigators at the University of Louisville Center for Dental Research exposed periodontal ligament cells obtained from extracted wisdom teeth to different amounts of smokeless tobacco extract, equivalent to moderate to heavy daily snuff usage. This exposure caused the disintegration of the periodontal ligament cells, which eventually in humans can lead to tooth loss, as attachment to the underlying bone is weakened.

Based on these findings, researchers at the Center concluded that using smokeless tobacco may lead to an increased risk of periodontal disease.

According to recent estimates, about 10 million Americans use smokeless tobacco, with 3 million of these users under 21 years of age. There has been evidence associating smokeless tobacco use with localized gingival recession, especially where the tobacco is habitually placed.