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Flow rate, amylase activity, and protein and sialic acid concentrations of saliva from children aged 18, 30 and 42 months attending a baby clinic

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Abstract

Whole-saliva samples were collected under slight suction from children enrolled in a dental-care programme at a baby clinic and analysed according to sex. The children were allocated to three age groups: 18, 30 and 42 months. No distinct sex difference was found for flow rate; there was a statistically non-significant tendency for an increased rate in females. In the males the flow rates of the 30- and 42-month groups were higher than those of the 18-month group; a similar result was obtained when findings for both sexes were combined. There was no difference in salivary protein concentration between the sexes or the age groups. Amylase activity increased significantly in males from 18 to 30 months of age. Sialic acid concentration (μmol/mg protein) showed a sex difference for the 18-month group; it was 38% higher in males than in females, and 48% higher than in the males of 42 months. This trend toward a reduction in sialic acid concentration with age suggests a reduced synthesis and secretion of glycoproteins. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Children; Saliva; Amylase activity; Sialic acid content

1. Introduction

Human salivary glands are completed morphologically in utero and their growth in childhood continues by proliferation of well-differentiated cells (Redman, 1987). Salivary secretions are important for oral health, accomplishing mechanical cleansing and protective functions through a number of physiological and biochemical mechanisms.

Salivary amylase may play an important part in the colonisation and metabolism of streptococci, leading to the formation of dental plaque and caries. It is a constituent of the acquired pellicle (Orstavik and Kraus, 1973; Al-Hashimi and Levine, 1989) and could, therefore, be available to act as a receptor for the adhesion of micro-organisms to tooth surfaces (Scanniepo et al., 1995). A feature of amylase is its ability to bind to bacterial surfaces and to hydrolyse starch, giving rise to products that are transformed into acids (Scanniepo et al., 1990). Also, the maltose and maltodextrins produced by amylase may be transformed to polysaccharides known as glucans by a glucosyltransferase located in the outer surface of mutans streptococci (Vacca-Smith et al., 1996). Amylase is produced primarily by the parotid gland and its activity has been proposed as a marker for parotid saliva (Sas and Dawes, 1997).

There are reports of low salivary amylase activity in infants 3–15 days old (Bellavia et al., 1979) and 1 year old (Ben-Aryeh et al., 1984). On the other hand, Rossiter et al. (1974) reported adult levels of salivary amylase activity in infants 6–12 months old. According to Collares et al. (1979), the salivary amylase activity of a group of 10 children was low at birth, increased to a maximum between 90 and 180 days and then decreased up to 12 months of age. On the other hand, Ben-Aryeh et al. (1990) found a significant ascending linear correlation with age for amylase activity from infants aged 7–11 months to adults up 25–63 years old.

Sialic acid is an important structural component of glycoproteins (Leach, 1963), playing a part in enhancing bacterial agglutination (Levine et al., 1978). Our purpose now

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was to examine salivary variables such as flow rate, amylase activity, and protein and sialic acid concentration in the saliva of children (18, 30 and 42 months of age) enrolled in a dental-care programme at a baby clinic.

2. Materials and methods

2.1. Participants

These were randomly selected from patients enrolled in a dental-care programme at the baby clinic of the State University of Londrina, at the age of 18, 30 and 42 months. The patients attending this clinic receive dental care (educative and preventative) from 1 year of age upwards. The children were divided into three age groups: (1) 32 infants, 18 months old (16 boys, 16 girls); (2) 31 toddlers, 30 months old (15 boys, 16 girls); (3) 31 aged 42 months (16 boys, 15 girls). The criteria for inclusion were that the patients should (a) be free of systemic disease and (b) should not have consumed any medication for at least 15 days preceding the saliva collection.

The research ethical committee of the Health Sciences Center, State University of Londrina, approved the study protocol. Parents/carers were given verbal and written information on the study's aim, after which written consent to their child's participation was obtained.

2.2. Collection of saliva

Those responsible for each child were instructed not to give him or her food or drink for 2 h before the saliva collection. To control for circadian rhythms, whole-saliva samples were collected between 9 and 11 a.m., by slight suction through a soft plastic catheter. During the collection, which was done in a well-ventilated and well-lit room, children aged 18 and 30 months were held by their mothers/carers (who were comfortably seated) and kept calm. The 42-month-old children were themselves seated. No stimulation was used, although the presence of the soft plastic catheter might have slightly stimulated the salivary flow. Saliva collected during the first 10 s was discarded; saliva was then collected for 2 min, so that the initial flow rate could be calculated. After this period the sampling continued until 5 ml of saliva had been collected. If a child was not able to rest, or cried during sampling, their samples were excluded. Immediately after collection the sample was frozen in dry ice, transported to the laboratory of the Oral Biology Research Center, São Paulo, and kept at −80 °C until analysed.

2.3. Analysis

The saliva was first thawed and centrifuged at 27,750 × *g* for 20 min in a refrigerated centrifuge. The supernatant was used for the analysis. Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard. Sialic acid was determined by the method of Warren (1959), after hydrolysis with 0.05 M sulphuric acid solution at 80 °C for 1 h. Amylase activity was determined by the method described by Fischer and Stein (1961), as modified by Bellavia et al. (1979), using a Beckman DB-G spectrophotometer. One enzyme unit is defined as the amount that catalyses the formation of 1 μmol of maltose under the specified conditions.

2.4. Statistical analysis

Data are presented as mean ± S.D. The Student's *t*-test was used to determine differences between the mean of each sex group of the same age and between each group of the same sex at different ages. The level of significance was taken as $P < 0.05$.

3. Results

The results of this investigation are expressed in Tables 1 and 2. Table 1 shows that the initial flow rates for male children aged 42 and 30 months were higher than those for male children aged 18 months. In the female group no such difference was observed. On combining these results for both sexes, the 42- and 30-month groups were found to have faster flow rates than the 18-month group. No differences between males and females were observed in any group. No difference was observed for protein concentration with either sex or age (Table 1).

Table 2 shows that amylase activity increased from 18 to 30 months of age in males. Although for females the mean amylase at 30 months was higher than at 18 months, this difference was not statistically significant. On combining the results for both sexes there was a statistically significant difference between 30 and 18 months of age. The value obtained for sialic acid in the male group of 18 months was,

Table 1
Flow rate and protein concentration of saliva from children attending a baby clinic, according to age and sex

Age (months)	Sex	*n*	Flow rate[a] (ml/min)	Protein[a] (mg/ml)
18	F	16	0.48 ± 0.21	1.01 ± 0.52
	M	16	0.47 ± 0.25 ab	0.93 ± 0.32
	F + M	32	0.47 ± 0.22 cd	0.97 ± 0.43
30	F	16	0.53 ± 0.27	0.88 ± 0.32
	M	15	0.73 ± 0.34 a	0.93 ± 0.23
	F + M	31	0.63 ± 0.32 c	0.91 ± 0.27
42	F	15	0.61 ± 0.30	0.98 ± 0.20
	M	16	0.71 ± 0.27 b	1.02 ± 0.48
	F + M	31	0.66 ± 0.28 d	1.00 ± 0.37

The same letter indicates statistical difference when compared with each other by Student's *t*-test ($P < 0.05$); *n*: the number of children in each group; F: female; M: male.

[a] Mean ± S.D.

Table 2
Amylase activity and sialic acid concentration of saliva from children attending a baby clinic, according to sex and age

Age (months)	Sex	*n*	Amylase[a] (μmol maltose/ mg protein)	Sialic acid[a] (μmol/ mg protein)
18	F	16	9.45 ± 4.37	0.211 ± 0.077 a
	M	16	9.47 ± 3.35 a	0.292 ± 0.132 ab
	F + M	32	9.47 ± 3.83 b	0.250 ± 0.114 c
30	F	16	12.02 ± 5.68	0.231 ± 0.160
	M	15	12.03 ± 2.89 a	0.217 ± 0.130
	F + M	31	12.01 ± 4.47 b	0.227 ± 0.148
42	F	15	9.98 ± 1.78	0.181 ± 0.072
	M	16	10.18 ± 3.28	0.197 ± 0.083 b
	F + M	31	10.08 ± 2.62	0.189 ± 0.077 c

The same letter indicates statistical difference when compared with each other by Student's *t*-test ($P < 0.05$); *n*: the number of children in each group; F: female; M: male.
[a] Mean ± S.D.

approximately 38% higher than that in the female group and 48% higher than that found for the 42-month male group. There was a tendency for the sialic acid concentration to decrease with age in both sexes. This becomes clear when the results from both sexes were combined: the sialic acid content decreased by 32% between 18 and 42 months of age.

4. Discussion

During infancy, salivary composition undergoes changes that reflect the development and maturation of the salivary glands (Ben-Aryeh et al., 1990). Later in this phase of life, changes such as the eruption of the primary teeth, the introduction of solid food and colonisation by a cariogenic microflora occur (Carlsson et al., 1975; Catalanotto et al., 1975; Berkowitz et al., 1981; Van Houte et al., 1982; Alaluusua and Renkonen, 1983; Köhler et al., 1988; Karn et al., 1998; Radford et al., 2000). Many studies have shown sex differences in salivary flow rate, although not always to the level of statistical significance (Heintze et al., 1983; Österberg et al., 1984; Heft and Baum, 1984; Pedersen et al., 1985). Higher salivary flow rates in boys than girls have also been reported in children and adolescents (Andersson, 1972; Andersson et al., 1974; Klock and Krasse, 1977; Crossner, 1984; Kedjarune et al., 1997).

Using standardised conditions for saliva collection, and the same suction device for all participants, we calculated the initial salivary flow rate. We used no stimulation for saliva collection, although the presence of the soft plastic catheter might have provided a slight stimulus. No sex differences in the initial flow rates were observed in any age group. For females there was a tendency for flow rate to increase with age, although this was not statistically significant. On the other hand, in the males this difference was significant. The groups aged 30 and 42 months had higher initial flow rates than those at 18 months. When data for both sexes were combined, initial flow rates were found to increase with age. The 42- and 30-month groups had higher initial flow rates than the 18-month group.

Working with children during the first year of life, Collares et al. (1979) found that their salivary flow rates were higher between 90 and 180 days of age, a period preceding the eruption of the primary teeth. At 1 year of age, the flow rates were about the same of those of children 20–36 months old but lower than those of adults. The values, expressed as confidence intervals, were from 0.26 to 0.55 ml/min for children aged 180 days and 0.28 to 0.40 ml/min for children aged 20–36 months. Andersson et al. (1974) report flow rates of 0.39 ± 0.21 ml/min for 10 years old and 0.39 ± 0.22 ml/min for 13 years old, while Negoro et al. (2000) report 0.68 ml/min for children aged 4.5–5.5 years. Our values for the initial flow rate varied from 0.47 to 0.73 ml/min and no sex differences were noted. Despite the presence of a soft catheter and slight suction, these values are of the same magnitude as those of Negoro et al. (2000) and agree with other reports of increased salivary flow rate with age, but no sex difference.

Although the Lowry method for protein determination (Lowry et al., 1951) has been used in all studies thus far, the reported mean protein concentrations in children's saliva show some variation. Tenovuo et al. (1986) demonstrated a behavioural effect on the composition of saliva: a significantly higher protein concentration in the saliva of crying (1.89 mg/ml) than non-crying children (1.13 mg/ml). Bellavia et al. (1979) report a protein concentration of 1.9 mg/ml for 3- to 15-day-old infants; Ben-Aryeh et al. (1984) found concentrations varying from 3.60 mg/ml for children aged 2–3 months to 1.19 mg/ml for children aged 7–12 months; Hyyppä et al. (1989) observed concentrations varying from 0.71 to 0.78 mg/ml in children aged 2–19 months. We observed no significant difference in the protein concentration in relation to age or sex in our participants. However, our results, varying from 0.88 to 1.02 mg/ml for children aged from 18 to 42 months, are compatible with some of those previously reported using the same Lowry method. The differences found in protein concentration might be due to geographic location, the nutritional habits of the individuals and the method of saliva collection used.

Amylase activity is influenced by dietary consistency and water content (Mazengo et al., 1994; Kurahashi and Inomata, 1999). Amylase may be present in heterotypic complexes with mucin MG1, and these complexes have the potential to enhance or diminish the biological properties of the individual components (Iontcheva et al., 1997). The activity of this enzyme varies considerably in infants, and it increases rapidly from low values at birth to, approximately two-thirds of adult values by 3 months (Sevenhuysen et al., 1984), reaching adult values by 12 months (Rossiter et al., 1974; Collares et al., 1979). The specific activity of amylase

in adult saliva is nine times higher than in the new-born (Bellavia et al., 1979); they also found electrophoretic differences between infant and adult amylase. We found no sex difference in the specific activity of amylase, although it increased significantly with age in males, and in the combined data for males and females. The values at 30 months of age were about 27% higher than at 18 months. One explanation for this difference could be that feeding with solid foods has been established by 30 months of age and the salivary glands are then influenced by the action of chewing.

In view of its abundant occurrence in exposed positions of molecules and its strong electronegative charge, sialic acid is thought to have important functions (see Section 1). Breast-fed infants reportedly have almost twice as much sialic acid in their saliva as formula-fed infants (Tram et al., 1997). The children of our 18-month group were no longer being breast-fed. We observed a sex difference in the content of sialic acid, when expressed as μmol/mg protein, in that group; the males showed about 38% more sialic acid than the females. This difference cannot be attributed either to the flow rate or the protein concentration, as no sex differences in these two variables were found. On the other hand, the concentration of sialic acid tended to decrease with age, and this becomes clear when we compare the values for the males of 18 and 42 months, with the 18-month group being 48% higher than the 42-month group. Similarly, when we combine the findings for both sexes, the value at 18 months is 32% higher than at 42 months. The decrease in sialic acid in children's saliva with age might suggest a decrease in the synthesis and secretion of glycoproteins.

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