PLAQUE pH RESPONSE TO SNACK FOODS IN CHILDREN WITH DIFFERENT LEVELS OF MUTANS STREPTOCOCCI

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Abstract. This study aimed to investigate the effects of some snack foods on plaque pH in children with different levels of mutans streptococci (MS). Six children, aged 9-12 years, with low ($<10^4$) and 6 children, aged 10-12 years, with high ($>10^6$) numbers of MS/ml saliva participated in the study. Dental plaque pH changes, after the consumption of milk chocolate, sweet biscuit, instant noodle, sticky rice with banana and a 10% sucrose positive control were measured using pH-electrode. The measurements of plaque pH were made on forty-eight-hour accumulated plaque, at baseline to determine the resting pH of the fasted plaque and at time intervals of 2, 5, 10, 20 and 30 minutes after food consumption. The plaque pH curves, delta pH values and area under curve for pH 6.0 for each test food were determined. Plaque acidogenicity was more pronounced for the high-MS group at almost all test periods compared to the low-MS group with all test foods. The test foods were ranked according to maximum pH drop in about the same order in both groups as follows: 10% sucrose > milk chocolate > sweet biscuit > sticky rice with banana > instant noodle. The plaque pH also stayed below pH 6.00 for a longer period in the high-MS group with sweet biscuit, milk chocolate, and sticky rice with banana. Findings suggest that pH responses were more acidic in high-MS group than low-MS group.

INTRODUCTION

The fermentation of dietary carbohydrates by oral microorganisms, particularly mutans streptococci (MS), in dental plaque plays a key role in the development of dental caries. These organisms have the ability, firstly, to produce acids even at a low pH and, secondly, to synthesize extracellular glucans. The extracellular polysaccharides favor plaque growth and may thereby increase the cariogenicity of dental plaque (van Houte, 1994). Furthermore, these polysaccharides promote the colonization of MS on the teeth and change the diffusion properties of the plaque matrix. As a result, the presence of extracellular glucans in dental plaque enhances its pH-lowering ability (Dibdin and Shellis, 1988; van Houte *et al*, 1989).

The relationship between low plaque pH and high caries activity was first observed by Stephan (1940). Previous study has shown a stronger plaque pH response in the high-caries group compared with the low-caries group after sucrose consumption (Lingstrom *et al*, 2000a). Little is known about whether exposure to different kinds of snack foods changes plaque acidogenicity and how it differs in individuals with different MS levels.

The aim of this study was to investigate the effects of some snack foods on plaque

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pH in children with different levels of MS.

MATERIALS AND METHODS

Subjects

Fifty-two children from primary schools in Chachoengsao Province, Thailand were screened in terms of the number of MS in their saliva. Twelve healthy subjects were selected and divided into 2 groups: 6 with MS $<10^4$ CFU per milliliter saliva (low-MS group) and 6 with MS $>10^6$ CFU per milliliter saliva (high-MS group) (Aranibar Quiroz *et al*, 2003) using spatula method as described by Kohler and Bratthall (1979). All selected subjects were required to show: co-operative behavior; normal pattern of growth and development; absence of congenital or systemic disease; absence of dental abscess; and absence of any medication therapy. The age (mean ± SD) of the two groups was 10.3 ± 1.0 and 10.5 ± 0.3 years, respectively. Informed consent was obtained from the children and their parents prior to the study. The study was approved by the ethics committee of Mahidol University (MU 2006-146).

Study design

Before each test period, the subjects received prophylaxis cleaning with pumice and rubber cup. They were asked to abstain from oral hygiene for 48 hours to allow accumulation of plaque and to fast overnight prior to sampling. Four snack foods, each with 5 g, were tested: milk chocolate, sweet biscuit, instant noodle and sticky rice with banana. A solution of 10% sucrose was used as positive control. The subjects were asked to rinse with sucrose solution for 2 minutes. For each of the four test foods, 1 minute was the given time for consumption. A washout period of at least one week elapsed between test periods. During the test periods, children consumed their regular diet and used fluoride toothpaste.

Plaque pH

Supragingival plaque samples were collected from buccal surfaces of maxillary posterior teeth using a spoon excavator, at baseline to determine the resting pH of the fasted plaque and at time intervals of 2, 5, 10, 20 and 30 minutes after consumption of each test food. The samples were immediately dispersed in 0.05 ml deionized water. Within 20 seconds after collection, the pH value was read with pH-electrode (IQ-Scientific, USA). The pH electrode was calibrated using pH 4.00 and 7.00 buffers before the start of each test and after every plaque pH determination of all samples.

The plaque pH curves, maximum pH drop (delta pH) values, and the area under curve for pH 6.0 (area between pH 6.0 and the pH curve) for each test food were determined. The area under curve for pH 6.0 was calculated using Microsoft Excel.

Statistical analysis

The mean and standard deviation of minimum pH, maximum pH drop, time point for maximum pH drop, and area of the response curve below pH 6.0 were determined. Two-way analysis of variance (ANOVA) was used to test the significance of differences between the low-MS group and high-MS group as well as among the different kinds of foods. When the ANOVA rejected the multi-sample hypothesis of equal means, multiple comparison testing was performed with Fisher's protected least significant difference test. A value of $p<0.05$ was considered statistically significant.

RESULTS

The results for plaque pH of the 2 groups are shown in Figs 1 and 2. Table 1 describes the resting pH, final pH, minimum pH, maximum pH drop (delta pH), time point for maximum pH drop and area under the curve for pH 6.0. Overall plaque acidogenicity was more pronounced for the high-MS group at all test periods compared to the low-MS group with all test foods. There was no statistical differ-

Table 1

Mean of resting pH, final pH, minimum pH, maximum pH drop, time point for maximum pH drop and area under the curve for pH 6.0 for the low- and high-MS groups after consumption of the test foods.

	Low-MS group					High-MS group				
	Milk chocolate	Sweet biscuit	Instant noodle	Sticky rice with banana	Sucrose	Milk chocolate	Sweet biscuit	Instant noodle	Sticky rice with banana	Sucrose
Resting pH	6.95±0.13	6.91±0.14	7.10±0.12	7.10±0.14	6.91±0.10	6.80±0.17	6.70±0.18	6.91±0.14	6.75±0.13	6.80±0.12
Final pH	6.91±0.17	7.03±0.13	7.03±0.16	7.13±0.10	7.10±0.10	6.71±0.09	6.45±0.16[a,b]	6.81±0.20[b]	6.80±0.16[b]	6.43±0.20[a,b]
Minimum pH	5.63±0.08[a]	5.75±0.16[a]	6.60±0.12[a]	6.33±0.13[a]	5.60±0.05[a]	5.30±0.22[a,b]	5.33±0.18[a,b]	6.03±0.13[a,b]	5.88±0.29[a,b]	5.15±0.18[a,b]
Maximum pH drop	1.21±0.17	0.95±0.13	0.41±0.08	0.60±0.08	1.21±0.17	1.48±0.23	1.33±0.10[b]	0.68±0.16[b]	0.93±0.17[b]	1.58±0.14[b]
Time point for maximum pH drop (min)	5.33±2.58	9.16±2.04	19.33±4.08	12.50±8.21	5.33±2.58	3.50±1.54	8.33±2.46	8.53±4.52[b]	7.50±2.73[b]	8.33±2.58[b]
Area under curve for pH 6.0 (min pH)	0.84±1.01	0.95±0.84	0	0	0.76±0.80	6.85±2.40[g]	8.47±2.62[g]	0.33±0.26	0.67±0.91	10.17±2.04[b]

[a]differs from resting pH; [g] differs from low-MS group with the same test food Results are mean ± SD of 6 subjects per group

ence when comparing resting pH between the two groups or between the test foods, while a significant difference was found for final pH.

When evaluating minimum pH, the lowest overall values were found after sucrose rinse (5.15±0.18), milk chocolate (5.30±0.22) and sweet biscuit (5.33±0.18) consumption for the high-MS group (Table 1). Statictically significant differences were found in both groups when comparing instant noodle or sticky rice with banana with other test foods. In the case of maximum pH drop, highest values were found after milk chocolate (1.48±0.23) and sweet biscuit (1.33±0.10) consumption for the high-MS group and after milk chocolate (1.21±0.17) consumption for the low-MS group, which were as high as the values of positive control sucrose solution. The test foods were ranked according to the maximum pH drop in about the same order in both groups as follows: 10% sucrose > milk chocolate > sweet biscuit > sticky rice with banana > instant noodle.

A variation in the time point for the maximum pH fall was found when comparing the two groups among the four test foods and sucrose rinse. The shortest time was found after milk chocolate consumption in both groups. The high-MS group reached the maximum pH fall faster than the low-MS group after sucrose rinse, instant noodle and sticky rice with banana consumption.

For area under curve for pH 6.0, sucrose rinse, milk chocolate or sweet biscuit consumption gave significant greater values than other test foods for the high-MS group.

DISCUSSION

It is obvious that it is impossible to standardize food consumption. Food preferences differ from person-to-person and from society-to-society. In general, people throughout the world, especially children, like to eat sweets and snack foods. As we know, foods

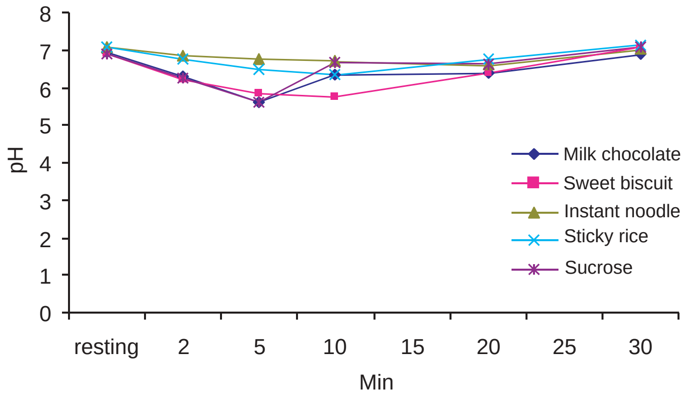

Fig1–Dental plaque pH changes for the low-MS group after consumption of test foods and sucrose rinse.

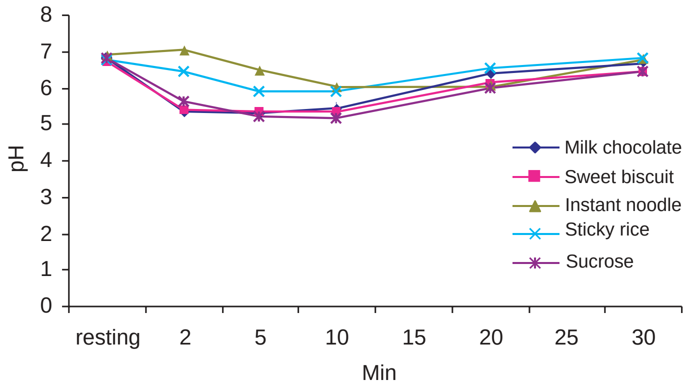

Fig 2–Dental plaque pH changes for the high-MS group after consumption of test foods and sucrose rinse.

that we consume may activate dental caries, which has led to a great deal of research on diet. Plaque pH measurements indicate the direct effect of food retained in the mouth. Many factors, such as salivary characteristics, genetic background, and food preferences that affect the plaque pH response. In addition, plaque pH response in children has been reported to be different from adults (Koparal *et al*, 1998). However, there are still little data on plaque pH measurements in children, although caries appears early in youngsters. Measuring the plaque pH changes after consuming snack foods in children, therefore, is a major concern.

In the present study, the most striking result was the difference in plaque pH response comparing the low- and high-MS groups. Although no difference in resting pH between the two groups could be seen, individuals with high numbers of MS had lower final pH compared with individuals with low numbers of MS after sweet biscuit, instant noodles and sticky rice with banana consumption. This finding is supported by the earlier study of Scheie *et al* (1992) who had not been able to discriminate the resting pH between children with a differing caries status, while others have found a difference (Turtola and Luoma, 1972; Abelson and Mandel, 1981). Moreover, the difference in the pH-lowering capacity of dental plaque between the two groups after consumption of the test foods found in the present study corresponds well to that found in a previous study in which a progressive decrease in plaque pH was found in relation to increased caries activity of individual (Lingstrom *et al*, 2000a).

An interesting observation in the present study was that the consumption of all test foods enhanced plaque acidogenicity to a higher extent for the high-MS group than for the low-MS group. Another interesting observation was the rapidity of the occurrence of the maximum pH decrease found for the individuals with high numbers of MS after consumption of test foods (Table 1). One explanation for this could be that the microorganisms living in dental plaque that utilized carbohydrates and formed lactic acid rapidly in the high-MS group. A previous study reported significantly higher levels of lactic acid in plaque from caries-positive subjects compared with caries-free subjects after sucrose rinse (Margolis and Moreno, 1992). However, an other study found a delay in the occurrence of the maximum pH decrease in the high-MS group (Aranibar Quiroz *et al*, 2003). This may reflect differences in the composition of the plaque microbiota and the types of food intake.

Regarding the types of food, milk chocolate and sweet biscuits were found to drop pH more than instant noodle and sticky rice with banana, in both groups. The reason for this could be the amount of sugar in milk chocolate and sweet biscuits that enhanced the decrease in plaque pH. Sugars are a form of fermentable carbohydrate that are found naturally in foods or added to foods during processing to alter the flavor, taste or texture of the food (Johnson and Frary, 2001). Plaque microflora utilize sugar or fermentable carbohydrate to produce acid, and so lower the plaque pH. This results in selective pressure for acidogenic and aciduric species (*eg* MS) to outgrow other resident plaque microflora and break down of microbial homeostasis in plaque; leading to dental caries. Apart from sugars, several dietary factors also affect the caries risk associated with individual foods. They are the following: food form, frequency of sugar consumption, retention time, nutrient composition, the potential of food to stimulate saliva, and the combinations of foods (Konig, 2000; Lingstrom *et al*, 2000b). The form of fermentable carbohydrate directly influences the duration of exposure and retention of the food on the teeth. Prolonged oral retention of cariogenic components of food may lead to extend periods of acid production and demineralization, and to shortened periods of remineralization.

In this study, a value of 6.0 which, is a typical drop in pH for children following an acidogenic challenge, was used as a critical pH level (Tahmassebi and Duggal, 1996). "Critical pH" is referred to as the pH at which the inorganic material of the tooth may start to dissolve. It is known that to define the critical pH as an absolute value is difficult, however, many researchers proposed the value ranging among 5.5 and 6.2 (Schachtele and Jensen, 1982; Tahmassebi and Duggal, 1996). Therefore, the area under curve for this pH value of 6.0 could be interpreted as being particularly important for potential cariogenicity, which indicates the retentiveness effect of food on salivary flow. Examining the area under curve in this study, the values for the high-MS group after sucrose rinse, consumption of milk chocolate, or sweet biscuits were greater than those for the low-MS group. This is the effects of a pH decrease during a longer period of time in the high-MS group. Moreover, for the high-MS group, consumption of sweet biscuit showed greater area under the curve than chocolate (Table 1). Our findings are supported by Kashket *et al* (1991), who found that particles of food with high contents of starch, such as cream sandwich cookies, were retained on teeth in larger amounts than foods that contained little starch, such as chocolate. Surprisingly, sticky rice with banana and noodle consumption did not decrease pH below 6.0 in the low-MS group while this could be demonstrated in the high-MS group.

In conclusion, this short-term study identifies some interesting trends between individuals with low and high MS counts in saliva. During a regular diet, individuals with high or low numbers of MS had the same level of plaque pH. However, when expose to fermentable carbohydrate, differences were observed. There was a more pronounced enhancement of plaque acidogenicity for the individuals with high MS numbers. The same relationship also holds true for the pH at all test periods with all test foods. Foods with high sugar content seem to have greater cariogenic potential than starchy food without sugar added.

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