Original

Effects on Plaque Acidogenicity of Salivary Factors and Frequency of Between-Meal Eating

歯垢の酸産生能に及ぼす唾液要因および
間食摂取頻度の影響について

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概要：糖摂取後の歯垢 pH の低下現象は、歯垢微生物による以外に他のいくつかの要因によって影響をうける。そこで今回、歯垢 pH に影響を及ぼす要因として全唾液を取上げ、安静および刺激唾液の pH、流出量、緩衝能のそれぞれについて測定し、歯垢 pH 変化に影響を及ぼす唾液要因について評価した。

男子歯科学学生84名（22～25歳）を対象とし、安静およびチュウイングガム刺激下で全唾液を採取し、pH、flow rate、buffer capacity を測定するとともに、*Lactobacilli* および *S. mutans* の菌数算定を行った。また、特定歯面から歯垢を可及的に一定量採取し、*in vitro* において8%シュクロース溶液を基質として15分間培養し、この結果を歯垢の酸産生能の指標とした。

歯垢の安静時 pH は安静全唾液の pH および緩衝能とそれぞれ有意な相関性を示し、同じく、刺激唾液の pH および緩衝能とも統計学的有意な相関関係を示した。

歯垢の酸産生能（final pH）と安静唾液の pH および緩衝能との間には有意な相関関係が得られた。しかし、刺激唾液の pH および緩衝能との間には有意な相関性が認められなかった。なお刺激唾液中の *Lactobacilli* と *S. mutans* 菌数は、両者とも歯垢の酸産生能と密接に関連することが見出された。

一方、質問票による間食回数の調査結果と歯垢の酸産生能との間には統計学的有意な正の相関関係が認められた。

以上の結果から、歯垢の acidogenicity に影響を及ぼす食餌性糖質ならびに唾液要因の意義が討論された。

Key words : Plaque acidogenicity, Salivary factors, Between-meal frequency, *S. mutans*, *Lactobacilli*

索引用語：歯垢の酸産生能、唾液要因、間食回数、*S. mutans*、*Lactobacilli*

Introduction

It is generally accepted that the immediate attack agents of dental caries are acids produced by dental plaque adhering to specific enamel surfaces. When various carbohydrates are ingested, a rapid decrease in plaque pH occurs following the production of organic acids by plaque bacteria followed by a slow gradual rise to the initial pH level. These changes in the pH of plaque are modified by the flow and composition of the oral fluids and may be influenced not only by the plaque flora itself but also by

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many other factors¹⁾.

The relationship between saliva, plaque and caries had been extensively discussed²⁻⁴⁾. However, how each of salivary factors influences the pH changes in plaque has not yet been critically assessed.

In the present report, we determined some salivary factors, i.e., pH, flow rate, and buffer effect, and attempted to analyze the relationship between each of the salivary parameters and plaque acidogenicity *in vitro*. In addition, we determined the numbers of *Lactobacilli* and *Streptococcus mutans* in saliva and analyzed the relation between their levels and the plaque pH.

Furthermore, we tried to evaluate the relative importance of the salivary factors and to get some information about the dynamic caries process in relation to saliva-plaque interactions.

Materials and Methods

Subjects: 84 male dental students aged 22-25 participated in this study. They were instructed to refrain from oral hygiene for one day. Tooth prophylaxis was not performed before the collection period started.

Plaque material: Plaque samples were collected from the buccal interdental areas of upper molars with the aid of a Gracy type curette under moisture control with cotton rolls.

Assessment of plaque acid production: The plaque activity was evaluated by measuring the pH of plaque collected in a sucrose solution *in vitro*. The materials and methods used in plaque pH determination have been reported elsewhere⁵⁾. Briefly, the reaction mixture contained approximately 1 mg plaque in an 8 % sucrose solution, total volume being 200 μ l. This was incubated aerobically for fifteen minutes at 36.5°C. The initial and final pH of the medium were read from a pH meter.

Saliva sample: The collection of saliva was performed between 10 and 11 a.m. Individual saliva samples were collected during the so-called resting state (collection time, 10 mins) and during the mastication of a bolus of sugarless chewing gum. Before collecting resting saliva, the subjects were asked to rinse once with 10 ml of distilled water and to expectorate the fluid in the mouth into a graduate chilled test tube.

Then, after the initial oral conditions were standardized by having the subjects chew a bolus of sugarless gum for one min, stimulated saliva was collected during the mastication of the chewing gum for 2 min.

Immediately after the saliva was collected, the pH, flow rate, and buffer effect were assessed. Buffer effect was evaluated by the buffer pH method⁶⁾. Briefly, the resulting pH of the saliva when 2.0 ml of saliva is titrated against 0.3 ml of 0.1 N lactic acid represents the buffer effect.

Microbiological procedures: The saliva samples were dispersed by using a Whirlimixer® for 60 sec and then diluted to 10⁻¹. One ml of the saliva samples was then taken and diluted in 10-fold steps with a 0.45 % sodium chloride solution. From each dilution, 0.1 ml was taken with a micropipette and cultured on Rogosa SL agar (Difco) for *Lactobacilli* and on MSB-agar for *S. mutans*⁷⁾.

All plates were incubated for 48 h at 37°C in 90 % N₂, 5 % CO₂ and 5 % H₂ (GasPac, BBL). The identification of *S. mutans* was confirmed by colony morphology and sugar fermentation tests. The saliva levels of both organisms were expressed as colony forming units (CFU) per ml saliva.

Clinical examination: The subjects were examined and scored for caries. The diagnosis of caries was made with mirror and explorer based on the WHO criteria for clinical caries⁸⁾.

Dietary questionnaire: A dietary questionnaire was given to each subject. The respondent recorded his frequency of between-meal snacks and/or beverages ingested during the ordinary entire day. The frequency pattern of subjects was scored on a progressive scale of 1 (none), 2 (once), 3 (2-3 times) and

Table 1 Plaque initial and final pH's at different *Lactobacilli* levels of stimulated whole saliva

Plaque pH	Lactobacilli (CFU/ml)			
	ND	10 ³	10 ⁴	10 ⁵ ≤
initial	6.38 ¹⁾	6.34	6.31	6.22
	±0.30	±0.28	±0.21	±0.27
final	5.59	5.31	5.25**	5.15***
	±0.47	±0.56	±0.53	±0.59
difference ΔpH	0.79	1.03	1.06	1.07

¹⁾ mean ± S. D.* ND : not detected (<10³ CFU/ml saliva)

** P < 0.05 *** P < 0.01

4 (more than 4 times).

Statistical analysis : The statistical significance of the difference between the two means was tested using Student's t-test. The relationship between the two variables was estimated by linear correlation analysis.

Results

The mean of initial plaque pH and that of final pH following a 15-min incubation with a sucrose solution in 84 subjects were pH 6.32 ± 0.26 (SD) and pH 5.32 ± 0.56, respectively.

Table 1 shows the mean final pH corresponding to the order of CFU/ml of *Lactobacilli* in saliva. The relationship between the salivary concentration of *S. mutans* and the final plaque pH is shown in Table 2. These results clearly indicate that the acidogenicity of the dental plaque in the present *in vitro* system increased with increasing orders of CFU/ml of both bacteria in stimulated whole saliva. The differences in final pH between the order of 10³ CFU/ml or less and those of 10⁴ CFU/ml, and 10⁵ CFU/ml or more of *Lactobacilli* in saliva were statistically significant. For *S. mutans*, the differences between the final pH of 10³ CFU/ml or less and those of 10⁴, 10⁵ and 10⁶ or more were also statistically significant.

The data showed a weak negative correlation between the final pH of plaque and the numbers of DMFT, indicating that the plaque pH response to sucrose reflected increased caries occurrence (Fig. 1).

The relationship between plaque pH and each of the salivary factors is shown in Table 3. The correlation between the plaque initial pH and the resting saliva pH and buffer effect was statistically significant. Likewise, the plaque initial pH was positively correlated with each of the stimulated saliva pH and buffer effects. On the other hand, the plaque final pH was positively correlated with the resting saliva pH and buffer effect. The plaque final pH was positively related to the stimulated saliva pH and

Table 2 Plaque initial and final pH's at different *S. mutans* levels of stimulated whole saliva

Plaque pH	Strep. mutans (CFU/ml)			
	≤10 ³	10 ⁴	10 ⁵	10 ⁶
initial	6.41 ¹⁾	6.33	6.23	6.27
	±0.27	±0.26	±0.27	±0.13
final	6.02	5.44*	5.17**	5.07**
	±0.23	±0.50	±0.62	±0.43
difference ¹⁾ ΔpH	0.39	0.89	1.06	1.20

¹⁾ mean ± S. D.

* P < 0.01 ** P < 0.001

Fig. 1 The relation between plaque pH after a 15 min. incubation and caries occurrence.

Table 3 Correlations between plaque pH, and pH, buffer pH and flow rate in resting and stimulated whole saliva

Plaque	Resting saliva			Stimulated saliva		
	pH	buffer pH	flow rate	pH	buffer pH	flow rate
initial pH	0.30***	0.27**	0.09	0.25**	0.25**	0.08
final pH	0.33****	0.31***	0.03	0.21*	0.19*	0.23*
*P<0.10 **P<0.02 ***P<0.01 ****P<0.001						

Table 4 Plaque final pH and between-meal frequency

	Between-meal frequency			
	0	1	2~3	4≤
Plaque final pH	5.52 ¹⁾	5.43	5.31	5.03
	±0.30	±0.59	±0.55	±0.56
n	4	22	48	10
¹⁾ mean ± S.D.				

Table 5 Correlations between plaque pH and between-meal frequency

Plaque pH	Between-meal frequency
initial	-0.13
final	-0.21*
* P<0.05	

buffer effect, but a statistical significance was not obtained in either. Table 4 shows the mean values of plaque final pH according to the reported frequency of between-meal snacks and/or sugar-containing beverages. The final pH decreased with the increase in the frequency. Table 5 shows the coefficients of correlation between the between-meal frequency and the plaque resting and final pH. The relation of the frequency to the plaque final pH was statistically significant.

A direct relationship between the initial and final pH of the plaque was found ($r=0.37$; $P<0.01$; data not shown).

Discussion

Stephan⁹⁾ demonstrated that a drop in plaque pH occurred after the ingestion of certain refined carbohydrates, and further he showed that pH minimum levels in plaque were inversely related to the caries activity in individuals¹⁰⁾. Englander *et al.*¹¹⁾ determined pH of dental plaque from the buccal surfaces of upper molars *in vitro* before and at varied minutes following a sucrose oral spray with and without saliva restriction, and noted the importance of saliva in preventing plaque pH values from falling below the level of pH 5.5.

We believe that the intensity and duration of the pH decrease depends not only on the plaque flora but also on the composition and frequency of the diet and on endogenous factors.

In the present study we have shown that the final plaque pH following a sucrose exposure is positively correlated with the pH and the buffer effect of resting saliva. Edgar¹²⁾ found a positive relation of the resting plaque pH and the minimum pH reached after sugar mouth rinse to the resting saliva pH levels. But the statistical significance of the correlation between the minimum pH and the resting saliva pH was not conclusive ($P<0.1$). From the relationships observed between the Stephan curves and the salivary responses, Edgar *et al.*¹³⁾ suggested that the pH of saliva is a major factor controlling plaque pH. The present study showed that the resting plaque pH was significantly correlated with the resting and the stimulated saliva pH, and moreover correlated with both salivary buffers (Table 3).

Recently, Tatevossian¹⁴⁾ showed that the difference between buffering in plaque fluid and saliva is quantitatively smaller above pH 6 and that the irreversible loss of buffer capacity above pH 6 may be due to loss of bicarbonate as CO₂ during the acid titration. He suggested that the salivary contamination of plaque can determine the buffering capacity of plaque in the range above pH 6¹⁴⁾, which, in turn, influences the pH lowering ability of plaque.

In this study, the final pH of plaque indicated a tendency toward a positive relation to the pH, buffer capacity, and flow rate of stimulated saliva. Especially, there was a relatively high value of correlation coefficient between the final pH of plaque and the flow rate. This may suggest direct and/or indirect effects on the amount and the composition of plaque of stimulated whole saliva. But the mechanism of this is not clear.

Kleinberg and Jenkins¹⁵⁾ observed that the pH of dental plaque in the different areas of mouth before and after meals reflected the flow and pH of resting saliva.

The present data further showed that the final pH of plaque was significantly related to the numbers of *S. mutans* and those of *Lactobacilli* in saliva.

Stralfors¹⁶⁾ found an inverse relationship between the pH minima produced by the ingestion of glucose and the *Lactobacilli* counts in the plaque on the maxillary anterior teeth. For *S. mutans*, Edga *et al.*¹³⁾ showed that an acidic Stephan curve characterized by a low resting pH and a low minimum pH is associated with higher numbers of *S. mutans* in plaque. McNamara *et al.*¹⁷⁾ observed that *Lactobacilli* were mainly found in the maxillary anterior approximal plaque, whereas the less acid Streptococci usually occurred in the labial maxillary anterior plaque.

In a human study, van Houte and Green¹⁸⁾ reported that the mean salivary concentration of naturally occurring *S. mutans* and *Lactobacilli* was 3.7×10^5 and 3.8×10^5 CFU per mL, respectively, and that both bacteria were highly localized on teeth.

Recent evidence suggests that the saliva level of *S. mutans* reflects the prevalence and proportion of this microorganism on the selected tooth surfaces^{19,20)}. On the other hand, Schei *et al.*²¹⁾ from an investigation of the effect of orthodontic treatment on the prevalence of *S. mutans*, concluded that creation of new retentive areas favors the local growth of the bacteria, which in turn increases the general infection level of this organism. In this respect, no significant correlation between the DMFT counts and the numbers of *S. mutans* and *Lactobacilli* in saliva has been obtained in this study. The orthodontic band placement has also been shown to induce a lowered resting pH of human incisor tooth plaque²²⁾.

There are numerous reports which indicate that frequent consumption of various fermentable carbohydrates (mainly, sucrose) can create an environment favorable to *Lactobacilli* as well as to *S. mutans*²³⁻²⁶⁾. From our dietary questionnaire, we found a significant positive correlation between frequency of between-meal eating and the pH lowering capacity of plaque. In general, dietary pattern influences the amount and type of plaque formation and the relative numbers of microorganisms on tooth surfaces. It has already been shown that the acidogenic composition of plaque reflects the dietary habits and caries status of an individual²⁷⁾. Griffiths²⁸⁾ showed that caries-free students had a significantly lower proportion of acidogenic organisms in their plaque than did caries-prone individuals and that this reduction was reflected in a lower rate of glycolysis. As such, it is conceivable that the changes in dietary pattern could influence the acid producing activity of plaque, perhaps by inducing the sucrose-splitting enzymes²⁹⁾. Invertase-like activity of dental plaque and oral fluid can be considered as an indicator of the cariogenicity of dental plaque in the presence of sucrose-containing diets³⁰⁾. Even so, it should not be ignored that the dietary habits could affect the response of salivary factors. However, the effect of diet on saliva is not yet definitive. Dawes³¹⁾ stated that the immediate local reflex effects of a diet on salivary flow rate

must be distinguished from the systemic effects of salivary flow rate on composition, which may take some time to develop. Tenovuo and Rekola⁸⁰⁾ observed a temporary drop in salivary pH and a decrease in oxidation-reduction potential of whole saliva after consumption of acid-containing beverages. It appears that the degree of functional stimulation of salivary glands by dietary intake may determine salivary flow rate⁸¹⁾ which related to the pH and buffect effect of saliva. Thus, it can be stated that the individual dietary habits may in part determine the levels of salivary pH and buffer effect and that their effects on saliva may be prolonged or temporary.

A low pH oral environment may promote the growth of *S. mutans*²⁵⁾ and also favor *Lactobacilli*³²⁾. The present results could provide information concerning the possible relations of salivary and dietary factors with the plaque pH response to a sugar exposure.

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