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The Effects of Xylitol-containing Chewing Gums on Dental Plaque and Acidogenic Potential

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Abstract. The aim of this study was to test the hypothesis that the chewing of xylitol- or xylitol/sorbitol-containing chewing gum reduces plaque formation and the acidogenic potential of dental plaque. Thirty healthy volunteers aged from 19 to 28 yrs were randomly allocated to one of three test groups, chewing either xylitol-, xylitol/sorbitol-, or sucrose-sweetened gums. A three-day plaque accumulation period of no oral hygiene was instituted prior to and at the termination of the chewing gum program, which lasted 33 days. Plaque quantity was assessed on the basis of protein content of individual plaque samples collected by a standardized technique. Acidogenic potential of individual baseline and test plaque samples was assessed by the quantity of various organic acids formed from D-(U-¹⁴C)glucose. Identification of extracellular and intracellular metabolites was performed by HPLC. Statistical evaluation of data was performed according to paired comparisons of individual baseline and post-chewing data. Plaque formation, acidogenic potential, and glycolytic profiles were similar at baseline and after the gum-chewing periods. Also, there was no intracellular accumulation of glycolytic metabolites within the plaque bacteria to indicate the inhibition of glycolysis. The study thus leads to the conclusion that, in young adults with low caries experience, exposure of the oral cavity to acceptable doses of xylitol or xylitol and sorbitol has no effect on the microbial deposits on the teeth.

Key words: chewing gum, dental plaque, glycolysis, organic acids, sorbitol, xylitol.

Introduction

The sugar alcohol xylitol has attracted much attention as an alternative sweetener, and xylitol-containing chewing gums are widely advertised with claims of beneficial effects on dental health. The Turku sugar study suggested that xylitol-containing chewing gums, chewed regularly between meals, had an "anticariogenic" effect (Scheinin *et al.*, 1975) and an inhibitory effect on dental plaque formation (Larmas *et al.*, 1976). Data from follow-up studies supported the concept that the chewing of xylitol- or xylitol/sorbitol-sweetened gums may reduce caries incidence (Isokangas, 1987; Isokangas *et al.*, 1988, 1989; Kandelman and Gagnon, 1990; Mäkinen *et al.*, 1995, 1996a,b) and dental plaque formation (Kandelman and Gagnon, 1990) when compared with the chewing of sucrose-containing gums or not chewing gum. However, the design of some of these studies, combined with inappropriate subgrouping of subjects in certain studies, has resulted in an interpretation of data which may lead to unsubstantiated conclusions.

Xylitol is not fermented by most oral micro-organisms, and caries-reducing effects have been attributed mainly to this lack of fermentation. However, "specific effects" on microbial growth and metabolism, on certain salivary factors, and on the physico-chemical processes of de- and re-mineralization have also been claimed (Mäkinen and Scheinin, 1976; Vadeboncoeur *et al.*, 1983; Mäkinen, 1985; Scheinin and Söderling, 1986; Söderling *et al.*, 1987; Bär, 1988; Mäkinen and Isokangas, 1988; Birkhed, 1994; Tanzer, 1995; Trahan, 1995; Mäkinen *et al.*, 1996b).

Sorbitol is another sugar alcohol frequently used as a sucrose substitute, because it is only slowly fermented by oral micro-organisms (Birkhed and Bär, 1991). Due to the relatively high cost of xylitol, sorbitol and xylitol are often combined, supposedly with a better clinical effect than with pure sorbitol (Birkhed *et al.*, 1983; Topitsoglou *et al.*, 1983). Moreover, it is claimed that a combination offers the same biological effects as when xylitol is used as the sole sweetener (Söderling *et al.*, 1989). *In vitro* studies even suggest that a combination of xylitol and sorbitol has enhanced anti-

microbial effects compared with xylitol alone (Assev and Rölla, 1986a).

The mechanisms of the xylitol-induced microbial growth inhibition have been studied mainly *in vitro*. They are claimed to involve induction of an energy-, phosphoenolpyruvate-, and ATP-consuming futile xylitol cycle, causing intracellular accumulation of xylitol-5-phosphate, and subsequent reduced acidogenic potential (Assev and Rölla, 1984, 1986b; Trahan et al., 1985). It has not been ascertained *in vivo*, however, whether xylitol- or xylitol/sorbitol-sweetened gums have the potential to reduce plaque quantity by virtue of such altered metabolic traits. With the increasing use of xylitol in various commodities, we have found it relevant to study, under controlled conditions, to what extent the presence of xylitol in the oral environment of today's young adult populations with a low caries experience can affect the biofilm on tooth surfaces. Therefore, the present aim was to test the hypothesis that the chewing of xylitol- or xylitol/sorbitol-sweetened chewing gum reduces plaque formation and the acidogenic potential of dental plaque.

Materials and methods

Study design

The study was performed according to a randomized, double-blind controlled design. Thirty healthy students (from 19 to 28 yrs old), with full dentitions, healthy gingival conditions, and no clinical signs of active dental caries, volunteered to participate. They all had more than 4 filled teeth, with an average DMFS of 6.8. The study protocol was approved by the local ethical committee, and informed written consent was obtained from the test subjects (Declaration of Helsinki, 1975).

The subjects were randomly allocated to one of three chewing-gum groups, chewing either xylitol- (X), xylitol/sorbitol- (X/S), or sucrose- (S) sweetened chewing gum. The content of sweeteners was as follows: (X) 0.40 g xylitol/piece, (X/S) 0.13 g xylitol and 0.31 g sorbitol/piece, and (S) 0.50 g sucrose/piece. The chewing gums were produced by an international chewing gum manufacturer, and the release of sweeteners was known. We chose the following chewing pattern to obtain maximum release of the sweeteners: Two pieces of chewing gum were chewed for 5 min 5X daily, giving a daily dose of 4 g xylitol or 1.3 g xylitol and 3.1 g sorbitol, respectively, for subjects chewing X and X/S gums. The test subjects adhered to their regular diet but avoided other xylitol and sorbitol products.

The test period started with a plaque accumulation period of 3 days, during which no oral hygiene was performed and no chewing gum was chewed. Baseline plaque samples were then collected for assessment of plaque quantity and for analyses of the acidogenic potential (see below). The subjects performed regular oral hygiene by using a standard fluoride dentifrice and followed the chewing gum regimen during the following 30 days. Oral hygiene was then discontinued and plaque was allowed to accumulate again during 3 days of continued exposure to chewing gums. Test plaque samples were collected for the assessment of the effects of the various chewing gums on

plaque quantity and on acidogenic potential.

Plaque quantity

Two baseline and two test plaque samples were collected from each test individual (buccal surfaces of teeth #17 and 16) by a standardized technique. A 3-mm-wide, bent copper band was scraped along the gingival margin, without coming into immediate gingival contact, so that mucosal damage would be prevented. The copper band was moved along the enamel to ensure that all clinically detectable dental plaque was harvested. The plaque was collected on both occasions after 3 days of undisturbed plaque accumulation when the subjects were performing no oral hygiene. The plaque samples were immediately frozen in liquid nitrogen and stored at -90°C until all samples could be assayed simultaneously. The plaque dry weight was determined, whereafter the protein content in the separate plaque samples was measured after extraction with 0.1% deoxycholate, by means of the Pierce BCA Protein Assay Reagent^R (Rockford, IL, USA). The concentration of deoxycholate that would give the maximum protein recovery from plaque samples had been determined in a pilot study.

Acidogenic potential

Plaque was collected for analyses of acidogenic potential after the 3 days of plaque accumulation periods prior to and after the chewing gum period. After saliva was removed with a gentle blast of air, a pooled plaque sample was collected separately from all tooth surfaces except from buccal surfaces of #17 and 16 by means of sterile P3/P4 Gracey curettes (Hu-Friedy, Chicago, IL, USA). Each sample was immediately suspended in 190 µL of reduced transport fluid (RTF) (Syed and Loesche, 1972), frozen in liquid nitrogen, and kept frozen until all samples had been collected and could be assayed simultaneously. Glycolysis was initiated by the addition of 10 µL D-(U-¹⁴C)glucose (final concentration, 2.5 mmol/L; specific activity, 10 µCi/µmol glucose; Amersham Int. plc, Amersham Labs, Buckinghamshire, UK) and allowed to proceed in a waterbath at 37°C for 60 min. Samples were taken out at 15, 30, 45, and 60 min. Further glycolysis was stopped by immediate centrifugation of the samples (11,000 × g, 3 min, MicroCentaur MSE, Sussex, UK), and the reaction mixture supernatants were run on an Aminex^R HPX-87H ion exclusion column with 0.012 mol/L H₂SO₄ as the mobile phase (flow rate, 0.6 mL/min; 65°C; Bio Rad, Richmond, CA, USA). The column was connected to a high-performance liquid chromatography pump (LKB 2150; LKB produkter, Bromma, Sweden) and an on-line radioactivity detector (model A110X; Radiometric Instruments and Chemicals, Tampa, FL, USA). The identity of extracellular metabolites was determined according to the retention times of standard reference samples of ¹⁴C-labeled metabolites: glucose, glucose-6-phosphate, fructose-1,2-diphosphate, lactate, pyruvate, formate, acetate, propionate, butyrate, succinate, and ethanol (Amersham International). The amounts of the various metabolites were calculated by integration of the area under the curve corresponding to the respective peaks in the chromatogram (Ramona v. 11.2; Nuclear Interface, Münster, Germany) and related to the amounts of protein in the respective plaque sample.

Intracellular glycolytic metabolites were analyzed in extracts of the 60-minute plaque samples. The plaque samples were washed twice with 1 mL RTF containing 2.5 mmol/L non-radioactive glucose and with centrifugation between each washing. The pellet was re-suspended in 100 μ L water and boiled for 30 min. The mixtures were centrifuged (10 min), and the supernatants containing intracellular metabolites were analyzed by high-performance liquid chromatography as described above for the extracellular metabolites.

Data analyses

It appeared from the analyses that there was remaining glucose in all samples at the 30-minute time point. These samples could therefore be considered as being incubated under conditions of excess glucose. The data given on the glycolytic potential are derived from the 30-minute samples. Each individual served as his/her own control, since baseline and test data were subjected to paired comparisons. Some of the parameters studied deviated from a normal distribution (plaque protein content). In these cases, paired comparisons were made according to the non-parametric Wilcoxon signed-rank test for matched pairs, and data are given as median values with a range. Otherwise, a paired Student's *t* test was applied (SigmaStat, Jandel Scientific Software), and mean values and standard errors of the means are given. The limit for rejection of the null hypothesis of no difference was set at 5%.

Results

Plaque quantity

Since no correlation was found between plaque dry weight and protein content of the baseline plaque samples, we considered the protein content of the sample to give a better reflection of the numbers of bacteria in the sample than its dry weight. Compared with baseline levels, there were no statistically significant differences in the plaque protein content during exposure to either X or S gums. The plaque samples from the subjects chewing X/S gums tended to contain more protein after the chewing period than at baseline ($p = 0.059$), but the amounts of protein in the plaque samples collected after the chewing gum periods were not statistically different among the three groups (Table 1).

Table 1. Plaque protein content (μ g) before and after the chewing of gum sweetened with either sucrose, xylitol, or xylitol and sorbitol^a

	Sucrose	n	Xylitol	n	Xylitol/Sorbitol	n
Before	67 ^b (6-1181) ^c	10	74 (15-204)	9	43 (8-176)	10
After	57 (12- 236)	10	70 (2-898)	9	74 (8-367)	10

^a See "Materials and methods" for details.

^b Median (based on the mean value of the two individual samples from each subject).

^c Range.

Acidogenic potential

Glucose consumption by plaque collected at baseline and after the chewing gum periods is shown in Table 2. None of the chewing gum regimens affected glucose consumption significantly as compared with baseline levels.

The glycolytic profiles of plaque samples collected at baseline and after the chewing gum periods are given in Table 3. Lactate, acetate, formate, propionate, and ethanol were the major glycolytic metabolites, with lactate and acetate being the two most abundant organic acids formed by plaque samples collected from all chewing gum groups both at baseline and after the chewing gum regimens. The identified metabolites represented between 40 and 60% of the glucose consumed. There were no statistically significant differences in amounts of any of the metabolites formed by plaque collected at baseline and after the chewing gum periods for any of the gums.

Intracellular accumulation of glycolytic metabolites was not detected in any of the plaque samples.

Discussion

In most studies reporting an effect of xylitol on plaque quantity, plaque has been assessed by plaque index measurements with comparisons of mean values (Mouton *et al.*, 1975; Larmas *et al.*, 1976; Kandelman and Gagnon, 1990), or by plaque wet weight (Mouton *et al.*, 1975; Söderling *et al.*, 1989, 1997). Both methods are linked with uncertainties and weaknesses. Plaque protein assessment is an accepted technique for studies involving metabolic parameters, such as acid formation by plaque bacteria and the number of bacteria in plaque samples (Distler *et al.*, 1987; Smit *et al.*, 1992; Sissons *et al.*, 1995), although variations in other components of dental plaque—*e.g.*, extracellular polysaccharides—will remain undetected by such an approach.

Table 2. Consumption of glucose (μ mol $\times 10^{-5}$ per μ g protein) by plaque before and after the chewing of gum sweetened with either sucrose, xylitol, or xylitol and sorbitol^a

	Sucrose	n	Xylitol	n	Xylitol/Sorbitol	n
Before	81.8 ^b (17.0) ^c	10	66.7 (16.5)	9	55.6 (9.6)	10
After	55.5 (8.2)	10	101.5 (27.3)	9	54.3 (5.4)	10

^a See "Materials and methods" for details.

^b Median (based on the mean value of the two individual samples from each subject).

^c Range.

Table 3. Glycolytic metabolites (μmol glucose equivalents $\times 10^{-5}$ per μg protein) in the plaque before and after the chewing of gum sweetened with either sucrose, xylitol, or xylitol and sorbitol^a

		Lactate	Acetate	Formate	Propionate	Ethanol
Sucrose	Before	23.2 ^b (7.5) ^c	10.9 (2.0)	1.0 (0.2)	5.0 (0.9)	0.9 (0.2)
	After	14.2 (2.8)	7.8 (0.8)	0.7 (0.1)	4.7 (1.0)	0.8 (0.1)
Xylitol	Before	12.0 (2.3)	7.6 (1.4)	0.8 (0.1)	4.3 (1.3)	0.7 (0.2)
	After	28.6 (8.5)	11.7 (2.7)	1.0 (0.3)	4.2 (1.7)	1.1 (0.2)
Xylitol/Sorbitol	Before	21.2 (4.5)	6.5 (1.0)	0.7 (0.2)	3.5 (1.5)	0.7 (0.1)
	After	17.3 (2.6)	7.7 (1.1)	0.7 (0.2)	4.4 (1.6)	0.7 (0.1)

^a See "Materials and methods" for details.

^b Median (based on the mean value of the two individual samples from each subject).

^c Range.

In the present study, since no correlation was found between plaque dry weight and plaque protein content of the baseline plaque samples, the protein content was used to reflect the numbers of plaque bacteria and thus plaque quantity. Thus, our finding of unaltered protein content cannot readily be compared with results found for plaque quantity in previous studies (Mouton *et al.*, 1975; Larmas *et al.*, 1976; Söderling *et al.*, 1989, 1997; Kandelman and Gagnon, 1990), but indicates that the numbers of plaque bacteria were unaltered by the chewing regimens.

The chewing of xylitol- or xylitol/sorbitol-sweetened chewing gum had no apparent effect on the acidogenic potential or on the glycolytic profile. Our findings, thus, are in contrast to the findings by Aguirre-Zero and co-workers (Aguirre-Zero *et al.*, 1993), who showed reduced plaque pH response to sucrose after two weeks of daily exposure to 9.1 g xylitol in chewing gums chewed 5 times daily, with the chewing period twice that used in the present experiment. Our results are also in contrast to results reported by Söderling and co-workers (Söderling *et al.*, 1989) and Wåler and co-workers (Wåler and Rölla, 1983), who showed reduced acid formation from glucose after xylitol exposure. One might infer that the inhibitory effect of xylitol has been related mainly to effects on mutans streptococci (Vadeboncoeur *et al.*, 1983), though effects on other oral micro-organisms have been reported (Assev *et al.*, 1983; Vadeboncoeur *et al.*, 1983). Our test subjects—who, from a caries point of view, represent today's young adult population—probably harbor low numbers of mutans streptococci (Axelsson *et al.*, 1987). Therefore, inhibitory effects on solely mutans streptococci might have been camouflaged. On the other hand, the present findings are in line with those of Wennerholm *et al.* (1994), who showed similar plaque formation and plaque pH response to sucrose in subjects after using chewing gums sweetened with either sorbitol or various amounts of xylitol. More recent findings have shown unaltered glycolytic patterns in dental plaque of subjects after 11 wks of chewing gums sweetened with amounts of xylitol similar to those used in the present study (Assev *et al.*, 1996).

Xylitol-induced microbial growth inhibition *in vitro* has

been suggested to be associated with an energy-, phosphoenol-pyruvate-, and ATP-consuming futile xylitol cycle involving intracellular accumulation of xylitol-5-phosphate with subsequent reduced acidogenic potential (Assev and Rölla, 1984, 1986b; Trahan *et al.*, 1985). Moreover, the accumulation of xylitol-5-phosphate in crude dental plaque exposed to xylitol has been interpreted as one of the main reasons for the cariostatic effect of xylitol (Waalder *et al.*, 1985; Wåler 1989; Waaler, 1992). No intracellular accumulation of glucose-6-phosphate was seen in the present plaque samples to support such a xylitol-5-phosphate-induced disturbance of the glycolysis. The differing results may be explained by different study designs. In one case, sucrose-induced plaque samples were incubated *in vitro* with xylitol as the sole external supply of substrate for an extended time period (from 6 to 24 hrs and 3 hrs, respectively) (Waalder *et al.*, 1985; Waaler, 1992). In the other case (Assev and Rölla, 1986b), a mono-culture of *Streptococcus sobrinus* OMZ 176 was incubated first for 3 hrs with high concentrations of xylitol (0.16 mol/L), after which the transport of only trace amounts of radioactive glucose (3.4 nmol/L) was assessed. Our data indicate that the very complex environmental conditions prevailing in the oral cavity may limit the value of extrapolations from simple laboratory experiments. The effect of xylitol on glycolysis at concentrations of glucose favoring its transport *via* phosphoenol-pyruvate-independent mechanisms (Carlsson and Hamilton, 1994), as probably prevails during food intake, may well be different.

The "therapeutic window" of xylitol has been a matter of discussion. It may be argued that the lack of effect of xylitol in the present study was due to the relatively low daily dose of xylitol (4 g in the xylitol group and 1.3 g in the xylitol/sorbitol group). In the longitudinal study by Kandelman and Gagnon (1990), both caries progression and plaque formation were reduced in the group chewing gums sweetened with either 1.1 g or 0.3 g xylitol *per piece* 3 times daily, compared with the no-chewing control group. These doses correspond to the doses supplied in the present study, and we therefore consider our findings to be of clinical significance. Moreover, since three of the test subjects in the

present xylitol group spontaneously complained of loose stool during the 3rd to 4th test wks, we consider this dose as a maximum.

In the Turku sugar study, the supply of 4.5 to 10.5 g xylitol daily from chewing gum reduced caries incidence (Scheinin *et al.*, 1975) and plaque formation compared with the sucrose control (Larmas *et al.*, 1976). Notably, significant effects on plaque formation were seen at the 12-month examination only, and not at the six-month examination. Moreover, the original Turku study indicates that the differences recorded may reflect differences in attitude and motivation among the test subjects rather than differences ascribed to the sweetener *per se*, since a general reduction in plaque formation was seen for both sucrose gum chewers and xylitol gum chewers throughout the study period of 1 yr (Larmas *et al.*, 1976). In the study by Isokangas (1987), the chewing of xylitol gums containing 3.5 g xylitol 3 times daily (total daily dose, 10.5 g) reduced caries incidence significantly, whereas 7 to 9 g of xylitol, chewed fewer than 3 times, gave no significant reduction in caries increment, indicating that the frequency of chewing may be more important than the actual daily dose of xylitol (Dodds *et al.*, 1991). The recent findings by Mäkinen *et al.* (1996a)—that xylitol- or sorbitol-sweetened chewing gums were equally efficacious in reducing caries incidence rates in children—support this contention. It is evident that chewing in itself increases salivary secretion rate and buffer capacity, as well as the oral clearance rate, of, for instance, microbial substrates and metabolic products, thereby decreasing the acidogenic potential of dental plaque (Dodds *et al.*, 1991). Moreover, the exposure to sucrose is reduced by a substitution with other sweeteners.

In summary, the present study indicated no reduction in plaque bacterial numbers by the chewing of xylitol- or xylitol/sorbitol-sweetened chewing gum, and no reduction of the acidogenic potential of dental plaque. We question, in fact, whether an effect on plaque growth and metabolism *in vivo* is to be expected in contemporary young adult populations. First, xylitol has been shown not to interfere with the metabolism of sucrose (Mühlemann *et al.*, 1977; Gauthier *et al.*, 1984), which is the most commonly consumed sugar. Second, xylitol is transported into the cells *via* a fructose PTS with anticipated K_m for xylitol of 54 mM compared with 27 μ M for fructose (Trahan *et al.*, 1991). In the oral cavity, from 4 to 10 μ mol/L glucose is always present in saliva (Carlsson and Hamilton, 1994). Thus, glucose and fructose are the preferred sugars, and xylitol will probably be transported in minimal amounts under *in vivo* conditions. Thus, xylitol-induced effects on plaque formation and plaque acidogenic potential, *in vivo*, may be marginal, and probably discernible only under extreme conditions. This contention is in line with recent reports on the effect of xylitol on plaque quantity, plaque metabolism, and plaque pH response (Wennerholm *et al.*, 1994; Park *et al.*, 1995; Assev *et al.*, 1996). Moreover, no significant difference in efficacy of a xylitol- and a sorbitol-sweetened chewing gum with respect to reduction in caries incidence rates in pre-school children was found (Mäkinen *et al.*, 1996). The study thus leads to the conclusion that, in young adults

with low caries experience, exposure of the oral cavity to acceptable doses of xylitol or xylitol and sorbitol has no effect on the microbial deposits on the teeth.

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