

Dental plaque formation and salivary mutans streptococci in schoolchildren after use of xylitol-containing chewing gum

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Objective. The aim of this study was to investigate the effect of a fixed daily dose of xylitol on mutans streptococci in saliva and the amount of visible dental plaque. A second aim was to explore if the possible effects differed between children with and without caries experience.

Methods. The study was designed as a double-blind randomized controlled trial with two parallel arms. All pupils ($n = 149$) in grades 1–6 in a comprehensive school in northern Sweden were invited, and 128 children (mean age = 12.7 years) consented to participate. The children were stratified as having caries experience (DMFS/dmfs ≥ 1) or not before the random allocation to a test or control group. The control group (A) was given two pellets containing sorbitol and maltitol three times daily for 4 weeks, and the test group (B) received corresponding

pellets with xylitol as single sweetener (total dose = 6.18 g day). Clinical scoring and saliva samples were collected at baseline and immediately after the test period. The outcome measures were visible plaque index, salivary mutans streptococci counts and salivary lactic acid production.

Results. The amount of visible plaque was significantly reduced in both groups after 4 weeks ($P < 0.05$). Likewise, the sucrose-induced lactic acid formation in saliva diminished in both groups ($P < 0.05$). The proportion of mutans streptococci decreased significantly in the test group compared to baseline, but not in the control group ($P < 0.05$). The alterations in the test group seemed most prominent among children without previous caries experience.

Conclusions. The results suggest that chewing gum with xylitol or sorbitol/maltitol can reduce the amount of dental plaque and acid production in saliva in schoolchildren, but only the xylitol-containing gum may also interfere with the microbial composition.

Introduction

Xylitol has been used as a substitute for refined white sugar for more than 30 years, and is thought to have an inhibitory action on the major causative bacteria in dental caries, *Streptococcus mutans* (for a review, see Maguire and Rugg-Gunn¹). It has been suggested that xylitol can decrease lactic acid production in dental plaque, resulting in a higher plaque pH, and can also promote an ecological shift, resulting in a less cariogenic environment^{2,3,16}. Habitual long-term xylitol consumption appears

to select for natural mutant cells of mutans streptococci, which lack the ability to absorb the sugar alcohol⁴. Xylitol is a common ingredient in sugar-free tablets, chewing gum, dentifrice and oral rinses. Chewing gum with xylitol has received special attention since mechanical cleaning together with saliva stimulation are very likely to be further benefits over and above the antibacterial effects of the polyol⁵.

The question of whether or not xylitol has a dose–response relationship has been relatively rarely evaluated in controlled trials. Clinical studies in child populations, however, have collectively suggested that a threshold level of 5–10 g day of xylitol in fractionated doses is required in order to obtain a significant anticaries effect^{6–9}. Furthermore, Milgrom and coworkers¹⁰ recently reinforced the need to achieve such levels in order to affect the

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mutans streptococci counts in plaque from adults. The present authors have previously described limited but slightly beneficial events in the oral ecology of children on low doses of xylitol^{3,11}, and therefore, it seemed reasonable to evaluate some selected variables after use of xylitol in a more appropriate dose regimen. The first aim of this study was to investigate the effect of xylitol on mutans streptococci and lactic acid formation in saliva, and the amount of visible dental plaque after a 4-week period of daily chewing on pellets with a total dose of 6.18 g xylitol. A second aim was to explore any possible differences between children with and without caries experience. The null hypothesis was that the outcome measures would not differ from those obtained after the use of a non-xylitol control gum sweetened with sorbitol and maltitol.

Subjects and methods

Study group

Based on a pilot study, a power calculation on salivary mutans streptococci levels displayed that 60 children in each group would be needed in order to avoid a type II error, with an estimated 30% difference between the groups ($\alpha = 0.05$, $\beta = 0.20$). Therefore, a total number of 149, 7–12-year-old children attending grades 1–6 in a comprehensive school situated in a small municipality in northern Sweden were invited to participate. Children with severe allergies or chronic diseases were excluded and informed consent was collected from 128 children (86% of the invited children, mean age = 10.2 ± 1.7 years) as well as from their parents. The school staff were also informed of the purpose and particulars of the project. All children were regular recall patients at the local public dental clinic and had received preventive-orientated non-operative and operative dental care since the age of 3 years. The participants claimed tooth brushing with fluoridated toothpaste at least once daily. The natural fluoride content in the piped drinking water was low (< 0.3 p.p.m.). None of the children were habitual consumers of xylitol products, but most of them had infrequent intakes of xylitol- or sorbitol-containing products.

Study design

The investigation had a randomized double-blind prospective design with two parallel arms that was approved by the regional ethical review board in Umeå, Sweden. The randomization was carried out on individual basis using a computer programme. Before the group allocation, all children were clinically examined and stratified as having or not having caries experience. The criteria for caries experience (CE) were that the subjects exhibited at least one filled and/or decayed (in dentine) primary or permanent tooth surface. Those without caries experience were marked as caries-free (CF), and this procedure was done in order to secure an equal number of children with caries experience in the test and the control groups.

After baseline clinical registrations and the collection of salivary samples, the participants were instructed to chew on either test gum containing xylitol, or control gum sweetened by sorbitol and maltitol three times a day for a period of 4 weeks. The outcome measures were: (1) visible plaque score, (2) salivary mutans streptococci counts and (3) salivary lactic acid production.

Chewing gum and regimen

The test and control gums were produced and supplied by Fennobon Oy (Karkkila, Finland). The ingredients of control chewing gum A were sorbitol (63.5 weight%), gum base, maltitol (4.5 weight%) flavours, humectant (E322), emulsifier (E422), artificial sweetener (E950), food colour (E171), acidity regulator (E296) and glazing agents (E903, E901 and E904). The mean weight of one pellet of chewing gum A was 1.08 g, of which sorbitol and maltitol constituted 0.69 and 0.05 g, respectively. Test chewing gum B contained xylitol (77.0 weight%), gum base, flavours, gum arabic, humectant (E322), emulsifier (E422), acidity regulator (E296) and glazing agents (E903, E901 and E904). Xylitol was the only sweetener, with one pellet of 1.34 g delivering 1.03 g of the polyol. The children were instructed to chew two pellets for 10 min three times a day (both on schooldays and non-schooldays), after breakfast and tooth-brushing, after lunch (weekdays, in school)

and in the evening after dinner. The test and the control gums were similar in form and colour, packed in identical boxes, and coded as 'A' or 'B'. The code was sealed by an independent monitor and not broken until the statistical calculations were finalized.

Clinical procedures

At baseline, the children were clinically examined in the classroom with the aid of a mirror, a probe and a strong torch. The presence of decayed, missed and filled surfaces was scored in the permanent (DMFS) as well as in the primary (dmfs) dentition according to World Health Organisation¹². No radiographs were exposed. If there was any doubt if a child had proximal or occlusal lesions or not, however, the dental records with radiographs from the local public dental clinic were consulted. Thereafter, a sample of stimulated whole saliva was collected by chewing one piece of paraffin (1.0 g) for 5 min or to when at least 3 mL saliva was collected. The stimulated saliva secretion rate was expressed as mL min⁻¹ and the samples were immediately transferred to the laboratory for further analyses.

The amount of visible dental plaque was checked after colouring the teeth with erythrosine (Rondell Red, Nordenta, Sweden). After a careful rinse with tap water, the buccal surfaces of 16, 11, 26, 36, 31 and 46 were examined, and the visible plaque of these six sites were scored 0–3 according to the Simplified Oral Debris Index (OHI-S) by Greene-Vermillion¹³. Teeth with fixed orthodontic appliances were excluded from the index calculations. After the clinical examinations, the oral hygiene habits were reinforced and all children received a new toothbrush together with a fluoridated dentifrice containing 1100 p.p.m. F. Immediately after the 4-week intervention period, the amount of visible dental plaque was reassessed and a second sample of stimulated saliva was collected.

Laboratory and microbial procedures

To determine the bacterial counts, 1.0 mL of saliva was serially diluted in M-DIL (NaCl, KCl, Na₂HPO₄ × 2H₂O, KH₂PO₄, sodium glycerophosphate × H₂O, MgCl₂ × 6 H₂) to obtain 5, 200,

4000 and 40 000 times dilutions. Then 50-μL aliquots of the 5× and 200× saliva suspensions were placed on a selective MSB agar plate for determination of the mutans streptococci counts¹⁴, while 50-μL aliquots of the 4000× and 40 000× suspensions were transferred to blood agar plates (Blood Agar Oral) for estimation of the total viable counts. All plates were cultivated at 37 °C in a micro-aerophilic environment in 5% CO₂ for 48 h. The number of colony-forming units (CFUs) were identified by morphology, counted in a stereomicroscope and expressed as CFU mL⁻¹.

The determination of lactic acid production in saliva was made enzymatically with the aid of a spectrophotometer (Ultrospec 100 Pro, Amersham Bioscience, Uppsala, Sweden). 0.5 mL saliva was pipetted into two Eppendorf tubes and acid production was initiated by the addition of 125 μL of 10-mM sucrose. The tubes were then incubated for 30 and 60 min, respectively, at 37 °C. The fermentation was stopped by a 10-min centrifugation (13 200 r.p.m.; Eppendorf Centrifuge 5415D, Eppendorf, Hamburg, Germany) and the supernatant was withdrawn and stored frozen until further analyses. After thawing, the L- and D-lactic acid concentration was determined using the commercial EnzyPlus D/L Kit (Diffchamb, Västra Frölunda, Sweden) and expressed as mM mL⁻¹.

Statistical methods

All data were processed by the SPSS, Version 12.0, computer software (SPSS Inc., Chicago, IL, USA). The index for dental plaque were scored and categorized before evaluated with chi-square tests. Bacterial and biochemical data were subjected to analysis of variance or Student's *t*-test. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

All 128 children who joined the study at baseline fulfilled the study protocol. The mean caries prevalence (DMFS + dmfs) for the total study group was 1.2 ± 2.3, while the corresponding value for those with caries experience (CE; *n* = 40) was 3.8 ± 2.7 (DMFS = 1.4 ± 1.0, dmfs = 2.4 ± 1.7). The DMFS + dmfs value for the

Table 1. Percentage distribution of sites with visible plaque scored according the Greene–Vermillion simplified oral hygiene index (OHI-S) in 128 children. The values are based on clinical assessment after erythrosine-staining of six predetermined sites (the buccal sites of teeth 16, 11, 26, 36, 31 and 46) from each participant.

Group and time point	Score			
	0	1	2	3
Group A (sorbitol-maltitol):				
baseline	30.1	41.8	23.1	5.0
4 weeks*	41.1	43.0	15.6	0.3
Group B (xylitol):				
baseline	24.6	47.8	25.1	2.5
4 weeks*	44.5	40.6	14.0	0.9

*Distribution significantly different from baseline, $P < 0.05$, Chi-square test.

caries-free group was 0. The salivary secretion rate varied from 0.5 to 2.3 mL min⁻¹.

Visible dental plaque

The baseline and immediate post-treatment plaque scores are shown in Table 1. A statistically significant reduction ($P < 0.05$) in the percentage distribution was disclosed in both the control (A) and the test (B) groups after 4 weeks, when compared to baseline. The number of sites with no visible plaque (score = 0) increased by approximately 10% in both groups. There was no difference between the groups after the 4-week study period and the plaque scores did not differ between children with and without caries experience.

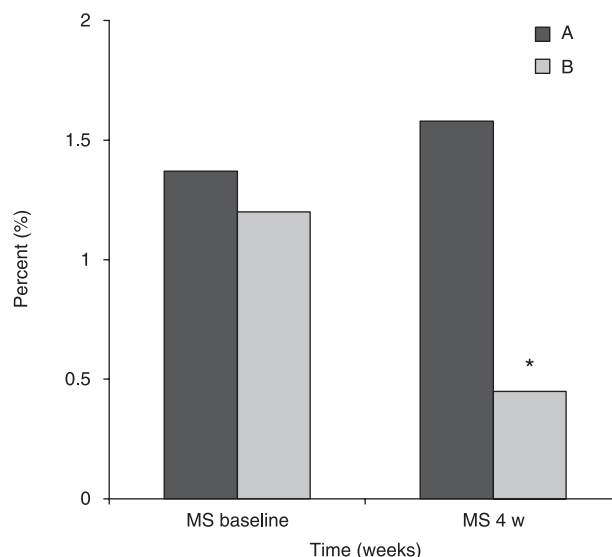


Fig. 1. Proportion of salivary mutans streptococci (MS) in relation to the total viable counts (TVCs) at baseline and after 4 weeks of daily chewing on xylitol-containing gum (group B, 6.18 g day⁻¹) or sorbitol/maltitol control gum (group A). A star denotes a statistically significant difference ($P < 0.01$) compared with the baseline.

Salivary mutans streptococci

At baseline, six children in the control group (A) and five children in the test group (B) had no detectable salivary mutans streptococci. The mean values of salivary mutans streptococci (MS) and total viable counts (TVCs) are presented in Table 2, and the proportion of MS in relation to TVC is shown in Fig. 1. The mean percentage dropped in group B from 1.2% at baseline to 0.45% after 4 weeks, and this difference was statistically significant ($P < 0.01$).

Table 2. Salivary mutans streptococci and total viable count (CFUs mL⁻¹, mean \pm SD) at baseline and after a 4-week period of daily chewing on xylitol-containing gum (group B, 6.18 g day⁻¹) or sorbitol/maltitol control gum (group A).*

Group	Number	Mutans streptococci		Total viable count		P-value†
		Baseline	4 weeks	Baseline	4 weeks	
Group A:						
all	64	$1.7 \times 10^4 \pm 3.7 \times 10^4$	$1.5 \times 10^4 \pm 3.5 \times 10^4$	$1.5 \times 10^6 \pm 1.2 \times 10^6$	$2.6 \times 10^6 \pm 3.7 \times 10^6$	NS
CE	20	$3.3 \times 10^4 \pm 5.7 \times 10^4$	$1.6 \times 10^4 \pm 3.6 \times 10^4$	$1.5 \times 10^6 \pm 1.1 \times 10^6$	$1.9 \times 10^6 \pm 1.4 \times 10^6$	NS
CF	44	$8.8 \times 10^3 \pm 1.8 \times 10^4$	$1.5 \times 10^4 \pm 3.4 \times 10^4$	$1.6 \times 10^6 \pm 1.2 \times 10^6$	$2.9 \times 10^6 \pm 4.5 \times 10^6$	NS
Group B:						
all	64	$2.1 \times 10^4 \pm 4.6 \times 10^4$	$8.4 \times 10^3 \pm 1.8 \times 10^4$	$2.0 \times 10^6 \pm 2.1 \times 10^6$	$2.4 \times 10^6 \pm 3.1 \times 10^6$	NS
CE	20	$1.4 \times 10^4 \pm 1.5 \times 10^4$	$1.1 \times 10^4 \pm 1.9 \times 10^4$	$2.2 \times 10^6 \pm 2.3 \times 10^6$	$1.8 \times 10^6 \pm 1.1 \times 10^6$	NS
CF	44	$2.4 \times 10^4 \pm 5.3 \times 10^4$	$7.4 \times 10^3 \pm 1.7 \times 10^4$	$2.0 \times 10^6 \pm 2.0 \times 10^6$	$2.6 \times 10^6 \pm 3.5 \times 10^6$	< 0.05

*Key: (NS) not significant; (CFUs) colony-forming units; (CE) caries experience; and (CF) caries-free. †Difference from baseline (analysis of variance).

Table 3. Lactic acid concentration (mM mL^{-1} , mean \pm SD) in sucrose-challenged saliva suspensions from 128 children at baseline and after 4 weeks of daily chewing of either xylitol pellets corresponding to 6.18 g of xylitol per day (group B), or control pellets sweetened by sorbitol and maltitol (group A).*

Group	Number	Lactic acid concentration		P-value†
		Baseline	4 weeks	
Group A:				
all	64	1.25 ± 0.79	0.97 ± 0.75	< 0.05
CE	20	1.18 ± 1.01	0.92 ± 0.69	NS
CF	44	1.29 ± 0.85	1.00 ± 0.77	< 0.05
Group B:				
all	64	1.29 ± 0.78	1.03 ± 0.75	< 0.05
CE	20	1.20 ± 0.81	1.19 ± 0.82	NS
CF	44	1.33 ± 0.77	0.94 ± 0.69	< 0.05

*Key: (NS) not significant; (CE) caries experience; and (CF) caries-free. †Differences tested against baseline (paired *t*-test).

The MS:TVC ratio also differed between groups A and B after 4 weeks in a statistically significant way ($P < 0.05$). In Table 2, the mean MS counts and TVC at baseline and after 4 weeks are detailed in relation to the caries experience. There was a significant reduction in salivary MS counts after 4 weeks among the caries-free children in the xylitol group B ($P < 0.05$), but this was not the case in any of the other subgroups.

Lactic acid formation

The concentration of lactic acid in the sucrose-challenged whole-saliva samples is presented in Table 3. The baseline levels were significantly reduced by approximately 25% ($P < 0.05$) after 4 weeks in both the xylitol and the sorbitol/maltitol groups. The decreased levels were most evident among subjects without any previous caries experience in both the test and the control groups.

Discussion

This study was performed to gain further knowledge on how a comparatively high daily dosage of xylitol may affect dental plaque formation and salivary mutans streptococci in schoolchildren. Since xylitol products are commonly advocated for patients with a high caries risk, the authors thought that it also would be

of interest to investigate if there were any differences in children with contrasting caries experience. The low prevalence of dental caries in the material forced them to use the presence of caries or fillings as a pivot point in order to avoid subgroups that were too small, but it should be noted that this was not necessarily a measure of present caries activity. The decision to use a sorbitol gum in the control group was, first of all, a prerequisite for the double-blind design, and secondly, a matter of compliance. An additional group with chewing on a neutral non-sweetened gum base would have been desirable in order to separate the effect of chewing, but still problematic with regard to the number of available children and awareness of chewing gum protocol. The clinical examinations were made by one examiner in the class room under field conditions. The study group was found to be representative for the corresponding age groups in the region with regard to the caries prevalence.

Before implementing this project, a pilot study was conducted to evaluate the compliance and look for possible side-effects (laxative or stomach ache) from the relatively high xylitol dose (6.18 g day^{-1}). The pilot study was performed identically to the final study and included one school class (17 children). Side-effects have been documented at lower doses¹⁵, even though other studies with equal or higher doses have not reported any adverse effects^{16,17}. The pilot study received positive feedback from children, parents and teachers, there was excellent compliance, and no side-effects from the chewing regimens were reported in either the pilot or the full-scale project.

For pragmatic reasons, the authors used a simplified oral debris index (the OHI-S) in the school setting, taking only the buccal surfaces of the 16, 11, 26, 36, 31 and 46 into account¹³. It has been debated whether a simplified index like this can be used to determine the plaque quantity in an adequate way since other methods, such as plaque weight and protein content, are considered to be superior^{18,19}. There are, however, direct comparisons indicating that plaque scoring produces results in line with other, more sophisticated methods^{17,20}, but the present results probably cannot be generalized to an interdental plaque situation.

An obvious and clear finding from this study was that both chewing gum regimes reduced the amount of visible plaque and lactic acid concentration in saliva, and this was achieved on top of reinforced instructions in daily tooth brushing. Thus, the null hypothesis could be rejected for these selected endpoints. On average, the children had relatively high plaque scores at baseline, and from a clinical point of view, they improved significantly during the chewing period. Apart from the mechanical cleaning properties of chewing, the ability of xylitol and sorbitol to reduce dental plaque has been discussed in many earlier studies^{17–19,21}, and both polyols have been proven to be hypo-acidogenic in telemetric plaque studies²². Söderling and co-workers²³ indicated that the mechanism of plaque reduction may differ between xylitol, and mixtures of xylitol and sorbitol. The latter performed equally well with respect to a reduction in the amount of plaque, but not the number of mutans streptococci. Since the reduction of plaque was significant in both test groups, but not in the control group with unsweetened placebo gum, the conclusion was that the polyols were additive to the chewing effect²³. Furthermore, Mäkinen *et al.*²⁴ demonstrated that plaque formed during frequent use of xylitol contained less polysaccharide compared to sorbitol-influenced plaque.

The total number of bacterial counts or mutans streptococci levels in saliva did not differ between the groups at either baseline or after 4 weeks. Moreover, there was no difference in mutans streptococci levels between children with and without experience of caries. Nevertheless, the proportion of mutans streptococci decreased significantly in the xylitol gum group in contrast to the sorbitol/maltitol gum group. This result agrees with several previous studies^{19,25–28}, indicating a small advantage of xylitol over sorbitol/maltitol. The decrease seemed to be most apparent in the caries-free children, but this observation must, of course, be interpreted with caution because of the generally low caries prevalence in the material. The xylitol influence was more pronounced here when compared to the present authors' previous report of lower daily xylitol doses from lozenges³, which reinforces the findings

of Milgrom *et al.*¹⁰, who suggested a dose-response relationship with a plateau effect for doses between 6 and 10 g. Collectively, this and other clinical and laboratory studies have shown that the biological properties of sorbitol and xylitol can differ with regard to their effect on microbial growth and metabolism. The present study did not, however, provide support for the concept that either xylitol- or sorbitol-based gums are especially beneficial for children with caries, but this issue needs to be further addressed in a population with a higher caries prevalence.

In conclusion, the results of this clinical study suggest that a 4-week protocol of daily chewing of pellets with xylitol or sorbitol/maltitol can reduce the amount of dental plaque and lactic acid production in saliva in school-children. In contrast to sorbitol/maltitol controls, xylitol-containing gum may also interfere with the microbial composition and decrease the proportion of salivary mutans streptococci.

What this paper adds

- This paper adds knowledge about how a specific amount of xylitol may affect dental plaque and salivary mutans streptococci in children.
- The results reinforce the theory that xylitol in chewing gum may have more effect than sorbitol.

Why this paper is important to paediatric dentists

- This paper provides important information for the use of xylitol in paediatric dentistry and shows that a high dose delivered in a fractioned way gives beneficial results with no side-effects.
- The findings suggest that xylitol can be used as a complement in preventive care in the 7–12-year-old age group.

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