Xylitol-induced changes of enamel microhardness paralleled by microradiographic observations

Arje Scheinin, Eva Söderling, Ulla Scheinin, Robert L. Glass and Marja-Leena Kallio

Departments of Cariology and Oral Biology, Institute of Dentistry, University of Turku, Turku, Finland, and Forsyth School for Research and Advanced Study in Dentistry, Boston, Massachusetts, USA

Scheinin A, Söderling E, Scheinin U, Glass RL, Kallio M-L. Xylitol-induced changes of enamel microhardness paralleled by microradiographic observations. Acta Odontol Scand 1993;51:241–246. Oslo. ISSN 0001–6357.

The study, aimed to analyze the feasibility of a prospective field study, was carried out in Polynesian children with rampant untreated caries. Slabs of bovine enamel were inserted for 8–12 days in cavities and subsequently replaced by permanent fillings. Before use, the surface of the slab was polished, and one half predemineralized and tested for microhardness. The follow-up in 30 subjects involved 54 slabs, 30 from negative controls with no added sweets and 24 from subjects receiving 20 g/day of xylitol in candy. The microhardness of the slabs was reassessed, and the difference between measurements calculated and tested for significance. The differences between the groups were highly significant, the predemineralized halves showing pronounced rehardening at exposure to xylitol. Parallel microradiographic observations conformed with the above findings. The results indicate that the use of a noncariogenic sweetener might be of value in high caries risk subjects. □ *Cariogenicity test; dental caries; dental enamel; remineralization; xylitol*

Arje Scheinin, Department of Cariology, Institute of Dentistry, University of Turku, Lemminkäisenkatu 2, SF-20520 Turku, Finland

The study was conducted to analyze the feasibility of a prospective WHO xylitol field study in French Polynesia (1), whose population is characterized by extremely high caries prevalence and incidence. Before the field study a short-term survey, involving use of xylitol-containing candy in comparison with a customary dietary regimen, was thus planned to be implemented in children with rampant caries.

Materials and methods

Preparation and storage of slabs

The intraoral test phase was modified from the model of Koulourides et al. (2). Bovine molars were used for preparation of the enamel slabs, size (1.5 to 2.5) × (2.0 to 4.0) mm^2. The slabs were prepared by polishing their outer surface and maintaining them in accordance with directions (2). The slabs were predemineralized before use; half of the enamel surface was thus covered with nail varnish, and the other half subjected to demineralization in 0.01 M Na-lactate buffer, pH 4.8 for 4 h at 37°C without stirring. After removal of the varnish with acetone the surface was tested for hardness with a Vickers indenter fitted to a Durimet microhardness tester (Ernst Leitz GmbH, Wetzlar, Germany) loaded with 200 g for 30 sec.

The slabs were not allowed to dry and were before insertion and after removal stored in separate tubes containing sterile, distilled water.

Adhesion of Streptococcus mutans

The colonization of *S. mutans* (ATCC 10499) on the predemineralized slabs was studied as follows. The bacteria were grown at 37°C in a 6% sucrose-containing medium (5 g trypticase, 5 g yeast extract, 60 g sucrose, 5 g K_2HPO_4, 4 g glucose, 4 ml Tween, 4 mg $MgSO_4 \cdot 7\ H_2O$, 0.2 mg $FeSO_4 \cdot 7\ H_2O$, and 0.09 mg $MnCl_2 \cdot 4\ H_2O$

in 1 l distilled water). The growth was interrupted in the logarithmic phase, and the cells were transferred to fresh medium, the absorbance (A_{540}) of the suspension being adjusted to 0.5. The enamel slabs were mounted in wax and positioned on a steel wire grid 2 cm from the bottom of the incubation vessel, at an angle of 45°. The bacteria were allowed to adhere to the slabs for 2 h without agitation. The grid with bacteria was transferred to a fresh medium every 2 h for a total of 16 h.

The slabs with the bacterial colonies were fixed in 2.5% glutaraldehyde in 0.1 M Na-phosphate buffer, pH 7.4, for 1 day and washed in 8% sucrose solution for 15 min. The samples were dehydrated in rising alcohol series up to 100% ethanol. The dehydrated specimens were dried in air and coated with a gold layer of approximately 20–30 nm in a vacuum evaporator and examined in a JSM-U3 scanning electron microscope at 15 kV. In the presence of 6% sucrose the bacteria colonized equally on the predemineralized and undecalcified sites (Fig. 1).

Subjects

A total of 30 subjects, 15 boys and 15 girls from the island of Moorea, aged 9–12 years, all with rampant untreated caries and no previous fillings, participated in the study.

Intraoral procedures

The slabs were to be placed temporarily in cavities, all prepared to save the teeth from imminent extraction owing to very deep carious lesions. All or most carious dentin was removed by using advanced portable equipment, any remaining softened dentin carefully excavated, a $CaOH_2$-based lining applied, and a slab inserted and fixed with a rapidly hardening temporary filling material. Whenever possible, the slab was placed deep enough to permit plaque retention on its oral surface. Slabs could be inserted in all the cavities without enlargement. The follow-up was planned for an analysis of 54 slabs, 30 from the comparison group ($n = 15$) and 24 from the xylitol group ($n = 15$). In the latter group two slabs were lost during the intraoral

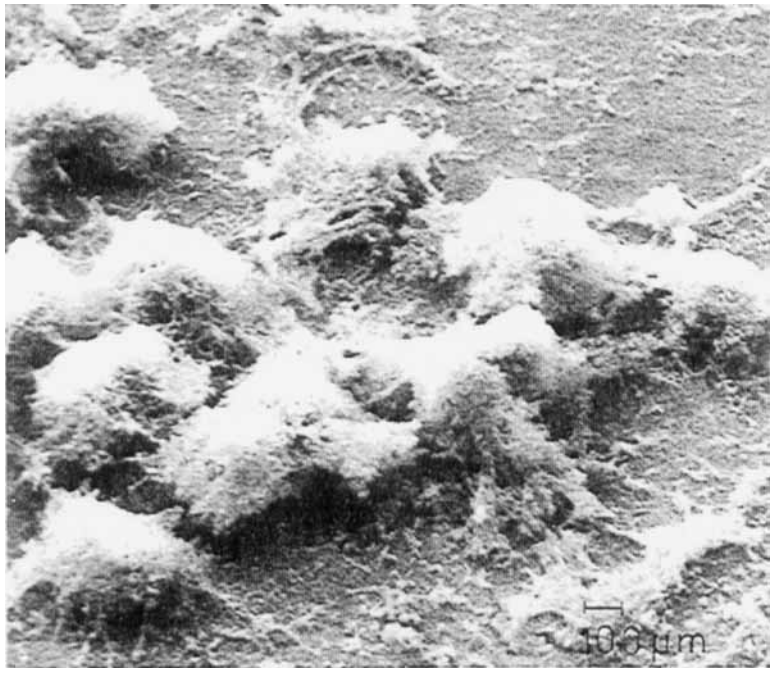

Fig. 1. *Streptococcus mutans*-induced plaque in the border zone between pre- and undemineralized bovine enamel.

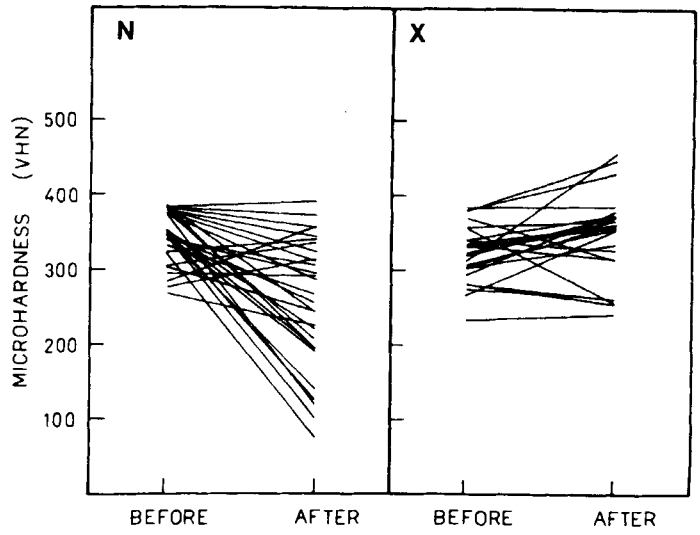

Fig. 2. Development of individual microhardness values (VHN) of undemineralized bovine enamel before and after the intraoral exposure phase (N = comparison group; X = xylitol group).

phase and three badly fractured at removal. After 9–12 days (in two cases, 8 days) the slabs were removed and permanent fillings inserted.

Dietary regimen

All subjects in the comparison and xylitol groups were instructed to maintain their normal diet. Each subject of the xylitol group was provided with a daily dose of 10 hard candies containing xylitol (maximum, 20 g/day) as the only sweetener. In the latter group the subjects were advised to maintain the sequence of any sugar-containing sweets to be immediately followed by a xylitol candy. Further supply was provided on request to family members. No sweets were given to the subjects in the comparison group.

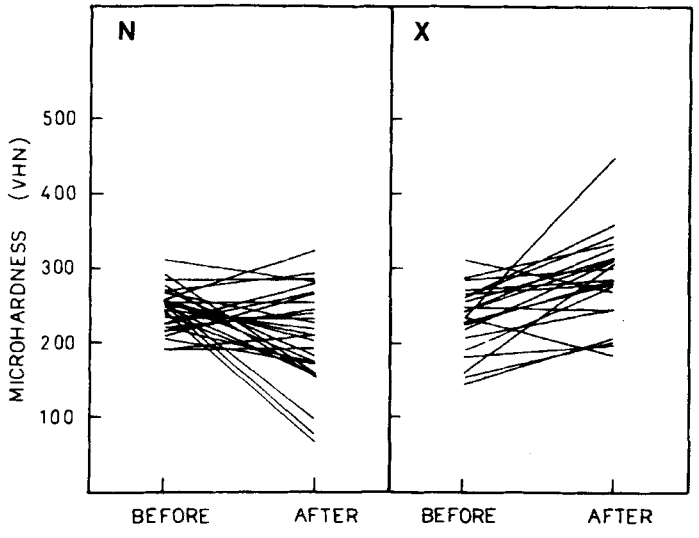

Fig. 3. Development of individual microhardness values (VHN) of predemineralized bovine enamel before and after the intraoral exposure phase (N = comparison group; X = xylitol group).

Microhardness measurements

The microhardness of the slabs was measured before insertion and after removal. The reported change in Vickers units was the difference between three pairs of consistently placed symmetric indentations from the demineralized and undemineralized halves. The significance of the difference was tested with Student's *t* test for paired samples.

Microradiography

The analyses were carried out by using sections of about 100 μm, embedded in methylmetacrylate. The latter was dissolved in chloroform. The microradiography (18 mA, 25 KV; exposure time, about 1.5 h) was carried out with Philips PV 1012/10 equipment.

Results

The five negative control slabs, stored in sterile distilled water for the duration of the test phase but otherwise handled as the slabs used in intraoral tests, showed no changes in microhardness during the study. The influence of the various dietary conditions on individual enamel slabs was analyzed through microhardness measurements carried out separately in unconditioned and predemineralized areas. The observations, shown individually for the subjects exposed to xylitol-containing sweets and the comparison group (Figs. 2 and 3), were used for analyses of the significance of the results. The observed differences between the comparison and xylitol groups were highly significant; the predemineralized halves of the latter slabs showed a stronger tendency toward remineralization than their matching halves without conditioning (Table 1).

The level of demineralization, however, was higher for the slabs not subjected to predemineralization. Generally, the slabs of the comparison group showed various degrees of softening, whereas in the xylitol group the reverse phenomenon was observed. The difference in the change in microhardness between the xylitol and control group was significant for both predemineralized and untreated sites of the slabs (Table 1).

The results of the microradiographic analyses conformed with the microhardness data, as indicated by representative micrographs (Fig. 4) from the negative controls, the comparison group, and the xylitol group, the latter showing a measurable increase in microhardness. In the slab from the comparison group the depth of the radiolucent area showed variation, presumably due to uneven distribution of plaque on the enamel surface. Only in a few slabs of the comparison group was the radiolucent area of subsurface nature; most of the slabs showed extensive radiolucency starting directly from the surface. In the xylitol group, however, an increase in radiopaqueness was observed along the predemineralized areas, but no changes were observed in the halves without pretreatment.

Discussion

Far-reaching conclusions about the described variations in microhardness and

Table 1. Xylitol-induced changes of microhardness (Vickers Hardness Number) in intact and partly demineralized slabs of bovine enamel inserted for 8–12 days in cavities prepared for permanent fillings in Polynesian children with rampant caries

Enamel	Comparison group, $n = 30$		Xylitol group, $n = 24$		Significance, C/X
	Change	SEM	Change	SEM	
Predemineralized	−37.3	±12.2	+50.4	±10.7	$p < 0.001$
Not demineralized	−91.1	±17.1	+23.4	±11.0	$p < 0.001$

radiopacity in bovine enamel should not be extrapolated as proof of an identical response pattern in man; the rate of lesion progress in bovine enamel has, for example, been shown to be threefold that in human permanent teeth (3). It is thus evident that less time will be required to produce lesions of the present nature in bovine enamel than in human teeth. A factor contributing to the demineralization of the slabs might be the polishing procedure (see Materials and methods); a partial removal of the highly mineralized surface layer might thus have facilitated the observed process of rehardening.

On the other hand, the reactions in human enamel, as observed in newly erupted extracted teeth when subjected to demineralization comparable to the present conditions (4, 5), seem to parallel the pattern we observed in bovine enamel. A further parallel is also indicated by the adhesion of *S. mutans* on bovine enamel and by rapid accumulation of a dense layer of plaque rapidly covering the microbiota (Fig. 1).

The present observations (Figs. 2–3, Table 1) show an increase of microhardness especially in the predemineralized halves of bovine enamel slabs at short-term use of xylitol-containing sweets. No conclusions are drawn about the possibility of the xylitol specificity of the observed rehardening; however, the results indicate that even in adverse conditions the use of a completely non-

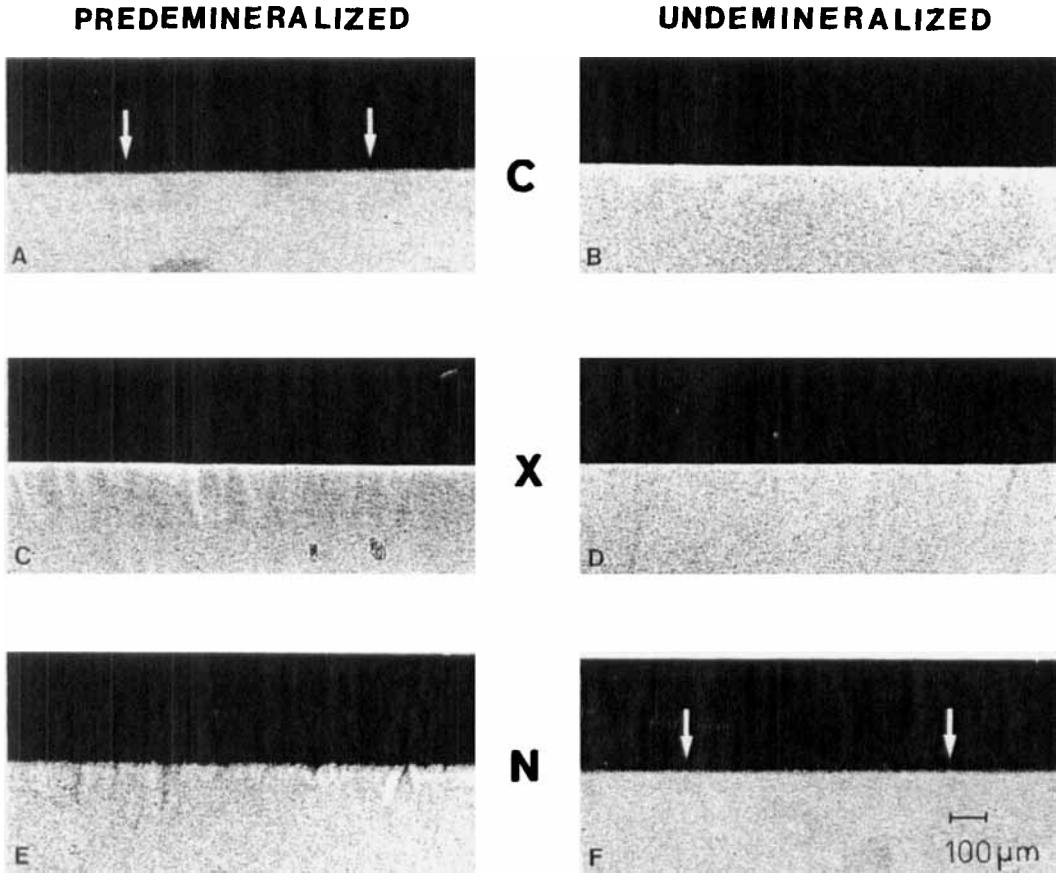

Fig. 4. Microradiographs of a negative control slab (C: A, B) and representative slabs from the xylitol (X: C, D) and comparison (N: E, F) groups after the intraoral exposure phase. The arrows indicate the enamel surface.

acidogenic sweetener might be of considerable value in high caries risk subjects.

References

1. Kandelman D, Bär A, Hefti A. Collaborative WHO xylitol field study in French Polynesia. Caries Res 1988;22:55–62.
2. Koulourides T, Bodden R, Keller S, Manson Hing L, Lastra J. Cariogenicity of nine sugars tested with an intraoral device in man. Caries Res 1976;10:427–41.
3. Featherstone JDB, Mellberg JR. Relative rates of progress of artificial caries lesions in bovine, ovine and human enamel. Caries Res 1981;15:109–14.
4. Hals E, Mörch T, Sand HF. Effect of lactate buffers on dental enamel in vitro. Acta Odontol Scand 1955;13:85–122.
5. Scheinin A. The effect of sodium fluoride and potassium fluoride solutions on the solubility of dental enamel. A chemical and histological study. Chapter VIII. Proc Finn Dent Soc 1958;54 suppl:63–88.

Received for publication 1 March 1993