Salivary mutans streptococci and dental caries in three-year-old children after maternal exposure to chewing gums containing combinations of xylitol, sorbitol, chlorhexidine, and fluoride

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The aim of this study was to evaluate the effect of maternal use of chewing gums containing combinations of xylitol, sorbitol, chlorhexidine, and fluoride on salivary mutans streptococci (MS) counts and caries prevalence in the mothers' 3-year-old children. After screening 416 women with newborn babies, 173 mothers with high counts of salivary MS were randomly assigned into 3 experimental chewing gum groups containing (A) xylitol ($n = 61$), (B) chlorhexidine/xylitol/sorbitol ($n = 55$), and (C) sodium fluoride/xylitol/sorbitol ($n = 57$). Mothers with low or medium MS counts formed a reference group (D) without any intervention ($n = 232$). The participants in the experimental groups were instructed to chew one piece of the gum for 5 min 3 times a day. The chewing regimen started when the child was 6 months old and was terminated 1 year later. The outcome measures were salivary MS counts and caries prevalence at the age of 3 years. Bacterial enumeration was carried out with a chair-side technique and caries (defs) was scored by clinical examination. Medium and high counts of salivary MS were found in 13%, 16%, and 22% in groups A, B, and C, respectively. The mean defs was 0.1 in group A, 0.2 in group B, and 0.4 in group C. The differences concerning salivary MS and caries were not statistically significant. The MS counts and caries prevalence in children of mothers with low MS counts (group D) were similar to those found in groups A and B. In conclusion, lower but non-significant levels of salivary MS and dental decay were observed in 3-year-old children to mothers who used high-content xylitol gums compared with those who used lower amounts of xylitol. The efficiency of this type of targeted intervention in a low-caries community may be questioned. □ *Caries; chlorhexidine; fluoride; mutans streptococci; xylitol*

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The early transmission of mutans streptococci (MS) from caregivers to their children is thought to play a significant part in early childhood caries (1), and consequently a preventive strategy to suppress maternal MS levels as a means of reducing colonization in the mothers' offspring has emerged. Clinical trials with antibacterial agents such as iodine and fluoride (2), chlorhexidine (3–5), and combinations thereof (6, 7) have been performed with mixed results. Screening and professional treatments are costly, however, and the concept would gain with a self-administered regimen. The potential effect of xylitol-containing chewing gums has been evaluated in studies in which the maternal use of xylitol gums during tooth eruption reduced MS acquisition and dental caries development in children up to 6 years of age in comparison with mothers who received professional applications of either a chlorhexidine or a fluoride varnish (8–10). Although the mechanism is not fully clear, habitual xylitol consumption may select for MS strains with impaired adhesive properties to enamel, but it has also been discussed whether or not the anti-caries properties were due to saliva stimulation (11–13). It is therefore of interest to evaluate the effect of xylitol, chlorhexidine, and fluoride in comparable chewing regimens and a randomized clinical trial with parallel arms was started in 1998. The 18-month results displayed a significant reduction of MS colonization in children of mothers in the xylitol and chlorhexidine/xylitol chewing gum groups compared with the fluoride chewing gum group (14).

The aim of the present communication was to describe the effect of maternal use of chewing gums containing xylitol, chlorhexidine/xylitol/sorbitol, and fluoride/xylitol/sorbitol on salivary MS counts and caries prevalence when the children were 3 years of age. The null hypothesis was that no difference in MS counts and caries prevalence would occur between the different experimental groups.

Materials and methods

Material and study design

A screening for the presence of salivary MS was conducted in 416 healthy women with newborn infants (213 girls and 203 boys) in the community of Varberg, a middle-sized city in southwest Sweden. Mean age of the mothers was 30.1 years, ranging between 17 and 44 years. The screening procedure was carried out by a midwife in

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connection with the regular well-being evaluation 3 months after the delivery. The levels of salivary MS in the mothers were estimated using the Strip mutans chair-side test (Dentocult-SM, Orion Diagnostica, Helsinki, Finland) following a standard procedure (15). Three experimental groups and one reference group were formed on the basis of the maternal MS counts. The mothers with high counts of salivary MS (≥150 colony forming units, CFU) were randomly assigned into three experimental groups (A–C) and instructed to chew one piece of chewing gum three times daily containing combinations of xylitol, sorbitol, chlorhexidine, and fluoride as described below. The reference group (D) consisted of mothers with low or medium counts of salivary MS (<150 CFU) who received no chewing gums. Maternal chewing was initiated when the children were 6 months of age and terminated 1 year later, at 18 months. Prior to randomization, all participants were given verbal and written information about the study and a consent form was signed. The ethics committee at Gothenburg University, Sweden, approved the study design.

A detailed flow chart of the participants is presented in Fig. 1. At screening, 184 mothers exhibited high counts of salivary MS; 11 of them did not want to proceed with any chewing gum, leaving 173 mother–child pairs in the 3 experimental groups. During the first months of the study, 22 (13%) mothers terminated their chewing regimen, 5 in group A, 11 in group B, and 6 in group C. The major reasons were taste and gastric problems, fatigue and pain in the jaws, and relocation to other communities. However, all drop-outs agreed to show up with their babies for the 3-year examination, forming a drop-out group (group E, $n = 22$). In the reference group, 2 subjects (0.9%) were lost due to relocation. According to the medical records, no marked differences in socio-economic characteristics were disclosed between any of the groups.

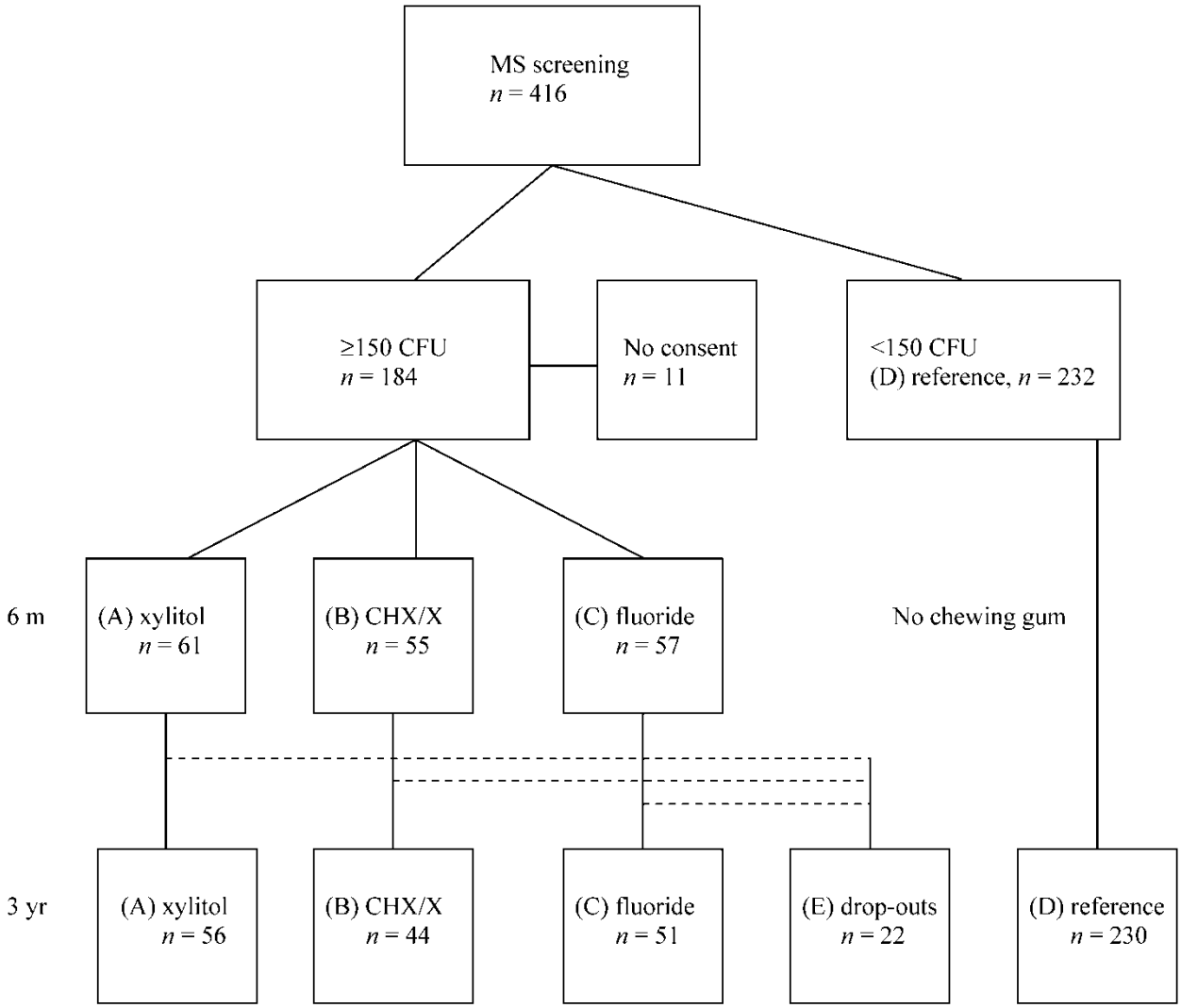

Fig. 1. Study design and flow chart. MS = mutans streptococci; CFU = colony forming units.

Table 1. Active ingredients and sweeteners of chewing gums used by the experimental groups

	Group A xyl gum	Group B CHX/xyl/sorb gum	Group C F/xyl/sorb gum
Xylitol	650 mg	532.5 mg	288.5 mg
Chlorhexidine	–	5.0 mg	–
Sorbitol	–	141.9 mg	188.8 mg
Sodium fluoride	–	–	0.55 mg
Total weight/gum	1050 mg	1120.1 mg	870 mg

Chewing gums

The active ingredients and sweeteners of the various chewing gums are presented in Table 1. The xylitol gum used by group A was a commercial product with each piece of gum containing 650 mg of xylitol (Xylifresh, Leaf, Turku, Finland). The chlorhexidine/xylitol/sorbitol gum (group B) was provided by Fertin Pharma A/S (Vejle, Denmark) and contained 5 mg chlorhexidine, 533 mg xylitol, and 141.9 mg sorbitol, whereas each of the gums of group C had 0.55 mg sodium fluoride, 289 mg xylitol, and 188.8 mg sorbitol (Fluorette, Fertin Pharma A/S). It should therefore be noted that the gums in groups B and C were sweetened by a combination of xylitol and sorbitol and those in groups A and B contained higher amounts of xylitol. All participants were instructed to chew one gum each day for 5 min in the morning, at noon, and in the evening. A supply of chewing gums was provided to cover a period of 3 months, after which a check on compliance was made before new chewing gums were delivered.

Bacterial sampling and cultivation

The level of salivary MS in the 3-year-olds was enumerated using the standard chair-side test, as described above. The children were asked to chew on a small piece of pre-warmed paraffin for 1 min and a plastic strip was thereafter rotated 5–10 times on the dorsum of the tongue and withdrawn through lightly closed lips. After cultivation at 37°C for 48 h, the bacteria were identified by morphology and enumerated as CFUs in a stereomicroscope on a predetermined area with the aid of a squared lattice (16). The following scores were used: **0** = no detectable CFU; **1** = 1–10 CFU (low counts); **2** = 11–99 CFU (medium counts); **3** = $\geq$100 CFU (high counts).

Caries examination

All children were examined in a dental chair by one trained examiner (IT) blind to which group the child belonged. Caries was scored according to the guidelines of the Public Dental Service in the County of Halland, which was mainly in harmony with the World Health Organisation criteria (17) and expressed as decayed (cavitated), extracted, and filled surfaces (defs). Non-cavitated lesions within the enamel, defined as white chalky areas on the smooth surfaces, were diagnosed and scored separately. No radiographs were exposed. Prior to and continuously during the examinations, the examiner was calibrated with an experienced colleague (BL) and uncertain cases were examined by both until consensus was reached.

Statistical methods

All data were processed using the SPSS software (11.5, SPSS Inc., Chicago, Ill., USA). Salivary MS counts were subjected to the Kruskal-Wallis non-parametric test, while caries data were tested by a proportional odds model for ordinary data. Associations were assessed with the Pearson correlation coefficient and odds ratio calculations were performed on the categorized data 'colonized' vs. 'non-colonized' and 'decayed' or 'non-decayed' subjects, respectively. A P value of less than 0.05 was considered as statistically significant.

Results

At the age of 3 years, 75% of all the children had non-detectable levels (score 0) of salivary MS. Distribution of the salivary MS counts in the different groups is given in Table 2. Fewer children in groups A and B exhibited medium and high counts of salivary MS than children in group C, but the differences were not statistically significant. The salivary MS levels in groups A and B were of

Table 2. Prevalence and distribution of salivary mutans streptococci (MS) counts in 3-year-old offspring of mothers undergoing intervention with various chewing gums

	Group A xyl (n = 56)	Group B CHX/xyl/sorb (n = 44)	Group C F/xyl/sorb (n = 51)	Group D reference (n = 230)	Group E drop-outs (n = 22)
Children's MS score (%)					
Score 0	78	70	64	80	54
Score 1	9	14	14	8	14
Score 2	4	9	12	6	23
Score 3	9	7	10	6	9

Statistically significant difference in MS distribution, A vs E ($P < 0.001$) and B vs E ($P < 0.05$).

Table 3. Caries prevalence in 3-year-old offspring of mothers undergoing intervention with various chewing gums

	Group A xyl ($n = 56$)	Group B CHX/xyl/sorb ($n = 44$)	Group C F/xyl/sorb ($n = 51$)	Group D reference ($n = 230$)	Group E drop-outs ($n = 22$)
Caries-free (%)	93	86	86	95	77
Non-cavitated lesions (%)	5	9	8	3	14
Cavitated lesions (%)	2	5	10	3	14
Defs (mean ± *s*)	0.11 ± 0.49	0.16 ± 0.43	0.37 ± 1.26	0.17 ± 1.11	0.82 ± 2.26

the same magnitude as those in the reference group (D). Caries prevalence was low and as few as 8% of all children had non-cavitated and/or cavitated lesions at the age of 3 years. The detailed figures in the different groups are presented in Table 3. An obvious tendency towards less caries in the high-xylitol groups A and B was found in comparison with group C, but again the differences failed to reach statistical significance. Caries prevalence in the reference group D was similar to those obtained in the intervention groups A and B.

The associations between early MS establishment and dental caries development for the children in the three intervention groups are presented in Table 4. The correlation coefficient was 0.60 with an odds ratio of 12.3 (95% CI: 3.8–40.0; $P < 0.01$). The corresponding values for the children of the non-intervention group D were 0.34 and 6.0 (95% CI: 1.6–58.6; $P < 0.05$), respectively.

The microbial data and clinical caries findings of the drop-out group (E) are given in Tables 2 and 3. This group exhibited statistically higher counts of salivary MS compared with the experimental groups A ($P < 0.01$) and B ($p < 0.05$), but not with group C. Moreover, the children in group E had significantly more caries ($P < 0.05$) than the children of group A.

Discussion

This primary object of study was to evaluate the possible influence of maternal xylitol consumption on the mother–child transmission of MS and caries development in the mothers' offspring. Previous studies have suggested a superior role of xylitol in this type of intervention, but it should be noted that control chewing gums were lacking (8–10). Likewise, as a non-chewing control group could not be included in the present study for ethical reasons, the xylitol and xylitol/sorbitol/chlorhexidine interventions had to be compared with a generally accepted and advocated chewing regime for risk patients containing fluoride. Although in agreement with Söderling et al. (8, 9) and Isokangas et al. (10), we found clear and similar trends following xylitol and chlorhexidine/xylitol/sorbitol consumption in the present study, we were unable to reject the null hypothesis. We demonstrated significantly less MS at the age of 18 months (14), but the differences in MS and caries were not statistically significant at the age of 3 years. These observations were more or less in agreement with the results of the recent study by Dasanayake and coworkers (5), who failed to demonstrate an anti-caries effect in children despite a significantly reduced mother–child transmission when the mothers were treated with a chlorhexidine-containing varnish. Compliance with the present chewing gum regime was checked during the course of the study and was considered to be acceptable. The total attrition rate was 13% and slightly more participants dropped out from group B than from groups A and C, because the taste was subjectively perceived as less attractive. Apart from the drop-out complaints, no side or adverse effects were noted with any of the chewing gums. The fact that the drop-outs agreed to the final examination gave us an opportunity to form an additional non-chewing group with children to mothers with high counts that did not comply with the program. This group represented mothers with very limited exposure to xylitol, sorbitol, chlorhexidine, and fluoride since they terminated the study protocol within 3 months. Interestingly, this group also exhibited the highest caries and salivary MS levels.

The possible main explanations for the non-significant findings were (i) the unexpectedly low prevalence of MS and caries (and large standard deviations) in the present study population, leaving the investigation with limited power to statistically verify possible differences between the groups, (ii) the xylitol dose was suboptimal, and (iii) the chewing regimen should have been extended past 18 months. Concerning the first point, the entire study group developed less caries (8% including non-cavitated lesions) than the 22% previously reported among Swedish 3-year-olds (18); this was not expected considering the selection of high-risk mothers. Also the proportion of children who harbored detectible levels of salivary MS was lower in our material when compared with our own previous findings

Table 4. Association between mutans streptococci (MS) colonization at the age of 18 months* and caries prevalence at the age of 3 years for the children in groups A, B, and C

	Initial and or manifest caries	
MS colonization	No	Yes
No	117	5
Yes	19	10

* Data from Thorild et al., 2003 (14).
Odds ratio = 12.3 (95% CI 3.8–40.0).

Table 5. Prevalence and distribution of salivary mutans streptococci (MS) counts and caries in the merged groups

	Group A + B High-xylitol gums ($n = 100$)	Group C + E Low-xylitol gums and drop-outs ($n = 73$)	P*
MS counts (%)			
Score 0	75	62	<0.05*
Score 1	13	14	
Score 2	6	15	
Score 3	6	8	
Defs (mean ± s)	0.13 ± 0.42	0.51 ± 1.61	<0.05*

* Kruskal-Wallis non-parametric test.

from the same city (19). In order to elucidate the impact of study group size with focus on xylitol, we merged the two high xylitol intervention groups (A and B; $n = 100$) and compared the findings with a merged low xylitol and drop-out group (C+E; $n = 73$). This could be justified by the fact that the gums in groups A and B contained more than 0.5 g xylitol each, while the fluoride gums had only half that amount and the drop-outs were non-complying high MS mothers that used xylitol gums for a very short period. The subsequent calculated results are given in Table 5. With these larger groups, a statistically significant difference ($P < 0.05$) in the children's MS counts and defs was obtained in favor of the high xylitol intervention. Nevertheless, the clinical differences were fairly small and our results might indicate that an antibacterial intervention directed to highly MS-colonized mothers with the aim of reducing caries in their children is not worth its cost in a low-caries community. On the other hand, it could be argued that the applied preventive measures in groups A and B were perhaps able to reduce the caries prevalence in the assumed high-risk groups to the same level as the assumed low-risk group, which will probably keep down future need and costs of dental care. Further and extended studies in high caries populations are needed to further elucidate this issue.

The second question regarding the suboptimal dose regime is still open. It has been suggested from clinical trials that approximately 5–10 g of xylitol per day in a fractioned administration is needed in order to prevent new cavities in the primary and young permanent dentition (12, 20, 21), and, clearly, the present xylitol dose of around 1–2 g was well below that. However, it can be argued that the reduced mother–child transmission of MS may be due to an ecological shift in the oral microflora from sticky xylitol-sensitive to less adhesive xylitol-resistant MS strains (22), and such shift has been demonstrated also with a low-dose regimen (23). Furthermore, significantly different but clinically small reductions of plaque acidogenicity and caries development have been described in preschool children with the same xylitol exposure as used here (24, 25). The present chewing gum intervention started when the children were 6 months of age and was terminated 1 year later. It could always be speculated whether this period should have been extended to 2 years of age, but the present very low general colonization rate may indicate that this would not have changed the results significantly.

The similar findings in groups A and B were logical and expected, since both gums contained the approximately same amount of xylitol. We found no indication that the addition of chlorhexidine to the gums either enhanced or compromised the transmission of MS or the anti-caries effect which was notable in the light of finding from previous mother–child studies utilizing chlorhexidine gels or varnishes (3–5). The reason for this is not clear, but may be explained by mechanisms of action. Chlorhexidine may primarily affect and suppress the metabolic activity of susceptible bacterial cells in the mothers and, secondarily, the adhesive properties of MS, while xylitol might affect the oral ecology by favoring the selection of xylitol-resistant and non-adhesive strains of oral streptococci with impaired colonizing abilities (22, 26, 27). Furthermore, the extent to which the presence of the nutrient sorbitol in groups B and C may have influenced the results remains an open question. The clear association between early MS acquisition and caries development demonstrated here was in agreement with previous findings (28, 29) and reinforces the concept that caries can be truly prevented by combating MS establishment in the oral cavity

In conclusion, the results of the present study could not demonstrate any statistically significant differences in the effects of maternal use of xylitol-, chlorhexidine/xylitol/sorbitol-, and fluoride/xylitol/sorbitol-containing chewing gums on MS and caries prevalence in 3-year-old children of mothers with high counts of salivary MS. Although a small clinical effect was seen as a result of the high xylitol regimes, it is questionable whether this type of targeted mother–child intervention is efficient in a low-caries community.

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