Composition of the salivary microflora during habitual consumption of fluoridated milk

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Engström K, Petersson LG, Sjöström I, Twetman S. Composition of the salivary microflora during habitual consumption of fluoridated milk. Acta Odontol Scand 2004;62:143–146. Oslo. ISSN 0001-6357.

The aim was to evaluate the effect of habitual consumption of fluoridated milk on the composition of the salivary microflora. The study group comprised 20 healthy schoolchildren and young adults with a mean age of 13.6 years and the investigation had a randomized double-blind crossover design with a washout period of 1 month. After professional tooth-cleaning at baseline, the subjects were supplied with either fluoridated (250 mL, 5 ppm F) or non-fluoridated milk for one daily intake during a period of 4 weeks. Salivary samples were collected immediately before tooth-cleaning and after 1, 2, and 4 weeks, respectively. The samples were immediately cultivated for total viable counts, oral streptococci, mutans streptococci, lactobacilli, and actinomyces spp. Bacterial counts were logarithmically transformed before statistical evaluation using ANOVA. No significant alterations of the salivary microflora were found during any of the milk regimens compared with baseline. There was a slight reduction in the proportion of mutans streptococci after 2 and 4 weeks during consumption with fluoridated milk but the difference failed to reach statistical significance. In conclusion, this study was unable to disclose any significant alteration of the composition of the salivary microflora following daily intake of fluoridated milk. □ *Actinomyces; fluoride milk; lactobacilli; mutans streptococci; saliva*

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It is generally understood that fluoride acts as an anticaries agent by interfering with the local balance between enamel demineralization and remineralization (1, 2). The most common source of fluoride is toothpaste, but alternative administration routes, such as fluoridated salt and milk, have gained motivated interest (3). Field studies utilizing fluoridated milk schemes for schoolchildren have been performed in several countries and with one exception (4), all demonstrated a substantial decrease in caries increment (5–7). In adjunct to the clinical studies, the pharmacokinetics of fluoride ingested with milk have been clarified (8).

Previous studies have suggested that habitual consumption of fluoridated milk results in fluoride levels in saliva and supragingival plaque that are equal to those obtained after a neutral sodium fluoride rinse (9–11). In addition, in vitro reports have suggested that milk with intrinsic fluoride may affect the oral ecology by increasing the pH in the oral biofilm and decreasing the demineralization of enamel (12, 13). The findings of Kertesz (14) & Pratten and co-workers (12) were of special interest, since they noted that fluoridated milk could reduce the counts of mutans streptococci in dental plaque. This possible influence on the bacterial composition of the oral biofilm merits further exploration in order to gain knowledge on the cariostatic events in connection with daily intake of fluoridated milk.

The aim of this study was to evaluate the effect of habitual consumption of fluoridated milk on the composition of the salivary microflora in schoolchildren and young adults. The null hypothesis was that the microflora would not differ from that obtained during consumption of non-fluoridated control milk.

Materials and methods

Subjects

The study group comprised 20 healthy schoolchildren and young adults (12 F, 8 M; 7–25 years) with a mean age of 13.6 years who volunteered to participate after they had been given verbal and written information. For subjects below the age of 18 years, the parents' consent was collected. The participants had good oral health, with very few fillings and no open cavities, and were inhabitants of a community with a low fluoride level in the piped drinking water (<0.1 ppm F).

Study design

The study had a randomized double-blind crossover design with a washout period of at least 1 month and was approved by the Ethics Committee at Umeå University. At baseline, the participants were professionally cleaned with a rotating brush and pumice paste, followed by thorough interdental flossing. Samples of chewing-stimulated whole saliva were collected immediately before cleaning and then after 1, 2, and 4 weeks. During this period, the participants were given 250 mL of either fluoridated (5 ppm F) or non-fluoridated (control) milk once daily together with the

DOI 10.1080/00016350410001487

evening meal in a randomized way. After the washout period, the procedure was repeated with the corresponding milk scheme. All subjects were encouraged to carry on with their normal diet and oral hygiene routines. During both experimental 4-week periods, the participants were provided with fluoride-free toothpaste (Weleda, Herbal tooth-gel; VEM) and instructed to use it in the normal way, while during the washout period the use of their regular fluoride-containing toothpaste was allowed. The outcome measure was total viable counts and the levels of oral streptococci, mutans streptococci, lactobacilli and actinomyces spp. during the two milk regimes as compared with baseline.

Preparation of milk

The designated milk was distributed to the participant twice a week during the experiment. From pre-prepared stock solutions, sodium fluoride (final concentration 5 ppm F) or non-fluoride aliquots were added to fresh low-fat milk (1.5%) in its original envelopes. The packages were marked with the subject's name to prevent their use by other family members. For the youngest subjects, the advocated intake was supervised by a custodian, but the participants were free to drink additional non-fluoridated milk ad lib during the experimental periods.

Saliva sampling and bacterial cultivation

Stimulated whole saliva was collected in the mornings, at least 2 h after breakfast and tooth-brushing. First, the subjects rinsed their mouth with water and then they were asked to chew on a piece of paraffin for 1 min. The obtained saliva was spat directly into a test tube that was immediately brought to the laboratory, thoroughly mixed and serial diluted in 10-fold steps in 0.05 M sodium phosphate buffer (pH 7.1). Thereafter 50 μL aliquots were placed in duplicate on the following plates: Trypticase/proteose-peptone-glucose agar (BBL, Boston, Mass., USA) supplemented with 4% horse blood, vitamin K and trace elements for detection of total viable counts, Mitis salivarius agar for total number of oral streptococci, Mitis salivarius bacitracin agar for enumeration of mutans streptococci (15), Rogosa SL agar for oral lactobacilli strains (16), and CNAC-20 agar for estimation of actinomyces (17). All agar plates were incubated at 37°C in aerobic conditions for an additional 3 days. The growth density was counted with the aid of a stereomicroscope (10–30× magnification). The strains were identified by morphological characteristics and expressed as colony forming units (CFU) per mL.

Statistical method

The data were subjected to analysis of variance for repeated measures and processed with SPSS (version 11.5, Chicago, Ill., USA). Bacterial counts were transformed into log 10 units before statistical analysis. The individual follow-up data were primarily compared with the baseline value within each group, but comparisons between the milk regimes were also made. A *P* value less than 0.05 was considered statistically significant.

Table 1. Baseline distribution streptococci, mutans streptococci, lactobacillus, and actinomyces (CFU/mL) in chewing stimulated saliva in 20 schoolchildren and young adults

	0	$>10^4$	$>10^5$	$>10^6$	$>10^7$	$\leq 10^7$
Streptococci spp.	–	–	2	7	8	3
Actinomyces spp.	–	2	12	6	–	–
Mutans streptococci	2	9	7	2	–	–
Lactobacilli	6	11	3	–	–	–

Results

The total viable growth in the stimulated saliva samples ranged from 7.3×10^5 to 8.9×10^{11} CFU/mL at baseline. Baseline data on the distribution of streptococci, actinomyces, and lactobacilli are given in Table 1. Two and 6 subjects displayed non-detectable counts of mutans streptococci and lactobacilli, respectively. The mean $\log_{10}$ values of oral streptococci and mutans streptococci at baseline and after 1, 2, and 4 weeks are presented in Table 2 and the corresponding figures concerning salivary lactobacilli and actinomyces levels are shown in Table 3. No statistically significant alterations of the composition of the salivary microflora were revealed in any group after 1, 2, and 4 weeks when compared to baseline and no differences existed between the fluoride and non-fluoride milk consumption periods. The proportion of salivary mutans streptococci in relation to the total number of oral streptococci ranged between 0 and 16.6% at baseline and the mean values are given in Table 4. There was a slight reduction of salivary mutans streptococci proportion after 2 and 4 weeks compared with baseline when the children were drinking fluoride milk, but the differences were not statistically significant. A slight reduction in the proportion of salivary mutans streptococci was observable also during the non-fluoride milk regime.

Table 2. Mean values ($\log_{10}$ CFU/mL $\pm$ *s*) of oral streptococci (OS) and mutans streptococci (MS) in stimulated whole saliva at baseline and after 1, 2, and 4 weeks during daily consumption of fluoridated or non-fluoridated milk

		Fluoride milk		Non-fluoride milk	
Time	*n*	OS	MS	OS	MS
Baseline	20	6.0 ± 1.2	2.5 ± 1.3	5.8 ± 0.5	2.8 ± 1.0
1 week	20	6.1 ± 0.6	3.2 ± 0.8	6.2 ± 0.5	3.1 ± 1.1
2 weeks	20	6.6 ± 0.4	3.0 ± 1.2	6.5 ± 0.3	3.0 ± 1.4
4 weeks	20	6.4 ± 0.5	3.0 ± 1.0	6.5 ± 0.3	2.8 ± 1.1

s = standard deviation.

Table 3. Mean values ($\log_{10}$ CFU/mL $\pm$ *s*) of oral lactobacilli (LB) and actinomyces (ACT) in stimulated saliva at baseline and after 1, 2, and 4 weeks during daily consumption of fluoridated- or non-fluoridated milk

		Fluoride milk		Non-fluoride milk	
Time	*n*	LB	ACT	LB	ACT
Baseline	20	1.4 ± 1.5	4.7 ± 0.5	1.8 ± 1.5	4.4 ± 0.6
1 week	20	1.7 ± 1.5	5.2 ± 0.6	1.7 ± 1.2	5.2 ± 0.8
2 weeks	20	1.0 ± 1.3	5.0 ± 0.5	1.7 ± 1.2	5.3 ± 0.5
4 weeks	20	1.5 ± 1.4	4.9 ± 0.6	1.8 ± 1.4	4.8 ± 0.7

s = standard deviation.

Discussion

This study was undertaken to examine the possible influence of daily consumption of fluoridated milk on the composition of the salivary microflora. Since milk fluoridation is mainly intended as a caries-preventive measure in school programs, we focused our evaluation of the salivary microflora on bacterial strains that are commonly associated with the carious process in childhood (18–20). Saliva samples were chosen to facilitate the collection of the follow-up sample in a standardized way and the cultivations were performed the very same day using conventional methods. To keep it simple, we applied selective media for identification of the target species, on the expectation that such media may only detect a limited fraction of the total cultivable flora. The milk intake was closely monitored by the children's custodians. Cooperation and compliance with the treatment protocol were excellent. No side or adverse effects were reported.

The main finding of this study was that the composition of the salivary microflora remained grossly unchanged during the fluoride milk consumption, and thus the null hypothesis could not be rejected. It is generally understood that the supragingival smooth surface plaque is dominated by streptococci and actinomyces species (21, 22) and consequently these bacteria were also most commonly recovered in our chewing stimulated saliva samples. The proportion of mutans streptococci in saliva was considered of special interest, since previous reports have shown a reduction of those bacteria in a biofilm model (12) and in dental plaque after intake of fluoridated (14) or probiotic milk (23). In our study, the total number of oral streptococci in saliva increased slightly with time during both the fluoride and non-fluoride milk periods as compared with baseline, while the mutans streptococci level remained constant. The proportion of salivary MS therefore decreased with time during both regimens, but the differences were not statistically significant compared to baseline. We were thus unable to verify the above-mentioned in vitro and in vivo findings, and we stress that the levels of the aciduric lactobacilli and actinomyces strains were unaltered throughout the study period.

Table 4. Proportion of salivary mutans streptococci in relation to total number of oral streptococci in stimulated saliva at baseline and after 1, 2, and 4 weeks during daily consumption of fluoridated- or non-fluoridated milk. Values are mean percent ± *s*.

Time	*n*	Fluoride milk	Non-fluoride milk
Baseline	20	1.9 ± 4.3	0.8 ± 1.3
1 week	20	0.4 ± 0.4	0.3 ± 0.4
2 weeks	20	0.2 ± 0.4	0.2 ± 0.4
4 weeks	20	0.2 ± 0.4	0.3 ± 0.4

s = standard deviation.

Recent clinical studies have suggested that cow's milk has a neutral or negative association with caries in children (24, 25) and its components of calcium, casein, and phosphoproteins may actively contribute to remineralization of lesions (26). Furthermore, the content of casein in milk may at the same time both prevent and enhance oral colonization of certain strains of streptococci and actinomyces (27). The addition of fluoride may very well add anti-caries properties to milk, but the present results suggest that this is due to other mechanisms than modifying the composition of the oral microflora. Although fluoride has certain antibacterial properties (28), there are reasons to assume that the fluoride concentration achieved in the oral cavity after the intake of fluoride milk were below those needed to slow down bacterial multiplication (29). However, our findings do not rule out the possibility that fluoride in milk may affect the metabolism or pathogenic potential of caries-associated bacteria in the dental plaque. Further studies on that matter are therefore justified.

In conclusion, this study was unable to disclose any significant alteration of the composition of the caries-associated salivary microflora following daily consumption of fluoridated milk, which may suggest that the anti-caries effect of fluoride in milk can be found elsewhere.

Acknowledgements.—The study was supported by grants from the Borrow Dental Milk Foundation, UK and the Swedish Patent Revenue Fund.

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Received for publication 25 February 2004
Accepted 15 April 2004