# Lactic Acid Formation in Supragingival Dental Plaque after Schoolchildren's Intake of Fluoridated Milk

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**Purpose:** Milk can be used as vehicle for fluoride administration. The aim of this study was to investigate the lactic acid formation in dental plaque after daily intake of fluoridated milk.

**Materials and Methods:** The study group consisted of 15 healthy schoolchildren, 6 – 15 years of age, in a double-blind crossover study design. After a one-week fluoride depletion period, 250 ml of fluoridated (5 ppm; total amount 1.25 mg F) standard milk or non-fluoridated control milk was consumed once daily together with an ordinary meal during 3 days of plaque accumulation with no oral hygiene. On the fourth day, plaque samples were collected immediately before a final milk intake and then after 30, 60 and 180 minutes. After a washout period of two weeks, the whole procedure was repeated with the corresponding fluoridated or non-fluoridated milk regimen. All samples were suspended and the sucrose-challenged lactic acid formation rate was determined enzymatically.

**Results:** The results showed a statistically significant ($p < 0.05$) increase of the lactic acid levels 30 min after the intake of the standard (control) milk while no such elevation was evident after the fluoride-containing milk. No differences were found after 60 and 180 min compared with baseline for any of the milks.

**Conclusion:** The findings suggest that fluoride added to milk may counteract the lactic acid formation that follows a non-fluoridated milk intake.

**Key words:** children, dental plaque, fluoride, lactic acid, milk

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It is evident that caries prevalence has declined during the recent decades in most industrialized countries (Bratthall et al, 1996). The main reason for this is probably the widespread use of fluorides that acts locally by decreasing enamel demineralization and enhancing enamel remineralization (Featherstone, 1999; ten Cate, 2000). The antimicrobial effect of fluoride on oral bacteria is also generally acknowledged although its clinical significance remains uncertain (Hamilton and Bowden, 1996). Without doubt toothpaste is the most common and important source of fluoride, but toothpaste is not available or is too expensive for the majority of the population in many countries and communities. Therefore, fluoridated milk schemes for schoolchildren have been implemented in a number of countries under the auspices of WHO, and field-studies in Hungary, Bulgaria and China have shown a substantial decrease in the increment of caries (for a review, see Bánóczy et al, 1996). In adjunct to the clinical studies, the pharmacokinetics of fluoride ingested with milk has been elucidated (Lennon et al, 1996).

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The anti-caries mechanism of fluoridated milk is not yet fully understood. Previous research has indicated that milk can be regarded as a vehicle of fluoride into the oral cavity (Phillips, 1991), and studies have been performed to investigate the concentration of fluoride in saliva and dental plaque in schoolchildren after standardized intake of fluoridated milk (Twetman et al, 1998; Petersson et al, 2002; Engström et al, 2002). The fluoride levels in saliva were found to equal those found after a sodium fluoride rinse with the same amount of fluoride, and the same relationship was found in newly-formed supragingival plaque (Petersson et al, 2002). Furthermore, recent findings suggest that plaque fluoride levels are directly related to the plaque calcium concentrations (Whitford et al, 2002), which is interesting in the light of the high content of calcium in milk. Thus, studies on plaque metabolism seem justified in order to gain further knowledge of the cariostatic events in connection with daily intake of fluoridated milk. Recent *in vitro* reports have suggested that milk with intrinsic fluoride may increase the pH in the oral biofilm and decrease the demineralization of enamel (Pratten et al, 2000; Kahama et al, 1998). The aim of the present *in vivo* study was to evaluate the metabolic activity in schoolchildren of early supragingival plaque formed under the influence of fluoridated milk. The null hypothesis was that lactic acid formation rates of plaque formed while fluoridated milk was ingested and of plaque formed while non-fluoridated milk was drunk would not differ.

## MATERIAL AND METHODS

### *Study Group*

The study group consisted of 15 healthy schoolchildren of both sexes (7 boys and 8 girls) that volunteered to participate. The mean age was 11.2 years with a range of 6 – 15 years. Consent from the children and their parents was obtained after providing written and verbal information. All the children had a non-compromised dental health with a mixed or permanent dentition with at least 20 erupted teeth. The participants lived in a community with low fluoride levels in the piped drinking water (< 0.2 ppm) and used fluoridated toothpaste (1,100 – 1,500 ppm F) once or twice a day. The Ethics Committee, Umeå University, approved the study protocol.

### *Study Design*

This experiment used a double-blind crossover design. After a fluoride-free period of 1 week, the participants were asked to refrain from all kinds of oral hygiene procedures for 3 days in order to accumulate supragingival plaque. During these three days, the participants drank either the fluoridated or non-fluoridated control milk once daily in a randomized order as described below. On the fourth day, the subjects were seen at the clinic two hours after their lunchtime meal. A baseline plaque sample was collected from the buccal surfaces of the upper incisors, canines and first premolars. The participants were then asked to drink their designated milk regimen and follow-up plaque samples were collected from the same sites after 30, 60 and 180 minutes respectively. After a washout period of two weeks with normal diet and oral hygiene routines, the fluoride-free week, 3-day plaque-accumulation period and the experimental procedure was repeated with the corresponding experimental or control milk regimen.

### *Sample Collection and Lactic Acid Assay*

Supragingival plaque samples were gently collected with a sterile explorer from the buccal surfaces of the selected teeth, pooled and transferred to plastic vials. The plaque samples were weighed on a microbalance, diluted in a modified Ringer solution (pH 6.2) and dispersed by sonication. Acid production was initiated by mixing equal volumes of the bacterial suspension with a MOPS reactive solution (3-[N-morpholino]propanesulfonic acid, pH 7.0), containing 2% sucrose, that was incubated for 10 min at 37°C and then stored on ice for an additional 5 min. The fermentation was stopped by centrifugation (2 min, 13,000 rpm) and the supernatant was withdrawn and stored frozen until further analyses. After thawing, L- and D-lactic acid concentration was determined enzymatically in a spectrophotometer (340 nm, Novaspec 11, Amersham pharmaciabiotech, Cambridge, UK) using the commercial Boehringer Mannheim kit (R-Biopharm GmbH, Germany) and expressed as formation rate, µmol/g/min.

### *Preparation and Intake of Milk*

The fluoridated milk was prepared by adding a concentrated aqueous solution of sodium fluoride to

**Table 1 Formation rate of lactic acid (μmol/g/min) in suspensions of supragingival dental plaque collected from 15 children at designated times before and after consumption of fluoridated (5 ppm F) and non-fluoridated milk**

| Time | Fluoridated milk | | Standard milk | |
|---|---|---|---|---|
| | mean | ± SD | mean | ± SD |
| baseline | 8.3 | 3.4 | 7.3 | 2.1 |
| 30 min | 8.0 | 3.2 | 8.8* | 3.6 |
| 60 min | 7.7 | 2.1 | 7.9 | 2.9 |
| 180 min | 7.2 | 3.9 | 7.6 | 3.7 |

* statistically significant from baseline, $p < 0.05$, Wilcoxon signed rank test

fresh standard milk (3% fat, 5% carbohydrates). During the 3-day plaque accumulation periods, 250 ml milk containing 1.25 mg F (5 ppm), or a non-fluoridated control, was served at home in a drinking glass together with the regular evening meal. The fluoridated and control milk were provided in the original packages and neither the participants nor the investigators were aware of its fluoride content. On the sampling days at the clinic, 250 ml of the milk was served without a meal immediately after the baseline plaque collection.

### *Statistical Method*

The lactic acid formation rate in the post-ingestion plaque samples was compared with the baseline value within each regimen with non-parametric Wilcoxon signed rank test using the SPSS 11.5 software program (SPSS inc. Chicago, IL, USA).

## RESULTS

The lactic acid formation rate at baseline and at the designated time intervals after the intake of the fluoridated and non-fluoridated (control) milk regimens is presented in Table 1. A statistically significant increase ($p < 0.05$) in lactic acid formation rate was recorded 30 minutes after the control milk intake whereas no such elevation took place after consumption of the fluoridated milk. The lactic acid formation diminished after 60 and 180 minutes following intake of both test and control milk although no differences were found compared with baseline. The event is illustrated graphically in Fig 1 in which the post-ingestion lactic acid formation was indexed to baseline. No effects regarding the order of intake were disclosed.

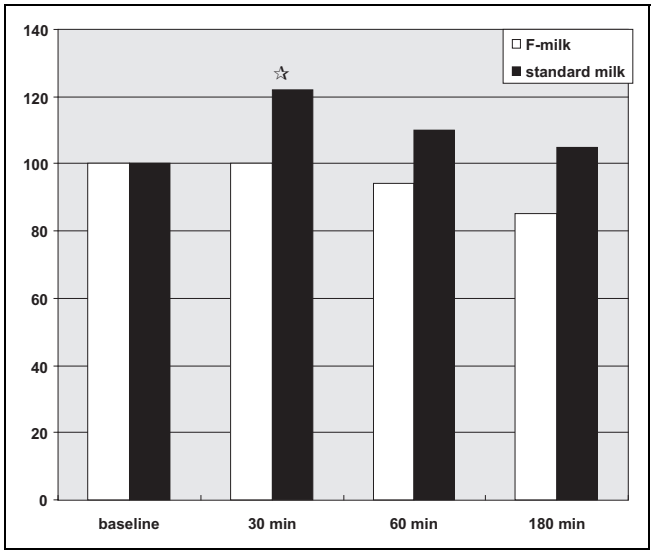


**Fig 1** Total formation rate of lactic acid in suspensions of supragingival dental plaque before (base- line) and after intake of fluoridated (5 ppm F) and non-fluoridated milk. Baseline values were indexed as 100. The star denotes a statistically significant difference compared to baseline ($p < 0.05$).

## DISCUSSION

The present study was undertaken to investigate the possible effect of the presence of fluoridated milk during plaque formulation on the acid formation rate of supragingival dental plaque exposed to sucrose. Prior to the samplings, plaque was allowed to accumulate during a 3-day period without any self-performed oral hygiene measures. The study group consisted of schoolchildren, which is the primary target group for a fluoridated milk scheme. The milk was consumed without any particular instructions in connection with a regular meal and the amount of fluoride in the milk was chosen to resemble the regimen of several clinical anti-caries studies (Gyurkovics et al, 1992; Pakhomov et al, 1995). The lactic acid assays were performed *in vitro* after sucrose addition according to a standard procedure, thereby reflecting the cariogenic potential of the plaque samples. We quantified both the L- and D-isomers of lactic acid but since both isomers exhibited the same pattern they were added in the present communication. It has previously been shown that the L-isomer is the dominating acid (approximately 2/3) in sugar-challenged plaque suspensions (Geddes, 1975; Borgström et al, 2000), which was in agreement with our findings (data not shown).

More lactic acid was formed in the plaque suspensions collected immediately after the intake of standard milk compared with those collected after the ingestion of fluoridated milk. This was a novel finding and the difference was statistically significant up to 30 minutes after intake. Thus, the null hypothesis was rejected. It seems reasonable to assume that the presence of fluoride in the plaque to some extent may affect the enolase activity, the key enzyme for bacterial glycolysis. Although it might be questioned if the fluoride concentration is high enough for enzyme inhibition, highly elevated plaque fluoride has been demonstrated in some individuals after intake of fluoridated milk (Engström et al, 2002). This initial slow-down of acid production may be of clinical importance since less acid formed means a diminished local pH drop and an increased time for the fluoride-mediated remineralization process. Thus, the findings of our experiment were mainly supportive to the *in vitro* findings of Kahama et al (1998) and Pratten et al (2000).

After 60 and 180 minutes, no differences in acid formation rate between the fluoridated and non-fluoridated milk regimens were observed, which was considered of less importance from a clinical point of view since the pH-drop normally recovers within 30 minutes after a meal. However, it must be emphasized that the individual variations were considerable, probably reflecting salivary clearance and the oral microflora and plaque composition of the individual. Thus, the 3-day plaque communities seemed to differ remarkably in complexity and succession although a community with few species and a high relative number of $\alpha$-haemolytic streptococci would have been expected (Socransky et al, 1977).

In conclusion, the present findings suggest that the presence of fluoride in milk may counteract carbohydrate-mediated lactic acid formation in suspensions of supragingival dental plaque, and that fluoridated milk may slightly alter the supragingival plaque ecology. Thus, the findings contribute to the possible explanations for the clinically established anti-caries properties of fluoridated milk programs for schoolchildren.

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## REFERENCES

1. Bánóczy J, Stephen KW, Pakhomov GN. Milk fluoridation for the prevention of dental caries. WHO, Geneva 1996:46-71.
2. Borgström MK, Edwardsson S, Svensäter G, Twetman S. Acid formation in sucrose-exposed dental plaque in relation to caries experience in schoolchildren. Clin Oral Invest 2000;4: 9-12.
3. Bratthall D, Hänsel-Petersson G, Sundberg H. Reasons for the caries decline: what do the experts believe? Eur J Oral Sci 1996;104:416-422.
4. ten Cate JM. Current concepts on the theories of the mechanism of fluoride. Acta Odontol Scand 2000;57:325-329.
5. Engström K, Petersson LG, Twetman S. Fluoride concentration in supragingival dental plaque after a single intake or habitual consumption of fluoridated milk. Acta Odontol Scand 2002;6:311-314.
6. Featherstone JDB. Prevention and reversal of dental caries: role of low level of fluoride. Community Dent Oral Epidemiol 1999;27:31-40.
7. Geddes DAM. Acid produced by human dental plaque in situ. Caries Res 1975;9:98-109.
8. Gyurkovics C, Zimmerman P, Hadas E, Bánóczy J. Effect of fluoridated milk on caries: 10-year results. J Clin Dent 1992; 3:121-124.
9. Hamilton IR, Bowden GHW. Fluoride effects on oral bacteria. In: Fejerskov O, Ekstrand J, Burt BA (eds). Fluoride in dentistry, 2nd ed. Copenhagen: Munksgaard 1996;230-251.

10. Kahama RW, Damen JJ, ten Cate JM. The effect of intrinsic fluoride in cow's milk on in vitro enamel demineralization. Caries Res 1998;32:200-203.
11. Lennon MA, Bian JY, Kolesnik AG, Kuzmina EM, Pakhomov GN, Phillips PC, Marthaler T, Villa A, Möller I. Legislation and community based aspects of the implementation of a milk fluoridation programme. Geneva: WHO 1996;72-96.
12. Pakhomov GN, Ivanova K, Möller IJ, Vrabcheva M. Dental caries-reducing effects of a milk fluoridation project in Bulgaria. J Publ Health Dent 1995;55:234-237.
13. Petersson LG, Arvidsson I, Lynch E, Engström K, Twetman S. Fluoride concentration in saliva and dental plaque in young children after intake of fluoridated milk. Caries Res 2002;36: 40-43.
14. Phillips PC. Fluoride availability in fluoridated milk systems. Caries Res 1991;25:237.
15. Pratten J, Bedi R, Wilson M. An in vitro study on the effect of fluoridated milk on oral bacterial biofilms. Appl Environ Microbiol 2000;66:1720-1723.
16. Socransky SS, Manganiello AD, Propas D, Oram V, van Houte J. Bacteriological studies of developing supragingival plaque. J Periodontol Res 1977;12:90-106.
17. Twetman S, Nederfors T, Petersson LG. Fluoride concentration in whole saliva and separate gland secretions in schoolchildren after intake of fluoridated milk. Caries Res 1998;32: 412-416.
18. Whitford GM, Wasdin JL, Schafer TE, Adair SM. Plaque fluoride concentrations are dependant on plaque calcium concentrations. Caries Res 2002;36:256-265.