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SHORT COMMUNICATION

# A randomized controlled trial: the efficacy of eluoride rinse combined with calcium pre-rinse to increase overnight salivary fluoride

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**Abstract**

***Background.*** Previous studies have shown that a calcium (Ca) pre-rinse given before a 228 ppm fluoride (F) rinse greatly increased salivary fluoride. ***Objectives.*** The aim of this randomized controlled trial is to examine if Ca pre-rinse could increase the fluoride concentration in the overnight unstimulated saliva after a 905 ppm F-rinse. ***Materials and methods.*** Pre-rinses containing 150 mM, 75 mM or 0 mM Ca-lactate prepared by a validated pharmaceutical cGPM procedure were tested by nine subjects in a randomized order immediately followed by a 905 ppm F-rinse. The fluoride concentration was measured in unstimulated saliva collected 10 h later. ***Results and conclusions.*** The Ca pre-treatment significantly increased F level in overnight saliva following the 905 ppm fluoride rinse by 1.7× relative to the 905 ppm F-rinse alone; however, a significant effect was only observed with the highest (150 mM) Ca concentration as pre-rinse. ***Clinical relevance.*** High concentration F rinses (905 ppm) are commonly recommended for patients at high-risk of caries. A pre-treatment with high levels of Ca may further improve the cariostatic effect of this ion.

**Key Words:** *calcium, fluoride, pre-rinse, saliva, RCT*

## Introduction

Fluoride (F) topical agents are a primary component of the non-invasive management of initial caries lesions in modern dentistry. The concentrations of fluoride in plaque fluids and saliva appear to be critical for enamel remineralization [1–4]. Because the formation of bioavailable stores of F that control these fluid F levels are dependent on the availability of calcium it is not unexpected that studies have shown that a calcium pre-rinse can elevate the fluoride levels in oral fluids after a 228 ppm F rinse [5–8]. Given the fast clearance of F after tooth brushing [9,10], the high F rinse (905 ppm) is recommended by the Swedish National Guidelines for Adult Dental Care since 2011 to patients showing an increased risk of developing caries or showing signs of an active caries disease [11]. Whether a calcium pre-rinse has any effect at the high concentration F rinse is unknown. The purpose of this randomized clinical trial (RCT) was to determine if calcium pre-rinse could increase salivary F after a 905 ppm F rinse above what could be obtained with the F rinse alone. A secondary aim was to assess differences in terms of taste and smell acceptability between calcium lactate rinses.

## Materials and methods

### *Study design*

The study was performed at the Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden and was approved by the Research Ethics Committee in Stockholm (2011/820-31/3) and by the Swedish Medical Products Agency (EudraCT 2011-001885-16).

The three calcium rinses were administered in the evening immediately before the fluoride rinse, using a double blind (observer, analyst and subject blind) cross-over design. Unstimulated saliva was then collected the following morning (as described below) ~10 h after the double rinse.

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*Test subjects*

The subjects were recruited by local advertisement at the Department of Dental Medicine in Huddinge, Sweden. Inclusion criteria were: ≥18 years of age, ≥10 natural teeth, willing to refrain from fluoride containing products (excluding toothpaste) from 3 days prior to and throughout the study, informed signed consent. Exclusion criteria were: less than 10 natural teeth, reduced cognitive capability, reduced understanding of Swedish, ongoing oral or systemic infections, pregnancy or breast-feeding. The number of subjects chosen for the study was based on an examination of data from similar studies found in the literature [12]. Nine volunteers (two men and seven women) aged between 29–64 years (mean = 48) were included in the final study.

*Pharmaceutical preparations and randomization procedure*

Three test pre-rinse solutions contained 150 mM, 75 mM or 0 mM of calcium lactate, respectively, supplemented with 0.1% methyl parahydroxybenzoate (E218) as a preservative, and were prepared under conditions and practices required by the cGMP regulations with quality assurance by Apotek Produktion & Laboratorier AB, Stockholm Sweden (MA 24:2010/505427). The three test solutions were identical in appearance, containing a clear colorless solution. The calcium lactate content was confirmed by ion chromatography according to the USP/IC standard. The solutions passed microbiology control for aerobic bacteria, fungi and E-coli. Bottles containing the test solutions were labeled and randomized in a cross-over design by the manufacturer so that the observer, the subjects and the analysts were unaware of the identity of the test materials. The 905 ppm F mouth rinse solution (Dentan 0.2% NaF) was provided by Meda, Solna Sweden. All rinse solutions were 20 mL in volume and the rinsing duration was 1 min.

*Rinse administration and sampling procedure*

The day before the study the subjects were instructed to avoid tea and fluoride toothpaste. Tooth brushing was performed, without dentifrice, in the morning and 2 h before the use of the rinses in the evening. No eating, drinking or oral hygiene measures were allowed after the last brushing and Ca pre-rinse/fluoride rinse until after saliva sampling the next morning. The subjects performed the rinsing procedures unsupervised, and were instructed to do this just before going to bed. Unstimulated saliva was collected in the morning by drooling into a cup for 5 min or until 0.5 ml was obtained. The salivary secretion rate was determined by weighing the cup before and after collection. The washout period between rinses was 3 or more days.

*Fluoride analysis*

The saliva was centrifuged (5 min, 2°C, 1,466 rad/s) and the clear supernatant was transferred to 500 μL vials (Eppendorff) and diluted 9:1 with TISAB III (Termo Fisher Scientific, Beverly, MA). Diluted samples were kept frozen until fluoride analysis. The fluoride concentration in saliva samples were analyzed using an inverted electrode apparatus [13].

*Questionnaire*

The participants filled in a questionnaire after each sampling occasion in which they were asked to rate the smell and taste of the test rinse by a 5-graded scale (very pleasant, pleasant, neither pleasant nor unpleasant, unpleasant or very unpleasant), and to state whether they would consider accepting a daily rinse (yes/no). The participants were also interviewed to insure compliance with rinse instructions and to inquire if they had experienced any symptoms or side-effects due to the use of these rinses.

*Statistics and data handling*

Fluoride levels in saliva were examined by a one-way ANOVA, repeated measures design. Results from the questionnaire were analyzed by Fisher's exact test. The level of significance was set to $p < 0.05$.

## Results

The salivary flow rate (Table I) ranged between 0.1–0.8 g/min and did not significantly differ between the sampling occasions. The overnight fluoride levels are shown in Figure 1. After the pre-rinse with control (0 mM Ca) and 905 ppm F-rinse, the salivary fluoride level increased by nearly 5-times. All fluoride rinses significantly increased salivary F with respect to baseline ($p < 0.001$), with the 150 mM Ca producing the greatest (8×) increase. The 150 mM Ca-lactate pre-rinse, but not the 75 mM test rinse, significantly increased salivary F relative to the control (ANOVA planned comparisons; $p = 0.01$) (Figure 1).

There were no significant differences in the scoring of taste, smell and acceptability of the pre-rinses (control, 75 and 150 mM Ca-lactate; $p > 0.05$). One subject rated the smell as unpleasant for the

Table I. Salivary mass (g/min).

| | Mean | SD |
|---|---|---|
| Baseline | 0.35 | 0.22 |
| Control | 0.36 | 0.28 |
| 75 mM | 0.38 | 0.29 |
| 150 mM | 0.40 | 0.29 |

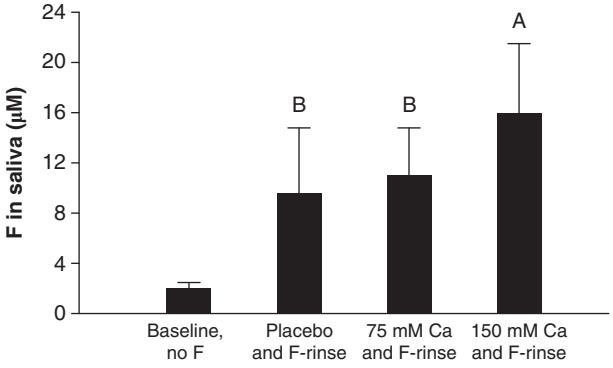


Figure 1. Fluoride in unstimulated centrifuged saliva at baseline or after pre-rinse with control rinse and F, 75 mM Ca lactate and F, or 150 mM Ca lactate and F. Significant differences ($p < 0.05$) indicated by capital letters. The error bars refer to the standard deviation.

75 and 150 mM solutions, whereas none rated the control as having an unpleasant smell. Three subjects rated the taste of the 75 and 150 mM solutions as unpleasant, compared to two for the control. Three subjects stated that they could not consider a regime of daily rinses for these solutions, whereas the corresponding number for the control was two. Two subjects were both critical of the taste and daily rinses.

## Discussion

The NaF (905 ppm F) preparation used here has become one of the most widely used mouth rinses in patients who are at high risk for dental caries in Sweden [11]. The 5-fold increase observed in overnight F with the control pre-rinse relative to baseline was very similar to the increase observed in another study [14]. More importantly, the data indicated that the F-concentration could be elevated to to 8-times over the baseline with the 150 mM calcium lactate pre-rinse. Given the inverse relationship between caries and fluoride concentration in the oral fluids [15–17], this suggests that a 150 mM Ca lactate pre-rinse should further improve the cariostatic effects of the 905 ppm F. However, the data also demonstrates, as noted in previous studies with a 228 ppm F rinse [12], that the increase in salivary F appears to be highly dependent on the pre-rinse Ca-concentration. Unfortunately, in comparison to the 5.5–9-fold F increases previously found with the 150 mM Ca pre-rinse [7,12], only a rather small, ~1.7× increase, was found here. The reason for this smaller effect is unclear, but it may be related to substances in the rinses (methylparahydroxibensoat and macrogol lauryl ether) that could have slowed the formation of oral F deposits that maintain salivary F levels and the fact that the ratio between the concentration of applied F and Ca pre-rinse increased from 1.5 to 6 units (ppm/mM) in the current study.

The mechanism behind the prolonged presence of fluoride when combined with calcium supply has been suggested to be the formation of Ca-F complexes, which would constitute a desirable slow release compartment of fluoride in the oral cavity as they attach to dental hard tissue [18–24], biofilm [24] and mucosa [20] after topical application.

The acceptability of the test rinses did not differ significantly between Ca-lactate concentrations and in fact the authors believe that flavor additives could be used to improve the acceptability of these pre-rinses.

Effective process validation contributes significantly to assuring drug quality. Here oral rinses of cGMP-standard were used throughout the study, which is an important strength in this study, and the randomization and cross-over design of test-rinses is an additional strength. Based on the result, we therefore suggest that the use of a concentrated calcium pre-rinse prior to a 905 ppm F-rinse increases the overnight concentration of salivary F relative to the 905 ppm F-rinse alone. Further research with a larger subject population is warranted and such research should also investigate the possibility of obtaining a higher overnight F concentration by using a higher Ca pre-rinse concentration and of utilizing preservatives carefully chosen to avoid potential interference. Ultimately, a RCT in caries-active patients will be needed in order to verify the cariostatic effect of combining calcium pre-rinse and fluoride.

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## References

[1] Arends J, Christoffersen J. Nature and role of loosely bound fluoride in dental caries. J Dent Res 1990;69:601–5; discussion 34–6.
[2] Featherstone JD, Glena R, Shariati M, Shields CP. Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. J Dent Res 1990;69:620–5; discussion 34–6.
[3] Margolis HC, Moreno EC. Physicochemical perspectives on the cariostatic mechanisms of systemic and topical fluorides. J Dent Res 1990;69:606–13; discussion 34–6.
[4] Ten Cate JM. In vitro studies on the effects of fluoride on de- and remineralization. J Dent Res 1990;69:614–19; discussion 34–6.

[5] Chow LC, Takagi S, Carey CM, Sieck BA. Remineralization effects of a two-solution fluoride mouthrinse: an *in situ* study. J Dent Res 2000;79:991–5.
[6] Vogel GL, Schumacher GE, Chow LC, Takagi S, Carey CM. Ca pre-rinse greatly increases plaque and plaque fluid F. J Dent Res 2008;87:466–9.
[7] Vogel GL, Chow LC, Carey CM. Calcium pre-rinse greatly increases overnight salivary fluoride after a 228 ppm fluoride rinse. Caries Res 2008;42:401–4.
[8] Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res 2010;89:1187–97.
[9] Mystikos C, Yoshino T, Ramberg P, Birkhed D. Effect of post-brushing mouthrinse solutions on salivary fluoride retention. Swed Dent J 2011;35:17–24.
[10] ten Cate JM. Contemporary perspective on the use of fluoride products in caries prevention. Br Dent J 2013;214:161–7.
[11] Lofstrom B, Backlin C, Sundstrom C, Hellstrom-Lindberg E, Ekbom A, Lundberg IE. Myeloid leukaemia in systemic lupus erythematosus–a nested case-control study based on Swedish registers. Rheumatology 2009;48:1222–6.
[12] Vogel GL, Chow LC, Carey CM, Schumacher GE, Takagi S. Effect of a calcium prerinse on salivary fluoride after a 228-ppm fluoride rinse. Caries Res 2006;40:178–80.
[13] Vogel GL, Carey CM, Chow LC, Ekstrand J. Fluoride analysis in nanoliter- and microliter-size fluid samples. J Dent Res 1990;69:522–8; discussion 56–7.
[14] Gabre P, Birkhed D, Gahnberg L. Fluoride retention of a mucosa adhesive paste compared with other home-care fluoride products. Caries Res 2008;42:240–6.
[15] Gaugler RW, Bruton WF. Fluoride concentration in dental plaque of naval recruits with and without caries. Arch Oral Biol 1982;27:269–72.
[16] Schamschula RG, Sugar E, Un PS, Toth K, Barmes DE, Adkins BL. Physiological indicators of fluoride exposure and utilization: an epidemiological study. Community Dent Oral Epidemiol 1985;13:104–7.
[17] Nobre dos Santos M, Melo dos Santos L, Francisco SB, Cury JA. Relationship among dental plaque composition, daily sugar exposure and caries in the primary dentition. Caries Res 2002;36:347–52.
[18] ten Cate JM, Featherstone JD. Mechanistic aspects of the interactions between fluoride and dental enamel. Crit Rev Oral Biol Med 1991;2:283–96.
[19] Ogaard B. CaF(2) formation: cariostatic properties and factors of enhancing the effect. Caries Res 2001;35:40–4.
[20] Vogel GL. Oral fluoride reservoirs and the prevention of dental caries. Monogr Oral Sci 2011;22:146–57.
[21] Rose RK, Shellis RP, Lee AR. The role of cation bridging in microbial fluoride binding. Caries Res 1996;30:458–64.
[22] Tenuta LM, Cerezetti RV, Del Bel Cury AA, Tabchoury CP, Cury JA. Fluoride release from CaF2 and enamel demineralization. J Dent Res 2008;87:1032–6.
[23] Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries: role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). J Clin Pediatr Dent 2004;28:203–14.
[24] Buzalaf MA, Pessan JP, Honorio HM, ten Cate JM. Mechanisms of action of fluoride for caries control. Monogr Oral Sci 2011;22:97–114.