Fluoride levels in saliva and dental plaque after consumption of snacks prepared with fluoridated salt

H. BJÖRNSTRÖM, S. NAJI, D. SIMIC, I. SJÖSTRÖM, S. TWETMAN

ABSTRACT. Aim To determine the concentration of fluoride in saliva and supragingival dental plaque at designated time intervals after consumption of snacks prepared with a standardised amount of fluoridated salt. Study design The investigation had a single blind prospective crossover design. Methods A group of 11 healthy young adults volunteered to participate after verbal and written information and consent. After a 1-week fluoride depletion period, the subjects consumed popcorn prepared with either fluoridated (250 mg/kg) or non-fluoridated salt during 30 minutes. Unstimulated whole saliva and samples of supragingival plaque were collected before consumption (baseline) and at 30, 60 and 120 minutes after the intake. Fluoride concentration was determined with a fluoride-specific electrode and the post-ingestion levels were compared with baseline by ANOVA. Results In saliva, the mean fluoride concentrations at baseline ranged from 0.021 to 0.027 mg/L and after the 30 minutes consumption of fluoride prepared snacks a 15-fold increase (p<0.001) was found. The same pattern was disclosed in the plaque samples. In both saliva and plaque, the fluoride levels remained significantly elevated after 2 hours (p<0.001 and p<0.05, respectively). Conclusion Consumption of snacks prepared with fluoridated table salt resulted in significantly increased fluoride levels in saliva and supragingival plaque for a period of at least two hours.

KEYWORDS: Fluoride, Plaque, Saliva, Salt, Snack consumption.

Introduction
Fluoridated table salt (F-salt) for domestic use is a well established caries preventive measure in many countries worldwide [WHO, 1994]. Recent clinical studies from Mexico and Jamaica have demonstrated significant caries reductions in children paralleled with the introduction of community based salt fluoridation programs [Irigoyen and Sanchez-Hinojosa, 2000; Estupiñán-Day et al., 2001; Meyer-Lueckel et al., 2002] and similar findings are available from Europe [Toth, 1978; de Crousaz et al., 1985; Cahen et al., 1993; Menghini et al., 1995; Fabien et al., 1996; Schulte et al., 2001]. Traditionally, F-salt has been regarded as a systemic and safe form of fluoride supplementation [Bergmann and Bergmann, 1995] and the urinary excretion of fluoride in children consuming F-salt (250 ppm F) is well established [Obry-Musset et al., 1992; Warpeha and Marthaler, 1995; Marthaler et al., 1995].

Less interest has, however, been focused on the local events following F-salt ingestion. In a recent study, Macpherson and Stephen [2001] have investigated the fluoride concentration in saliva after consumption of baked food products prepared with F-salt. They found small but significant elevations of fluoride for a limited period of time, indicating that frequent intake of fluoridated food might help to sustain potentially cariostatic fluoride levels in the oral cavity. Such an eating habit is strongly encouraged by snacks such as potato chips and popcorn that are very popular among children and adolescents. Our hypothesis was, therefore, that if such snacks were salted with F-salt, a rapid fluoride increase could be prolonged over an extended period of time. The aim of this study was therefore to investigate the concentration of fluoride in saliva and dental plaque at designated time intervals after ingestion of popcorn prepared with a standardised amount of F-salt. The null hypothesis was that fluoride levels would not differ from those obtained after the same snack prepared with non-F-salt.
Materials and methods
A group of 11 healthy young adults (6 males, 5 females, aged 21-27 years) volunteered to participate after verbal and written information. They all had a non-compromised dental health and were free from chronic medication. They were all inhabitants of a community with low fluoride levels (<0.3 ppm) in the piped drinking water.

Study design. The investigation had a single blind prospective crossover design as shown in Figure 1, and the study protocol was approved by the local Ethic Research Committee at Umeå University. Two weeks before the experiment, each participant was supplied with a fluoride-free tooth paste (Weleda, Herbal tooth-gel, VEM) with instructions to use it for a 7-day run-in period. The subjects were also asked to avoid fluoride-rich food products such as tea, fish and mineral water. Two days before the experimental day the participants were asked to refrain from all kind of oral hygiene in order to accumulate supragingival dental plaque. At baseline, an unstimulated saliva sample and a plaque sample were collected. Thereafter, the subjects were given a bowl of popcorn salted with either fluoride-containing salt (Heide Jodsalz mit Fluorid, Kali und Salz GmbH, Kassel, Germany: 250 mg/kg potassium fluoride ±15%) or non-fluoridated normal iodine salt. The popcorn was prepared from 1.0 dl of non-popped corn and two teaspoons (6.0 g) of salt and 15 mL of vegetable oil. The subjects were told to eat the entire content of the bowl during a 30 minutes period, after which follow-up samples of unstimulated saliva and plaque were collected. The sampling procedure was repeated after 60 and 120 minutes. The experimental periods were separated by a washout period of at least 10 days and the participants were unaware of which kind of salt they had consumed.

Saliva and plaque sampling. Unstimulated (resting) whole saliva was collected over a 5-minute period. The subjects were asked to sit in a relaxed position and let the saliva accumulate in the floor of the mouth and, as passively as possible, spit it out into a plastic tube. The supragingival plaque was collected with the aid of a dental explorer and pooled from one quadrant at a time. The quadrants were chosen at random, so one was used for the baseline and the three remaining for the follow-up sampling. All samples were weighed in a microbalance and stored frozen in -70ºC until further analysis.

Fluoride determinations. 1.0 mL of the collected saliva was transferred with a pipette into an Eppendorf tube together with 100 µL TISAB buffer. After mixing, the samples were let to rest for 15 minutes and centrifuged for 5 minutes (13,000 rpm).

Fig. 1 - Diagram showing crossover study design to assess fluoride concentrations in saliva and plaque after ingestion of fluoridated and non-fluoridate salt when ingested with popcorn.
The clear supernatant was then used for the fluoride determination. The concentration of fluoride in saliva was quantified with a fluoride sensitive electrode (96-09, Orion Research, Cambridge, MA, USA), standardised in the range of 0.1-5.0 µmol/L F according to Ekstrand [1977]. The precision of the method, expressed as coefficient of variation, was 7.0%.

In the plaque samples, the fluoride content was determined by a diffusion method described by Taves [1968]. After thawing, the sample was suspended in 2.0 ml buffer and placed in a diffusion vessel with constant stirring for 6 hours at room temperature in sealed Petri dishes together with 2.0 mL of 4 mol/L HClO4 saturated with hexamethyldisiloxane (HF). HF was adsorbed to the lids that were spotted with 50 mL 0.5 mol/L NaOH. Following diffusion, the lids were dried with a dessicator. The dried layer was dissolved with 50 mL 0.5 mol/L HCl and 100 mL HAc buffer (pH 5.0) and fluoride concentration was then measured with the electrode and expressed as mg F/L, equivalent to ppm. All determinations were made in duplicate. The precision of the method was verified by repeated measurements and was calculated to ±3% above 10 mg F, ±5% between 2.5-10 mg F and ±10% at concentrations below 2.5 mg F.

Statistical method. The obtained data were processed with the SPSS 11.0 software program (Chicago, Illinois, USA). The post ingestion values were compared with the baseline levels with analysis of variance (ANOVA) for repeated measures.

<table>
<thead>
<tr>
<th>Time</th>
<th>standard salt mean ±SE</th>
<th>fluoridated salt mean ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.027 0.007</td>
<td>0.021 0.004</td>
</tr>
<tr>
<td>30 min</td>
<td>0.033 0.004</td>
<td>0.338* 0.054</td>
</tr>
<tr>
<td>60 min</td>
<td>0.017 0.002</td>
<td>0.073** 0.014</td>
</tr>
<tr>
<td>120 min</td>
<td>0.019 0.003</td>
<td>0.051* 0.006</td>
</tr>
</tbody>
</table>

*significantly different from baseline, p<0.001. This value is based on 10 subjects since one male outlier with 1.990 mg/L was discarded. **significantly different from baseline, p<0.01

**Table 1 -** Mean fluoride concentration (SE) in saliva (mg/L, equivalent to ppm) from 11 young adults at baseline and designated times after consumption of popcorn prepared with fluoridated and non-fluoridated iodine table salt.

The fluoride concentrations in unstimulated whole saliva at baseline and at the designated time intervals are presented in Table 1. At baseline, the mean fluoride concentrations ranged from 0.021 to 0.027 mg/L for the test and control salts, but no significant differences were found. Immediately after the 30-minutes consumption of F-salt, a statistically significant (p<0.001) increase, up to over 0.30 mg/L, was registered in saliva while no such increase was evident for the non-fluoridated salt. The fluoride levels were still significantly elevated after 1 and 2 hours (p<0.01). The same pattern was seen in the supragingival plaque samples. Here, the baseline values varied around 2-4 mg/L and increased 3 times immediately after the intake of fluoridated salt (p<0.05) and remained elevated throughout the designated follow-up period. The detailed figures are presented in Table 2.

**Results**

The fluoride concentrations in unstimulated whole saliva at baseline and at the designated time intervals are presented in Table 1. At baseline, the mean fluoride concentrations ranged from 0.021 to 0.027 mg/L for the test and control salts, but no significant differences were found. Immediately after the 30-minutes consumption of F-salt, a statistically significant (p<0.001) increase, up to over 0.30 mg/L, was registered in saliva while no such increase was evident for the non-fluoridated salt. The fluoride levels were still significantly elevated after 1 and 2 hours (p<0.01). The same pattern was seen in the supragingival plaque samples. Here, the baseline values varied around 2-4 mg/L and increased 3 times immediately after the intake of fluoridated salt (p<0.05) and remained elevated throughout the designated follow-up period. The detailed figures are presented in Table 2.

**Discussion**

In a Swedish child population, approximately 10-15% is thought to exhibit increased caries risk. These patients are often characterized by irregular, non-daily tooth brushing and with a preference for frequent consumption of carbohydrate-based foodstuff (so called “junk food”) such as potato chips, popcorn and sweets. It has been shown that snacking and light meals are very common among Nordic adolescents, contributing 25-35% of the daily energy intake [Samuelsson, 2000]. The aim of this study was
therefore to examine the potential fluoride levels in plaque and saliva following a 30-minute consumption period of snacks prepared with fluoridated salt. Limited information seems to be available on the local events in the oral cavity after consumption of fluoridated salt, which definitely is of interest as the local effect of fluoride has been upgraded in recent years at the expense of the systemic effect, which is believed to play only a minor role in the cariostatic action of fluoride [ten Cate, 1999]. The participants volunteered after verbal and written examination and they must be considered as representative for adolescents and young adults living in a low-caries community. The compliance with the experimental plan was excellent as it was carefully monitored during the run-in, wash-out and experimental periods. The 30-minute intake period was chosen to make it possible to consume the assigned amount of popcorn in a realistic way. However, a bias was that the salt was loosely bound to the popcorn, which meant that the exact amount of salt that really was ingested might have varied from person to person. The given salt dose corresponded to the estimated average daily intake of domestic salt in developed countries [Murray and Naylor, 1996]. It should also be noted that the experimental salt contained potassium chloride, which is preferable and less causative for high blood pressure compared with sodium chloride table salts.

The results from this study were novel and clear-cut. Consumption of popcorn prepared with F-salt increased the concentration of fluoride in saliva and dental plaque significantly compared with baseline and in contrast to the levels found after intake of the non-fluoridated control salt. Thus, the null hypothesis was rejected. The recorded fluoride levels were in general higher compared with those that were established previously after other topical fluoride supplements, for example fluoride mouth rinses, varnishes and toothpaste [Zero et al., 1992; Seppä et al., 1997; Twetman et al., 1999].

In this experiment, we found significantly elevated fluoride levels in saliva and plaque even 120 minutes after snack ingestion. In the study by Macpherson and Stephen [2001], increased levels were registered for less than 20 minutes but the salt in their experiment was included in bread and bakeries. How long a period the increased values persisted in the present study is still an open question. Our conclusion so far is that elevated levels are present for at least 2 hours, but an extended follow-up period is needed to clarify this issue. Theoretically, if the plaque levels are not back to baseline after 24 hours, an accumulation could take place during 4-9 days if the salt is ingested in sufficient amounts at least once daily.

One important aspect must be emphasised regarding the whole saliva. The fluoride concentration measured here was not directly reflecting the amount of fluoride that was secreted through the glands. It is possible that a second delayed peak may exist after F-salt ingestion when the fluoride reaches the salivary gland via the blood. To be able to detect such a peak value, more frequent follow-up samplings would be required. Our measurements in whole saliva reflected the local situation and, therefore, any efforts to calculate the salivary output (fluoride concentration multiplied with the secretion rate) were not motivated. All subjects had unstimulated secretion rates within normal limits (0.25-0.45 mL/min). The crossover design used in this experiment compensated to a high extent for the individual variations in saliva secretion rate as each subject was having both the test and control salt intake.

**Conclusion**

We have demonstrated increased fluoride concentrations in saliva and plaque following consumption of fluoridated table salt. As the concentrations obtained were of the same magnitude or even higher compared with other commonly used topical fluorides, it may very well be of clinical significance. The findings can partly explain the cariostatic action of fluoridated salt that has been observed in several countries around the world. In low-caries communities with a skewed distribution, fluoridated salt may be considered as a targeted measure to secure a daily fluoride input in risk patients with irregular or unsatisfactory tooth brushing habits.

**Acknowledgement**

The authors would like to thank Mr. Can Yurdunuseven, Karolinska Institute, Huddinge, Sweden, for his skilled analytical work.

**References**


