Fluoride Levels in Saliva after Tea Intake

Authors: Nargis Nasiri, Markus Domäng

Tutor: Christina Stecksén-Blicks
ABSTRACT

Tea contains fluoride but its effect as a caries-preventive measure is not fully understood. The aim of this study was to evaluate the fluoride levels in saliva after tea-intake.

Part one of the study analyzed the fluoride content of teas and one black and one green tea was thereafter selected for the clinical part of the study (part two). Ten healthy adults participated in part two which was designed as a prospective, crossover study where the salivary fluoride levels were analyzed after tea-intake at designated follow-ups.

The fluoride level in saliva increased after tea-intake (0.04 ± 0.2 vs 0.97 ± 0.32 mg/L, (p < 0.01)). After intake of the black tea fluoride levels remained elevated compared to baseline at 5 minutes (0.04 ± 0.02 vs 0.12 ± 0.08 mg/L, (p < 0.01)) and 10 minutes (0.04 ± 0.02 vs 0.09 ± 0.07 mg/L, (p < 0.05) but not at 20 minutes (0.04 ± 0.02 vs 0.05 ± 0.03 mg/L, (p = 0.056)).

The green tea had elevated fluoride levels up to 5 minutes (0.05 ± 0.03 vs 0.14 ± 0.09 mg/L, (p < 0.01)) but not at 10 minutes (0.05 ± 0.03 vs 0.09 ± 0.08 mg/L, (p > 0.05)).

The fluoride level in saliva is elevated after tea-intake which may suggest a caries-preventive effect, but not for a long period of time due to its rapid clearance in saliva.
INTRODUCTION

Fluorine is an atom and in its pure form fluorine is a gas but it’s very reactive and is usually found in compounds as an ion called fluoride.

Supplementation of fluoride has been a central part in the prevention of dental caries since the middle of the 20th century. Fluoride has been used in water fluoridation, in toothpastes, mouth rinses, tablets, chewing gums, gels and varnishes (Koch and Poulsen, 2009). The introduction of fluoride toothpaste has been ascribed a decrease in the prevalence of caries, not least among children and adolescents (Twetman et al., 2003). Also the process of water fluoridation of community water has been proven to be an important caries-reducing measure (McDonagh et al., 2000). Groundwater is naturally fluoridated to various degrees due to regional conditions such as soils lacking calcium as in areas with high levels of gneiss or granite, proximity to the ocean and volcanic activities as well as from industrial waste products. Groundwater collected locally, for instance from local wells, can due to the endemic bedrock have a different fluoride concentration level compared with public water where levels of constituent minerals in the water can be modified (Denbesten and Li, 2011). Naturally fluoridated water can depending on the fluoride levels provide a caries-reducing effect (Chen, 1989).

The caries-reducing effect of fluoride has a dose-response relation which is well documented through different measures of delivering fluoride to the oral cavity (McDonagh et al., 2000; Rugg-Gunn and Bánóczy, 2013; Twetman et al., 2003). The fluoride content in fluoridated products is often measured in parts per million (ppm) which means one millionth of the same unit, for example 1 mg/kg which translates to 0.000001 grams per gram. When speaking of naturally occurring fluoride or fluoride as a part of biological processes the content is often referred to as a concentration (mg/L). The connection between ppm and mg/L is that they translate to each other in almost direct proportion, 1 ppm is equal to 1 mg/L.

The therapeutic spectrum for fluoride is narrow, however. Acute toxic doses may occur when intakes of 5 mg F/kg body weight are ingested and lethal dose is approximately 15mg F/kg body weight (Koch and Poulsen, 2009). Consequently, side effects related to over-dosage has been much discussed. If larger doses of fluoride are ingested side effects can be seen such as enamel fluorosis (McDonagh et al., 2000). Fluorosis is a developmental disturbance of the enamel that makes it more porous due to hypomineralization (Denbesten and Li, 2011). The damages can to various degrees be of qualitative or quantitative nature (Thylstrup
Mild fluorosis may occur in connection with an ingested amount of approximately 0.1 mg F/kg body weight per day during the period of tooth formation (Koch and Poulsen, 2009).

Except for water, fluorides are found in varying amounts in beverages, in some foods as seafood and plants such as tea (Thippeswamy et al., 2010). Consuming large amounts of dietary fluoride in adult life may give bone higher density. However, if consumption is excessive, the bone may also become more fragile and prone to fracture (Shen et al., 2009), this is called skeletal fluorosis. Cases of skeletal fluorosis have been reported and they are often associated with excessive habitual tea consumption (Whyte et al., 2008).

Tea leaves are derived from the tea plant *Camellia sinensis*. When brewing tea leaves the inherent fluoride is released to the water and hence the tea water becomes fluoridated. The fluoride content varies in different teas (Emekli-Alturfan et al., 2009) and also with tea quality with higher levels in low quality teas (Lu et al., 2004). The potential caries preventive effect of tea drinking has not been investigated. The aim of the present study is therefore to assess the fluoride concentrations in saliva after tea intake.

**MATERIALS AND METHODS**

**Study design**

The study had two parts. In part one analysis of the fluoride content of teas was performed and one black and one green tea were selected for the clinical part of the study (part two). Part two was designed as prospective, crossover study were the salivary fluoride levels were analyzed after tea intake at designated follow-ups.

**Selection of teas**

Twelve different bags of teas from a common tea brand in Sweden (Lipton) were selected; ten black teas and two green teas from the same batch to avoid any variation between batches. Each tea was brewed according to a standardized method and in this procedure each tea bag was put in 2 dl boiling water (100 °C) for 2 minutes. Thereafter the tea bags were removed and the teas were allowed to cool to room temperature before analysis of fluoride concentration. One black tea and one green tea with high fluoride contents were chosen for the clinical part of the study (Lipton Yellow Label Tea, 2.59 mg F/L and Lipton Green Tea
Citrus, 1.64 mg F/L). Ten tea bags of the same type were taken from the same batch for use in the clinical part of the study.

**Participants and clinical procedures**

Ten healthy adult dental students (22-26 years of age) at Umeå University volunteered to participate in the study.

Inclusion criteria were that they should be healthy and have normal saliva secretion (> 0.7 ml / min stimulated saliva). To ensure that participants had normal saliva chewing stimulated secretion samples were taken before inclusion.

Participants were informed to avoid all oral hygiene procedures, food and drink in at least 1 hour before the experimental session. This information was given verbally and in writing and confirmed before sampling took place. There were no dropouts.

First, a pilot study was performed where one of the teas was tested. One of the researchers drank 2 dl tea and sampled 1 ml saliva in a test tube at 0, 1, 2, 3, 5, 10, 15 and 20 minutes after the intake. The researcher had before the intake met the inclusion criteria and a chewing stimulated saliva samples were taken as control and was used as a baseline. Based on the pilot tests, it was decided that samples should be taken 0, 1, 2, 5, 10 and 20 minutes after intake of tea.

At the clinical session, first the participants were asked to provide a baseline sample of chewing stimulated saliva in a test tube. They were then asked to drink 2 dl of the selected teas and spit 1 ml of saliva in a test tube at designated follow-ups at 0, 1, 2, 5, 10 and 20 minutes after the intake.

The participants received no instructions on how they should drink the tea, but were encouraged to drink it as they usually drink tea in a habitual way. Each set of experiment was performed at different days for each participant.

**Fluoride analysis**

After collection of the saliva samples fluoride analysis was done using an Orion Research Microprocessor ion analyzer/901 (Orion Research Inc. Cambridge, Mass. U.S.) fluoride specific ion electrode. Distilled water and fluoride-standards of 0.01, 0.1, 1 and 10 mg F/L respectively was used for calibration of the ion analyzer. Frozen samples were thawed to
room temperature and 1000 µl of each sample was added to 100 µl of TISAB (ion stabilizer solution) and put in a test tube and placed in the ion analyzer until a stable millivolt-value (mV) was achieved. After each analysis the electrode was cleaned with distilled water. Before each set of measurement, the routine with standards was repeated to calibrate the analyzer to obtain the daily values of the analyzer’s fluoride sensitivity.

The program FLUOR.BAS (in Excel) developed and validated by Rolf Sjöström at the biochemical laboratory (at the Department of Odontology, Umeå University) was used to translate mV value to fluoride concentration in mg/L.

Samples that were not analyzed the same day were frozen at -20 °C for later analysis.

**Ethical aspects**

The participants were informed both orally and in written to ensure that they were in agreement with that their participation was voluntary and the possibility to cancel participation at any time without reason. All participants gave their informed written consent. No invasive procedures were performed and no pain was associated with participation. The new knowledge that the project should gain was considered to outweigh the encroachment in the privacy that was associated with participation in the project. Each participant was given a code number and each sample the order of the samples.

The Ethics Forum at the Department of odontology found that appropriate ethical considerations had been integrated into this degree project and gave their approval. There was no sponsoring or communication with any cooperation.

**Statistical methods**

Data were computerized in Excel (Microsoft Inc.) and converted to SPSS version 22 (IBM Inc.) for analysis. The fluoride concentration of the teas was presented as descriptive statistics. Differences in the fluoride concentrations between the teas were analyzed with a one-way ANOVA. For comparison of saliva samples a paired sample T-test was used. A p-value of < 0.05 was considered as statistically significant.

**Literature search**

We used articles from the PubMed-database, keywords used was “fluoride tea saliva”, “fluorosis tea”, “skeletal fluorosis tea”, “bone mass tea”, “caries preventive effect fluoride
toothpaste systematic review” and “fluoride content beverages”. The selection of articles were first based on screening of the titles by relevance, abstracts of the articles with titles relevant for the studied topic was read and either included or excluded based on relevance for the research question. In this process we focused on research on teas used in Europe.

**Websites:**


**RESULTS**

The mean fluoride concentration in the 12 different teas were $1.85 \pm 0.84$ mg/L, ranging from a highest value of $3.01$ mg/L to a lowest value of $0.03$ mg/L. The fluoride concentration level for each tea is presented in Table 1. The black teas had generally a higher fluoride concentration than the green teas, the mean fluoride concentration for the black teas was $1.91 \pm 0.92$ mg/L compared to $1.58 \pm 0.08$ mg/L for the green teas, but the difference was not statistically significant ($p > 0.05$). When Lipton Blue Fruit Tea which had $0.03$ mg F/L was excluded from the analysis black teas had a mean of $2.11 \pm 0.68$ mg F/L and green teas $1.58 \pm 0.08$ mg F/L but the difference between the teas was not statistically significant ($p > 0.05$).

Two teas were selected for the clinical part of the study, Lipton Yellow Label (Tea 1) which had a fluoride concentration of $2.59$ mg/L and Lipton Green Tea Citrus (Tea 2) $1.64$ mg/L. All 10 participants completed the clinical part of the study. The fluoride concentration levels in saliva at baseline averaged around $0.04 \pm 0.03$ mg/L. Fluoride concentration values are presented in Table 2 and Figure 1. After drinking Tea 1 a significant increase in fluoride level in saliva compared to baseline was shown ($0.04 \pm 0.02$ vs $1.04 \pm 0.32$ mg/L, $p < 0.01$), this increase remained significant for 5 minutes ($0.04 \pm 0.02$ vs $0.12 \pm 0.08$ mg/L, $p < 0.01$) and up to 10 minutes ($0.04 \pm 0.02$ vs $0.09 \pm 0.07$ mg/L, $p < 0.05$). At 20 minutes after intake of Tea 1 the elevated fluoride level in saliva was, compared to baseline, close to but no longer statistically significantly different ($0.04 \pm 0.02$ vs $0.05 \pm 0.03$ mg/L, $p = 0.056$). After intake of Tea 2, there was a statistically significant increase compared to baseline ($0.05 \pm 0.03$ vs $0.91 \pm 0.33$ mg/L, $p < 0.01$) which lasted up to 5 minutes ($0.05 \pm 0.03$ vs $0.14 \pm 0.09$ mg/L, $p < 0.01$) but not after 10 minutes ($0.05 \pm 0.03$ vs $0.09 \pm 0.08$ mg/L, $p > 0.05$). When comparing
Tea 1 with Tea 2, there were no statistically significant differences in the fluoride levels between the two teas at any designated follow-up (p > 0.05).

When the two teas were analyzed together, the highest fluoride concentration in saliva was found at 0 minutes after tea intake (0.97 ± 0.32 mg/L). Compared with baseline (0.04 ± 0.03 mg/L) there was a statistically significant different fluoride level at each designated follow-up, up to 10 minutes (0.04 ± 0.03 vs 0.09 ± 0.07 mg/L, (p < 0.01) and after 20 minutes the difference in fluoride level was still found to be statistically significant (0.04 ± 0.03 vs 0.06 ± 0.04 mg/L, (p < 0.05).

DISCUSSION

This study explores the fluoride level in saliva after tea intake and a higher concentration is obtained up to 20 minutes after an intake. It also shows that the elevation of fluoride level is steadily decreased with time after tea intake.

The outcome differed between the two selected teas. For the green tea (Tea 2) a statistically significantly higher fluoride level was found 5 minutes after intake, while for the black tea (Tea 1) a statistically significant elevation in saliva was found at 10 minutes and close to statistically significant at 20 minutes post intake. Though the difference between the two teas was not statistically significant the findings are in line with the current knowledge that oral administrations with higher fluoride concentrations give longer periods of elevated fluoride level in the oral cavity (Mason et al., 2010). Our finding with a longer period of elevated fluoride level in saliva when results from the two teas were analyzed together may depend on statistical power with a larger sample size for statistical analysis.

Among examined teas, the black teas seemed to have a higher fluoride concentration than the green teas but the difference was not statistically significant. Lipton Blue Fruit Tea had a lower level of fluoride compared to all the other teas and the level was close to the fluoride concentration found in the community water in Umeå (0.24 mg F/L) (UMEVA, 2011). Since the selection of teas was quite limited especially for the group of green teas where only two different teas were tested no definite conclusion can be drawn from the findings in this study, further studies are required concerning fluoride concentrations in green and black teas. Furthermore, the selected teas came from the same batch which may have been of importance for the findings since the fluoride content of teas from different batches of the same brand
may vary. Most teas used in western countries come from the same type of tea plant, *Camellia sinensis*. In the production of tea the fluoride content may vary due to different factors in the production and the preparation of the tea (Lu *et al.*, 2004; Lung *et al.*, 2008).

The study group was small and all participants were dental students. Individuals working with dental care may have other oral hygiene habits than the general public, by using more or less oral hygiene products the baseline salivary fluoride level may differ. The majority of the participants were females (70%) and therefore the results from this study are more representative for a female population, due to a known difference between genders in saliva secretion with effects on the fluoride clearance in saliva (Inoue *et al.*, 2006). Since there was no control group to compare with the study groups, it’s impossible to say how much of the salivary fluoride increase came from the tea and how much came from the drinking water in which it was boiled. By using a series of drinking water as control the internal validity of the study would have been raised. The brewing time was standardized in our study but it is know that brewing time may have some impact on the fluoride levels for some infusion teas (Emekli-Alturfan *et al.*, 2009).

The participants performed the tea intake and saliva sampling under the observation of one of the researchers who also collected the samples, therefore blinding was affected. The supervised saliva sampling in a clinical environment may have had a negative effect on saliva secretion due to stress. Efforts were made to complete the sampling under relaxed conditions and the baseline-sample taken before tea-intake showed that all participants had a stimulated saliva secretion above 1 mL/min. As the subjects had to sample a considerably amount of saliva and at narrow intervals, sometimes they had to rub on the soft tissues of the mouth to achieve saliva production. Although, it can only be speculated on the impact of this might be that during periods of time only certain areas of the mouth was cleared of fluoride. This may have caused a small increase in the measured fluoride level at the later follow-ups (10 and 20 min) when secretion was no longer forced and a proposed more pooled saliva sample from the whole oral cavity was achieved. Studies have suggested that retention of fluoride varies in different parts of the oral cavity such as in saliva, in the soft tissues and in dental proximal plaque (Zero *et al.*, 1992; Duckworth, 2013). It has been suggested that fluoride from tea accumulates in dental plaque but not in saliva (Yue *et al.*, 1998).

The fluoride analyses were not repeated therefore reproducibility of the fluoride analyses may be questioned. However, for every series of samples the trend of a rapid increase in fluoride
level followed by a steady decrease which stabilized towards a value at/or close to baseline was observed. During the course of the fluoride analysis, at calibration of the analyzer, the standards showed a small but progressive difference in the mV value at each session which indicate that the used method of measuring fluoride concentrations is very sensitive and maybe not totally reliable.

Considering tea drinking as a caries preventive measure, there may be beneficial effects after intake, however from this study any caries prophylactic effect from tea intake can only be speculated on. Our finding indicates a brief initial effect but since the levels are considerably lower than those used in topical fluorides the clinical importance may be negligible. When assessing the caries preventive effect of a fluoridated product, both fluoride concentration and frequency of exposure need to be considered. In addition to the fluoride content in teas other beneficial caries preventive factors in teas are antimicrobial substances (Goenka et al., 2013; Gazzani et al., 2012), which might be of interest for future trials.

There is no officially set lower limit to how much fluoride that is needed to prevent caries but it is established that there is a dose-response relation between fluoride concentration and effect on caries (Rugg-Gunn and Bánóczy, 2013). Estimating fluoride exposure is very complex considering all sources of fluoride. It has been stated that a fluoride concentration in drinking water of 1 mg F/L is optimal for reduction of caries in children when risk of dental fluorosis is considered (Institute of Medicine (US), 1997). As for the potential risk of dental fluorosis, it has been suggested that the daily intake of fluoride should not exceed 0.1 mg/kg body weight during periods of tooth development (Koch and Poulsen, 2009). For a child with a body weight of 10 kg, an intake of 2 dl tea with a fluoride concentration of 2-3 mg F/L would result in a 0.04-0.06 mg F/kg body weight which is below the proposed threshold for mild fluorosis. However, intakes from other sources as water and toothpaste have to be added which together with the fluoride from tea would result in an intake above the threshold value. An appropriate recommendation for young children would be to restrict tea drinking in particular during the first 2-4 years of life. It’s the obligation as a dental practitioner to present such recommendations to parents of young children in an effort to minimize the risks of fluorosis.

For the adult population, there have been of discussion the preventive effects of tea-drinking on age-related bone loss. Studies have shown a positive association between tea-drinking and bone density in postmenopausal women (Hegarty et al., 2000; Devine et al., 2007). Though
the daily dose of fluoride needed to achieve such health benefits is not yet determined, the effects might not be entirely dose-dependent (Hegarty et al., 2000). There are few studies conducted to determine the maximal intake for beneficial health effects. An intake of about 20 mg F/day during the majority of adult life is expected to cause symptomatic skeletal fluorosis (Izuora et al., 2011). For teas with a fluoride concentration of 2-3 mg F/L this would require a daily intake of 7-10 liters of tea, which far exceeds the regular consumption for the majority of habitual tea-drinkers.

In conclusion this study indicates that the fluoride level in saliva after tea intake is elevated for up to 10 minutes for black teas and 5 minutes for green teas, though at low levels. Tea drinking should be restricted for children during tooth development to minimize the risks of fluorosis.

ACKNOWLEDGEMENTS

We would like to thank our tutor, Professor Christina Stecksén-Blicks, for her much appreciated guidance and time. Special thanks also go to laboratory technician Elisabeth Granström for her help and support during the lab work as well as all the participants for their time, patience and interest.
REFERENCES


Table 1. Fluoride in mg F/L in brewed teas.

<table>
<thead>
<tr>
<th>Teas</th>
<th>mg F/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Black teas</strong></td>
<td></td>
</tr>
<tr>
<td>Lipton Kericho Estate Tea</td>
<td>3.01</td>
</tr>
<tr>
<td>Lipton Russian Earl Grey</td>
<td>2.93</td>
</tr>
<tr>
<td>Lipton Yellow Label Tea <em>(Tea 1)</em></td>
<td>2.59</td>
</tr>
<tr>
<td>Lipton Tea Black Currant</td>
<td>2.49</td>
</tr>
<tr>
<td>Lipton Lemon Tea</td>
<td>1.91</td>
</tr>
<tr>
<td>Lipton Forest Fruits Tea</td>
<td>1.87</td>
</tr>
<tr>
<td>Lipton Earl Grey</td>
<td>1.84</td>
</tr>
<tr>
<td>Lipton Darjeeling</td>
<td>1.27</td>
</tr>
<tr>
<td>Lipton Vanilla Tea</td>
<td>1.11</td>
</tr>
<tr>
<td>Lipton Blue Fruit Tea</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Green teas</strong></td>
<td></td>
</tr>
<tr>
<td>Lipton Green Tea Citrus <em>(Tea 2)</em></td>
<td>1.64</td>
</tr>
<tr>
<td>Lipton Green Tea Orient</td>
<td>1.52</td>
</tr>
</tbody>
</table>
Table 2. Fluoride concentrations in mg F/L in saliva (mean ± standard deviation, range) for samples based on Tea 1 (Lipton Yellow Label Tea), Tea 2 (Lipton Green Tea Citrus) and pooled values at designated follow-ups.

<table>
<thead>
<tr>
<th>Time</th>
<th>Baseline</th>
<th>0 min</th>
<th>1 min</th>
<th>2 min</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
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<tbody>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Tea 1</td>
<td>N=10</td>
<td>0.04±0.02</td>
<td>1.04±0.32*</td>
<td>0.38±0.18*</td>
<td>0.22±0.12*</td>
<td>0.12±0.08*</td>
<td>0.09±0.07*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01-0.06</td>
<td>0.51-1.48</td>
<td>0.16-0.74</td>
<td>0.06-0.44</td>
<td>0.03-0.26</td>
<td>0.03-0.25</td>
</tr>
<tr>
<td>Tea 2</td>
<td>N=10</td>
<td>0.05±0.03</td>
<td>0.91±0.33*</td>
<td>0.34±0.15*</td>
<td>0.19±0.10*</td>
<td>0.14±0.09*</td>
<td>0.09±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01-0.10</td>
<td>0.36-1.28</td>
<td>0.13-0.56</td>
<td>0.08-0.40</td>
<td>0.04-0.34</td>
<td>0.03-0.29</td>
</tr>
<tr>
<td>Tea 1+2</td>
<td>N=20</td>
<td>0.04±0.03</td>
<td>0.97±0.32*</td>
<td>0.36±0.16*</td>
<td>0.20±0.11*</td>
<td>0.13±0.08*</td>
<td>0.09±0.07*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01-0.10</td>
<td>0.36-1.48</td>
<td>0.13-0.74</td>
<td>0.06-0.44</td>
<td>0.03-0.34</td>
<td>0.03-0.29</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to baseline. Paired sample T-test.
Figure 1. Comparison of mean fluoride concentration levels in mg F/L after tea-intake for Tea 1, Tea 2 and pooled values at designated follow-ups.