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ORIGINAL ARTICLE

Saliva fluoride before and during 3 years of supervised use of fluoride toothpaste

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Abstract
Objectives The purpose of the study was to examine pre-brushing saliva fluoride concentrations before and during a large, 3-year, prospective toothpaste study on the effect of post-brushing rinsing on dental caries. The aims were to study saliva fluoride over time and the effect of rinsing on saliva fluoride and to relate saliva fluoride to caries increments and accumulation of plaque.
Materials and methods Saliva samples (baseline and 1, 2, and 3 years) were collected from 11-year-old children attending two schools (A and B) in Kaunas, Lithuania, who refrained from brushing the evening and morning before saliva collection. Numbers of saliva samples collected varied from 264 at baseline to 188 at the 3-year follow-up. Children in school A rinsed with water after daily brushing, while children in school B did not rinse. Total caries and visible plaque were registered at baseline and after 3 years.
Results Mean saliva fluoride concentrations at baseline and after 1, 2, and 3 years from school A (rinsing) were 0.014, 0.026, 0.029, and 0.034 ppm and from school B (no rinsing) were 0.013, 0.028, 0.031, and 0.031 ppm, respectively. Increases in saliva fluoride from baseline were significant (Wilcoxon's test, $p<0.001$), but the increase from baseline to year 1 was not statistically significantly different between schools. Saliva fluoride did not increase beyond year 1 and did at no time point differ between schools. Reductions in numbers of tooth surfaces with dental plaque were significantly positively related to the number of caries reversals over the 3 years.
Conclusions Background saliva fluoride concentration is increased by brushing at least once daily on schooldays, does not increase further over 3 years, and is not affected by rinsing after brushing.
Clinical relevance Continuous use of fluoride toothpaste produces ambient saliva fluoride levels similar to saliva fluoride in areas with fluoridated water.

Keywords Saliva fluoride · Fluoride dentifrice · Toothbrushing · Water rinsing

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Introduction

Whole saliva fluoride concentrations may be related to concentrations of fluoride at the plaque/tooth interface, which is the site of action for the caries-controlling effect of fluoride. After use of fluoride toothpastes, fluoride concentrations in whole saliva are, initially, increased 100-fold or more, but these high concentrations drop exponentially to pre-brushing levels within about 2 h [1–3], which makes it difficult to understand the considerable effect of fluoride toothpastes on caries as most caries challenges will occur later than 2 h after brushing.

However, it has also been demonstrated in two studies, involving a few adult subjects, that continuous use of a fluoride toothpaste, twice daily for 2 weeks, increases the background concentrations of fluoride in saliva (i.e., concentrations up to 18 h after the last toothbrushing) [4, 5], and such increases are related to the concentration of fluoride in

the toothpaste used [4]. Increased ambient concentrations of fluoride in saliva caused by toothpaste are similar to previously reported 0.02 ppm higher fluoride concentrations found in saliva from areas with 1 ppm or more in drinking water compared to areas with low fluoride concentrations in water [6–8]. This may explain why it has been possible, on a population basis, to demonstrate significantly higher mean saliva fluoride levels in caries-free subjects as compared to subjects with high caries prevalence [9, 10], irrespective of fluoride concentrations in water.

As the studies on saliva fluoride after toothpaste cited above only lasted 2–4 weeks, it is not known whether further increases in fluoride concentration in saliva occur after 4 weeks. As the use of fluoride toothpaste is ubiquitous in many countries, it is seldom possible to measure saliva fluoride levels prior to exposure to fluoride toothpaste.

We had the opportunity to further investigate the various factors discussed above using saliva collected from children, with a history of a low frequency of exposure to fluoride toothpaste, who participated in a previously described, 3-year, prospective, supervised trial of the effect of post-brushing rinsing on dental caries which used a toothpaste containing 1,500 ppm F [11, 12].

The aims of this study were to (1) examine mixed whole saliva fluoride concentrations in relation to the reported frequency of toothbrushing at baseline, (2) examine possible changes in saliva after 1, 2, and 3 years of supervised toothbrushing at school, (3) test whether saliva fluoride is affected by rinsing after brushing, (4) test whether background levels of fluoride in saliva are correlated with caries increments, and (5) test plaque accumulation related to caries increments.

Subjects and methods

The original study on the effect of post-brushing mouth rinsing with water involved 407 children (mean age, 11.8 years) attending three schools in Kaunas, Lithuania, where the fluoride concentration in drinking water is <0.16 ppm). In the following subsections, only details of the two intervention schools that are relevant to this paper are summarized. For further details on the study design, sample size, compliance, etc., see Machiulskiene et al. [11].

Subjects

A total of 287 children aged 10–14 years (mean=11.3; 95 % CI=[11.2; 11.4]) attending two secondary schools in Kaunas participated in the present 3-year study of the effect of supervised toothbrushing on the salivary fluoride levels.

The parents of the participating children had given written informed consent for the study, which was approved by ethical committees in Denmark and Lithuania (Independent Ethical Committee, Kaunas Medical Academy, February 1995).

Clinical examinations

At baseline and after 3 years, the children were given a clinical caries examination using the caries diagnostic criteria described by Nyvad et al. [13]. Prior to the caries examinations, plaque was scored as absent or present according to the presence of visible plaque collected on the probe from all surfaces of teeth nos. 16, 11, 31, and 36. Thus, there was a yes–no decision for each of the 18 tooth surfaces. These teeth constitute a subset of the Ramfjord selection of teeth, which has been found to validly represent the full-mouth score [14, 15]. At both the start of the study and at the 3-year follow-up examination, the children were asked to categorize their normal toothbrushing frequency as: <1/week, <1/day, 1/day, or >1/day and their use of toothpaste as: with fluoride, nonfluoride, or no toothpaste. Toothpastes of unknown fluoride content were recorded as nonfluoride.

Intervention

The toothpaste used for this study was a commercially available toothpaste (Colgate—Great Regular Flavor) containing 1,500 ppm F (500 as NaF, 1,000 as MFP). The toothpaste and toothbrushes were supplied once a month to the children and their families for home use. Supervised toothbrushing started 2 months after the first saliva sampling. The children were assembled by classes in the school canteen between 9:00 am and 12:00 am every schoolday for the 3 years of the study. A school dentist/dental surgery assistant controlled that the children applied sufficient toothpaste to cover the heads of the standard toothbrushes used for the study. After this, the children were observed while they brushed for a fixed time period of 3 min, which was also controlled by the dental staff who then supervised the post-brushing behavior of the children. Children in school A were instructed to rinse their mouth thoroughly using a beaker of tap water (150 ml), whereas in school B, mouth rinsing was not permitted and the children were only allowed to spit out once.

Saliva sampling

Saliva was collected four times: at the outset of the study and 1, 2, and 3 years later. Children were asked to refrain from toothbrushing the evening before and on the day of saliva sampling. Unstimulated whole saliva (minimum, 1 ml) was collected by drooling into plastic vials. All samples were collected during morning classes and frozen until analysis.

Fluoride analysis

Portions (0.5 ml) of the whole saliva were acidulated, without prior centrifugation [16], by addition of 25 μl of 1.0 M acetic acid prior to direct measurement with a fluoride ion-sensitive electrode (Model 96-09, Orion Res. Inc., Cambridge, MA, USA).

Statistical analysis

A stratified Wilcoxon rank–sum test was used to determine if the baseline saliva fluoride concentrations, the baseline decayed/missing/filled surface (DMFS) counts, the baseline number of unerupted surfaces, or the baseline plaque scores differed between children in each school who were clinically examined at the 3-year follow-up examination and those who were not. Since the saliva fluoride concentrations showed pronounced skewness, they were log-transformed prior to all subsequent statistical analyses. A repeated-measures two-way analysis of variance (ANOVA) was used to determine if the log saliva fluoride levels differed over time after baseline and differed between children in the two schools. The influence of baseline plaque score, toothbrushing frequency, and toothpaste type on the log baseline salivary fluoride level was explored using multiple linear regression analysis. Multiple linear regression analysis was also used to determine the influence on the 3-year net DMFS increment and the number of caries reversals of the individual log mean saliva fluoride concentration across years 1 to 3 and the plaque score reduction from baseline to year 3. The relationship between the plaque score reduction from baseline to year 3 and the log mean saliva fluoride concentration across years 1 to 3 was also investigated using linear regression analysis.

Results

The number of children available for clinical examination at baseline was 143 and 144 in schools A and B, respectively. At the 3-year follow-up, 90 and 103 of these children from schools A and B, respectively, were clinically examined, and follow-up clinical data were thus available for a total of 193 children. The number of children providing salivary samples varied over the years, from a total of 264 at baseline to 188 at the 3-year follow-up. A total of 127 children provided all 4 saliva samples, whereas 193 children provided at least 1 sample during years 1–3. At baseline, only a small number of children (33 out of 130 in school A and 42 out of 134 in school B) had saliva fluoride concentrations at or above the detection limit (0.01 ppm F) of the method (Fig. 1). To facilitate data handling, saliva samples containing 0.01 ppm F or less at the first collection were assumed to contain this concentration. In both schools, the cumulative percentage of children with more than 0.02 ppm F in saliva increased from about 10 % at baseline to about 55 % after year 1 and remained at this level for the remaining period.

The salivary fluoride concentrations increased from baseline to year 1; thereafter, they remained stable (Table 1). Repeated-measures ANOVA showed that the differences between schools were not statistically significant, whether we considered only the subset of children who had provided all four saliva samples ($n=127$) or the subset of children who had just provided at least one saliva sample at year 1, 2,

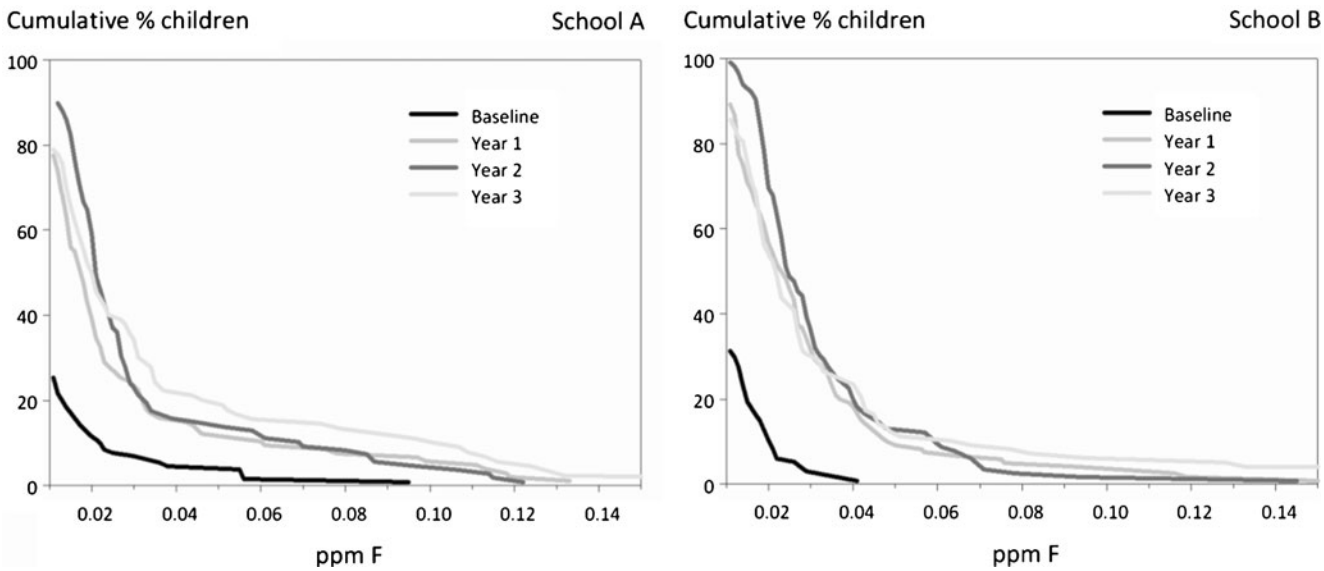

Fig. 1 The distribution at baseline and years 1, 2, and 3 of the salivary fluoride levels among children in the two schools, based on all children ($n=$ 90 and 103) who were clinically examined at both baseline and 3-year follow-up and provided at least one saliva sample during years 1–3

Table 1 Numbers of children with saliva samples during the 3 years in schools A and B for different subsets of children

Child characteristic		School A, no. of children	School B, no. of children	School A salivary fluoride, ppm, mean (95 % CI)	School B salivary fluoride, ppm, mean (95 % CI)	*p* value[a]
All children with saliva samples	Baseline	130	134	0.014 [0.012; 0.016]	0.013 [0.012; 0.013]	0.35
	Year 1	107	120	0.026 [0.021; 0.031]	0.028 [0.024; 0.033]	0.01
	Year 2	108	115	0.029 [0.025; 0.034]	0.031 [0.027; 0.034]	0.01
	Year 3	90	98	0.034 [0.026; 0.041]	0.031 [0.025; 0.037]	0.57
Children with all four saliva samples and both baseline and follow-up examination	Baseline	56	71	0.014 [0.011; 0.017]	0.013 [0.012; 0.015]	0.75
	Year 1	56	71	0.031 [0.022; 0.039]	0.027 [0.022; 0.033]	0.61
	Year 2	56	71	0.031 [0.024; 0.038]	0.030 [0.025; 0.034]	0.21
	Year 3	56	71	0.033 [0.025; 0.041]	0.027 [0.020; 0.033]	0.48
Children with at least one saliva sample at years 1–3 and both baseline and follow-up examination	Baseline	79	95	0.013 [0.011; 0.016]	0.013 [0.012; 0.014]	0.18
	Year 1	72	90	0.028 [0.021; 0.035]	0.030 [0.025; 0.035]	0.10
	Year 2	81	95	0.029 [0.024; 0.034]	0.030 [0.026; 0.034]	0.02
	Year 3	81	92	0.034 [0.027; 0.042]	0.031 [0.025; 0.038]	0.98

Also given are the mean salivary fluoride concentrations for each sampling period and school

[a] Wilcoxon rank–sum test of school A versus school B

or 3 (n=193) (Table 1). Repeated-measures ANOVA also showed no statistically significant change over time from years 1 to 3, whether we considered only the subset of children who had provided all four saliva samples (n=127) or the subset of children who had just provided at least one saliva sample at year 1, 2, or 3 (n=193) (Tables 1 and 2). In order to be able to extract as much information as possible, we, therefore, based all subsequent analyses on the latter 193 children.

The baseline DMFS was significantly higher (p<0.02) among children who were not clinically examined at year 3 than among those who were. This means that the children who did not attend the final clinical examination had more caries than the other children when the study started. Neither the baseline saliva fluoride levels nor the number of unerupted surfaces nor the baseline plaque score differed statistically significantly between those children in each school who completed the 3-year study and those who did not (Table 3).

Table 2 The mean, median, and interquartile range (IQR) of the salivary fluoride level across years 1–3 for three different subsets of children

Child subset	School A, no. of children	School B, no. of children	Statistical estimate	School A salivary fluoride, ppm, years 1–3	School B salivary fluoride, ppm, years 1–3
All children who had four saliva samples and both clinical examinations	56	71	Mean	0.031	0.028
			Median	0.023	0.023
			IQR	0.017–0.036	0.018–0.031
All children who had last three saliva samples and both clinical examinations	59	74	Mean	0.031	0.028
			Median	0.023	0.024
			IQR	0.017–0.034	0.018–0.031
All children who had at least one of last three saliva samples and both clinical examinations	90	103	Mean	0.031	0.032
			Median	0.022	0.026
			IQR	0.016–0.033	0.018–0.037

Table 3 The mean values (and SD) of selected baseline clinical and salivary parameters for children who completed the 3-year study and those who did not (given by school)

Baseline parameter	School A		School B	
	Completed, n=90	Dropped out, n=53	Completed, n=103	Dropped out, n=41
Salivary fluoride (ppm)[a]	0.013 (0.011)	0.014 (0.011)	0.013 (0.005)	0.012 (0.005)
DMFS[b]	16.8 (10.5)	24.7 (23.7)	17.4 (7.7)	20.4 (12.2)
Unerupted surfaces	21.1 (19.6)	17.4 (19.2)	23.8 (20.1)	18.2 (15.4)
Surfaces with plaque	12.7 (4.9)	11.5 (5.6)	12.8 (3.9)	12.9 (4.5)

[a] The number of children with baseline salivary samples were 79, 51, 95, and 39, respectively, in the four groups

[b] Statistically significant difference between dropouts and completers (stratified Wilcoxon rank–sum test)

The frequency of brushing recorded and use of fluoride toothpaste are shown in Table 4. Prior to the study, about one fourth of the children in both schools brushed their teeth less than once daily and a similar proportion used toothpaste recorded as nonfluoride (without fluoride or unknown fluoride content). At the 3-year follow-up, around 90 % of the children brushed their teeth more than once daily, and most children used fluoride toothpaste. Multiple linear regression analysis showed that the baseline toothbrushing habits, the type of toothpaste used, or the baseline plaque score did not influence the baseline salivary fluoride levels.

The various caries parameters after 3 years did not differ between the schools (Table 5). The caries development for each child was not associated with the mean saliva fluoride concentrations across years 1, 2, and 3. This finding is exemplified by the scatter plot of DMFS increments versus saliva fluoride shown in Fig. 2. In both schools, there was a large and significant reduction after 3 years in the numbers of tooth surfaces with plaque (Table 5).

Linear regression analysis showed that neither the mean salivary fluoride level across years 1 to 3 nor the plaque score reduction influenced the net DMFS increment. However, the greater the reduction in the number of sites with plaque over the 3 years, the greater the number of reversals (caries lesions disappearing between baseline and year 3; regression coefficient b=0.11, 95 % CI for b=[0.01; 0.21]), and this relationship was statistically significant.

Discussion

This study showed that using a toothpaste containing 1,500 ppm F increases ambient whole saliva fluoride concentrations by about 0.02 ppm and the increased concentrations, measured after 1 year, do not increase further after 2 and 3 years use of the toothpaste. Also, the study showed that ambient saliva fluoride concentrations are not significantly affected by rinsing after toothbrushing nor are they related to caries increments after 3 years. Reductions in numbers of tooth surfaces with plaque were shown to be related to the number of caries reversals over the 3 years.

The increase in saliva fluoride concentrations recorded in this study is in close agreement with previous studies in a few adults who used fluoride toothpaste for 2–4 weeks [4, 5]. Although the increased saliva fluoride levels reflect the exposure to fluoride toothpaste among our subjects, the lack

Table 4 Reported frequency of toothbrushing and type of toothpaste used at baseline and at 3-year follow-up in 193 12-year-old Lithuanian children

	Baseline		Year 3	
	School A, n=90	School B, n=103	School A, n=90	School B, n=103
Toothbrushing frequency				
<1/week	4 (4 %)	2 (2 %)	–	–
<1/day	22 (24 %)	20 (19 %)	1 (1 %)	2 (2 %)
1/day	41 (46 %)	50 (49 %)	1 (1 %)	6 (6 %)
>1/day	23 (26 %)	31 (30 %)	88 (92 %)	95 (95 %)
Type of toothpaste used				
Fluoride	69 (77 %)	69 (67 %)	89 (99 %)	101 (98 %)
Nonfluoride	18 (20 %)	33 (32 %)	1 (1 %)	1 (1 %)
No paste	3 (3 %)	1 (1 %)	0 (0 %)	1 (1 %)

Table 5 Caries experience at baseline and caries increments for children examined both at baseline and at 3-year follow-up

	Quantity counted	School A (n=90), mean [95 % CI]	School B (n=103), mean [95 % CI]	Difference, mean [95 % CI]
At baseline	DMFS	16.6 [14.4; 18.8]	17.4 [15.9; 18.9]	−0.8 [−3.4; 1.8]
	Unerupted surfaces	1.8 [1.3; 2.4]	2.1 [1.5; 2.7]	−0.3 [−1.0; 0.5]
Changes among surfaces present at baseline	New DMFS	9.4 [8.1; 10.7]	8.6 [7.5; 9.7]	0.8 [−0.9; 2.5]
	Reversals	4.0 [3.4; 4.6]	4.4 [3.8; 5.1]	−0.4 [−1.3; 0.5]
	Net DMFS increment	5.4 [4.0; 6.8]	4.2 [2.8; 5.6]	1.2 [−0.8; 3.2]
Changes among surfaces unerupted at baseline	Unerupted surfaces	21.0 [16.8; 25.1]	23.8 [19.9; 27.7]	−2.8 [−8.5; 2.8]
	Carious at follow-up	1.8 [1.3; 2.4]	2.1 [1.5; 2.7]	−0.3 [−1.0; 0.5]
Baseline	Surfaces with plaque[a]	12.7 [11.6; 13.7]	12.8 [12.1; 13.6]	−0.2 [−1.4; 1.1]
At 3-year examination	Surfaces with plaque[a]	1.9 [1.3; 2.5]	2.1 [1.5; 2.7]	−0.3 [−1.1; 0.6]
Change from baseline to year 3	Surfaces with plaque[a]	10.8 [9.7; 11.9]	10.7 [9.7; 11.6]	0.1 [−1.3; 1.5]

Also given are the estimates of the number of surfaces (of 18 possible) with plaque at baseline and at follow-up

[a] Counted over a maximum of 18 surfaces

of an association between the occurrence of caries and the fluoride level may be explained by variations in other factors such as plaque and intake of carbohydrate.

The extremely low whole saliva fluoride concentrations recorded at the outset of our study may reflect the low levels of fluoride in drinking water in Kaunas (0.16 ppm) and especially the history of infrequent use of fluoride toothpaste reported by about half of the children. Such low levels may have been common before the use of fluoride toothpaste became so widespread.

Some authors have reported higher baseline saliva fluoride concentrations than those concentrations measured in our study. For example, Zero et al. [17] recorded mean fluoride concentrations of 0.05 ppm in adults living in an area with fluoridated water (Rochester, NY) who had used a nonfluoride toothpaste 24 h before baseline sampling.

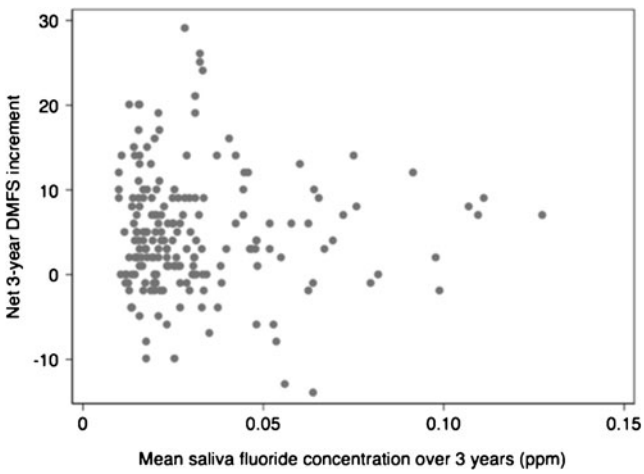

Fig. 2 Net DMFS increments after 3 years plotted against mean saliva fluoride concentrations over years 1 to 3 for all children (n=193) who were clinically examined at both baseline and 3-year follow-up and provided at least one saliva sample during years 1–3

We speculated that the higher saliva fluoride concentrations in such studies might be explained by continuous exposure to fluoride toothpaste for more than the 2–4 weeks previously studied, which could result in increases in saliva fluoride levels caused by accumulation of fluoride in the body (in bone) with time [18]. However, our data show that, at least up to 3 years of toothpaste use, there is no significant increase from the first year. Although exposure to fluoride toothpaste for longer than the 3 years used in our study may cause time-related increases in saliva fluoride, as discussed by Ismail [19], we expect that the role of systemic fluoride contributing to the fluoride concentration of saliva is probably very small compared to the fluoride added directly to whole saliva by regular use of fluoride toothpaste.

Some, more recent, studies have reported high baseline saliva fluoride levels, from 0.3 to 7 ppm [20, 21]. Of the total of 902 saliva samples collected for the present study, <3 % had fluoride concentrations of 0.1 ppm or more. We presume that these subjects had failed to comply with our instructions about not brushing teeth from the evening before sampling and that very high concentrations recorded in some studies emphasize the need for standardization of fluoride measurements highlighted in a recent study by Martinez-Mier et al. [22] comparing between-laboratory measurements of fluoride.

We speculated that the supervised nonrinsing on schooldays throughout the 3 years of this study might produce higher saliva fluoride levels in the nonrinsing subjects, who may have ingested more fluoride, but this was not the case. In relation to this, we would like to emphasize that, although we previously demonstrated a profound caries reduction among these children compared to a control school, we did not record a difference in caries between rinsing and nonrinsing.

This study has clearly demonstrated that regular use of fluoride toothpaste by individuals with a previously low level of exposure to fluoride results in ambient saliva fluoride concentrations similar to those previously recorded in subjects from areas with fluoridated water.

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Conflict of interest The authors declare that they have no conflict of interest.

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