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Urinary fluoride excretion in preschool children after intake of fluoridated milk and use of fluoride-containing toothpaste

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Objective: To assess the urinary fluoride excretion in preschool children after drinking fluoridated milk with 0.185 mg F and 0.375 mg F and to study the impact of use of fluoride toothpaste. ***Basic research design:*** Double-blind cross-over study. ***Participants:*** Nine healthy children, 2.5-4.5 years of age. ***Intervention:*** In a randomized order, participants drank 1.5 dl milk once daily for 7 days with no fluoride added (control), 0.185 mg fluoride added and 0.375 mg fluoride added. The experiment was performed twice with (Part I) and without (Part II) parental tooth brushing with 1,000 ppm fluoride toothpaste. The fluoride content in the piped drinking water was 0.5 mg F/L. ***Main outcome measure****:* Urinary fluoride excretion. ***Results:*** The 24-hour urinary fluoride excretion/kg body weight varied from 0.014 mg F for the placebo intervention and non-fluoride toothpaste to 0.027 mg F for the 0.375 mg intervention with use of 1,000 ppm fluoride toothpaste. The difference compared with the placebo intervention was not statistically significant for any of the interventions when fluoride toothpaste was used ($p>0.05$) while it was statistically significantly different when non-fluoride toothpaste was used ($p<0.05$). ***Conclusions:*** All sources of fluoride must be considered when designing community programs. With 0.5 mg F/L in the drinking water and daily use of fluoride toothpaste, most children had a fluoride intake optimal for dental health. In this setting, additional intake of fluoride milk was within safe limits up to 0.185 mg/day while conclusions about the safety of 0.375 mg/day were uncertain.

Key words: *caries prevention, children, fluoride excretion, fluoride toothpaste, milk fluoridation, Sweden*

Introduction

The role of fluoride in caries prevention is well established and an adequate, regular intake of fluoride is beneficial for maintenance of dental health. The caries-preventive role of fluoride is mainly topical whereas excessive fluoride intake during tooth formation can cause dental fluorosis which is a systemic side-effect. This is manifested as a developmental disturbance that makes enamel more porous. In mild fluorosis, there are white opaque striations across the enamel surface, whereas in more severe cases, the porous regions increase in size with enamel pitting and secondary discoloration of the enamel surface (Bronckers *et al.,* 2009). A total daily intake of 0.05 to 0.07 mg F/kg body weight is suggested as optimal for dental health during tooth formation ages while a daily intake of 0.1 mg F/kg body weight is defined as an upper safe level (Burt, 1992). Fluoride is ingested from water, foods, toothpaste, and other agents. It is estimated that around 90% of the ingested fluoride is absorbed by healthy children of which 55% is retained in the body when the total daily intake is above 0.5 mg (Villa *et al.,* 2010, Zohoori *et al.,* 2013). The metabolism of fluoride may be affected by many factors including genetics, diet, urinary flow rate and renal tubular fluid pH (Whitford, 1966). Urinary fluoride excretion is the most important metabolic pathway for fluoride elimination from the body in children and adults and it is estimated that approximately 35 % of ingested fluoride is excreted in the urine of children (Rugg-Gunn *et al.,* 2011; Villa *et al.,* 2010). The World Health Organization, WHO (2014) has issued standards for 24-hour urinary fluoride excretion with an optimal fluoride intake. These values range between 0.30 to 0.41 mg F/24h for the age group 2-4 years and between 0.33 and 0.45 mg F/24h for children aged 3-5 years.

Milk has been recognized as a vehicle for community based administration of fluoride to reduce the burden of dental caries in children (Banoczy *et al.,* 2013; Cagetti *et al.,* 2013; Petersen *et al.,* 2015; Yeung *et al.,* 2015). The amount of fluoride added to milk should depend on background fluoride exposure and age of the children and fluoride has been added to milk in caries preventive programmes in the range of 0.5 to 1.0 mg per day (Banoczy *et al*., 2013). In a study by Ketley *et al*. (2002) children with a low level of fluoride in the drinking water (<0.1 mg/L) drank fluoridated milk with 0.5 mg F. When data were corrected for age and fluoride excretion from toothpaste the fluoride intake in the children drinking fluoridated school milk was somewhere between those living in an optimally fluoridated area and those in a low fluoride area. As dental caries experience is cumulative from an early age (André Kramer *et al.,* 2014) there are clear benefits of including children soon after tooth eruption in such milk programmes. This, however, increases the concern for fluoride toxicity for the individual child and a lower fluoride concentration may be more appropriate for the youngest age groups. The aim of the present study was to assess the urinary fluoride excretion in children aged 2.5-4.5 years after a daily intake of fluoridated milk with 0.185 mg F and 0.375 mg F and to compare the levels with and without use of fluoride toothpaste.

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Material and methods

Participants were 9 healthy children aged 31-56 months, mean age 44.4 (SD 9.5) months. Their mean body weight was 18.4 (2.0) kg, range 15-22 kg. The fluoride content in their piped drinking water was 0.5 mg F/L. The study was approved by the Regional Ethical Review Board in Umeå, Sweden (Dnr 2013-39-32M). Parental signed informed consent was received before participation.

The study had three arms and was conducted with a double-blind crossover design (Figure 1) with two different toothpaste regimens; Part I with parental tooth brushing with 1,000 ppm fluoride toothpaste two times per day, and Part II with parental tooth brushing with non-fluoride toothpaste two times per day. No other fluoride supplements were given during or between the test periods. After a one week run-in period, the children were served 1.5 dl milk once daily at home for 7 days with no fluoride added, 0.185 mg fluoride added and 0.375 mg fluoride added. The milk was served in a randomized order in a double-blind way and none of the parents, the providers or the analyst knew the fluoride content of the milk. The allocation concealment was secured through an independent monitor at the university. The three test sessions were separated by one-week wash-out periods when the children used toothpaste with 1,000 ppm sodium fluoride toothpaste (Part I) or non-fluoride toothpaste (Part II). Both toothpaste products were provided by the investigators. Parents were instructed to use a pea-sized amount of toothpaste for each brushing. Fluoride for milk supplementation was provided in standardized color-coded capsules containing milk-powder and the content was added and mixed to 150 ml fresh milk immediately before drinking. At day seven in each test session, parents sampled a 24-hour urine collection in plastic vials. The total urine volume, after mixing, was measured and recorded and two test tubes were filled; one was sent to the laboratory and one was kept frozen, as a reserve. During each test session parents filled in a logbook with data on body weight of the child, intake of milk, tooth brushing occasions and the use of toothpaste. On day seven, the number of urinations and the total sampled urine volume was registered. One child displayed incomplete urine collection during Part I in the 0.375 mg fluoride test session due to stomach flu and was therefore excluded from analysis.

At arrival at the laboratory, 6 ml urine was used to analyze of the urinary creatinine level at the biochemical laboratory at Umeå university Hospital according to validated standards. The remaining urine was centrifuged at 4,000G (Beckman Allegra centrifuge) for 10 minutes and the precipitate was removed. Thereafter, the urine samples were kept frozen at -20 C° until all samplings had been collected. For analysis of the urine fluoride content, 1 ml urine was mixed with 1 ml TISAB II and the fluoride content was read with an ion-sensitive electrode (Orion research Inc. Cambridge, Mass, USA). The electrode was calibrated with distilled ionized water and standards of 0.01, 0.1, 1.0 and 10 mg F/L. Standard curves were performed before each reading session. All readings were performed in duplicate and the mean of the two readings was calculated. To translate the mV value to mg/L the program FLUORBAS was used. The program has been developed and validated by Mr. Rolf Sjöström at the biochemical laboratory at the Department of Odontology, Umeå University.

Data were analysed using PASW v22.0 statistics software. The total ingested fluoride/24 hour (TDFI) was calculated according to the formula by Villa *et al*. (2010) where Daily Urinary Fluoride Excretion (DUFE) = Total Daily Fluoride Intake (TDFI) x 0.35 + 0.03. The contribution of fluoride from toothpaste was calculated as the difference between the total estimated ingested fluoride with and without fluoride toothpaste usage in the placebo test group. The paired sample t-test was used for comparisons between test groups and Pearson correlation coefficient was calculated on the correlation between total ingested fluoride and ingested fluoride from toothpaste. A *p*-value of less than 0.05 was considered as statistically significant.

Results

The compliance with the interventions was excellent according to the logbooks. During Part I when 1,000 ppm toothpaste was used, eight children provided three valid 24-hour samples and one child two valid samples. In Part II when non-fluoride toothpaste was used, all participating children provided three valid 24-hour samples. Urinary volumes ranged between 300 ml to 760 ml. Urinary creatinine values ranged between 9.0 to 22.6 mg/kg body weight and were fairly stable in each individual indicating valid samplings.

The urinary fluoride excretion per 24 hour and per kg body weight is summarized in Table 1. When 1,000 ppm fluoride toothpaste was used with the placebo intervention, the mean urinary fluoride excretion was 0.402 mg F/24 hour and with non-fluoride toothpaste it was 0.272 mg F/24 hour. After the 0.185 mg fluoride intervention, the corresponding values were 0.480 mg F/24 hour and 0.336 mg F/24 hour, respectively, and after the 0.375 mg fluoride intervention it was 0.503 mg F/24 hours with fluoride toothpaste and 0.404 mg F/24 hour with non-fluoride toothpaste. The differences in 24-hour fluoride excretion did not reach statistical significance compared with the placebo regimen for any of the interventions when fluoride toothpaste was used ($p>0.05$),

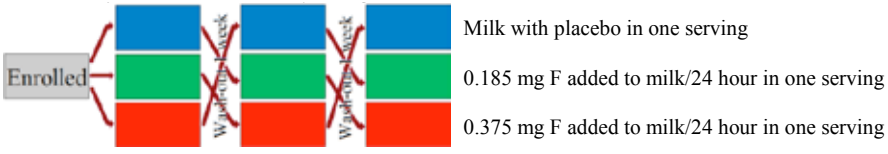

Figure 1. *Overview of the cross-over study design*
NB: The set-up was repeated twice; Part I with twice daily tooth brushing with 1,000 ppm fluoride toothpaste, and Part II with twice daily tooth brushing with non-fluoride toothpaste.

Table 1. Mean and standard deviation (sd) for 24 hour fluoride excretion and estimated total ingested F/24 h with and without use of fluoridated toothpaste with 1,000 ppm twice daily and fluoride level in drinking water 0.5 mg F/L in children aged 2.5-4.5 years.

	Placebo *1.5 dl milk daily with 0 mgF/L*		*Intervention with 0.185 mg F* *1.5 dl milk daily with 1.25 mg F/L*		*Intervention with 0.375 mg F* *1.5 dl milk daily with 2.5 mg F/L*	
	Toothpaste 1,000 ppm n=9, mean (SD)	*Non-fluoride toothpaste n=9, mean (SD)*	*Toothpaste 1,000 ppm n=9, mean (SD)*	*Non-fluoride toothpaste n=9, mean (SD)*	*Toothpaste 1,000 ppm n=8, mean (SD)*	*Non-fluoride toothpaste n=9, mean (SD)*
Urinary fluoride excretion, mg F/24h	0.402 (0.151)	0.272 (0.096)	0.480 ns (0.138)	0.336[1] (0.095)	0.503 ns (0.114)	0.404[1] (0.090)
mg F/kg body weight/24h	0.022 (0.008)	0.014 (0.004)	0.026 ns (0.007)	0.018[1] (0.003)	0.027 ns (0.010)	0.021[1] (0.004)
Estimated total F intake mg/24h, TDFI[2]	1.065 (0.430)	0.694 (0.275)	1.285 ns (0.394)	0.875[1] (0.272)	1.351 ns (0.324)	1.114[1] (0.278)

ns = not statistically significant compared to placebo and use of fluoride toothpaste; [1] $p<0.05$ compared to placebo and use of non-fluoride toothpaste; [2] calculated according to the formula by Villa *et al.* (2010)

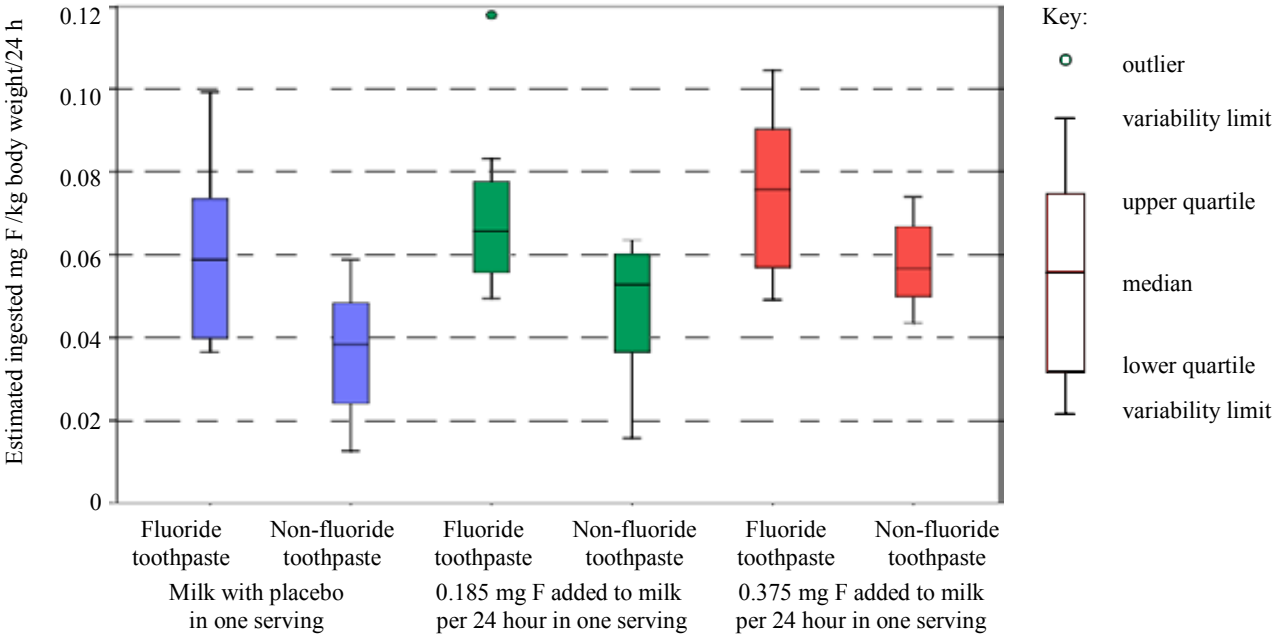

Figure 2. *Box-plots of estimated ingested fluoride (F/24 hour/kg body weight) in children aged 2.5-4.5*
NB: The fluoride level in drinking water was 0.5 mg F/L; Variability limit whiskers mark IQRx1.5±Q1/Q3

while the differences were statistically significant when non-fluoride toothpaste was used ($p<0.05$). The 24-hour urinary fluoride excretion/kg body weight varied from 0.014 mg F for the placebo intervention and non-fluoride toothpaste to 0.027 mg F for the 0.375 mg intervention with use of 1,000 ppm fluoride toothpaste. The differences compared with the placebo intervention were not statistically significant for any of the interventions when fluoride toothpaste was used ($p>0.05$) while it was statistically significantly different when non-fluoride toothpaste was used ($p<0.05$).

The estimated mean 24-hour ingestion of fluoride is presented in Table 1 and per kg body weight in Figure 2. The values ranged from 0.01 mg/kg body weight to 0.12 mg/ kg body weight across the intervention groups and toothpaste regimens. When the control milk interventions during Part I and Part II were compared, the contribution of fluoride from toothpaste in relation to the estimated total ingested amount of fluoride was 23%. As shown in Figure 3, there was a statistically significant correlation between the estimated ingested amount of fluoride and the percentage contribution of fluoride from toothpaste (r=0.845; $p<0.01$).

Discussion

The distribution of dental caries in many countries is unequal with a strong socio-economic gradient (Piovesan *et al*., 2014; Schwendicke *et al.,* 2015; Stecksén-Blicks *et al*., 2014; Sutthavong *et al*., 2010). To reduce dental health inequalities among children, development of population based preventive strategies are fundamental and there is scientific evidence that supports fluoride technologies (Twetman, 2015). However, monitoring fluoride exposure and excretion is an important part of community-based fluoride supplementation programmes and fluoride excretion in urine can be considered a useful biomarker of fluoride exposure, as urine is the most important metabolic pathway for removal of absorbed fluoride from the body (Maguire and Zohoori, 2013).

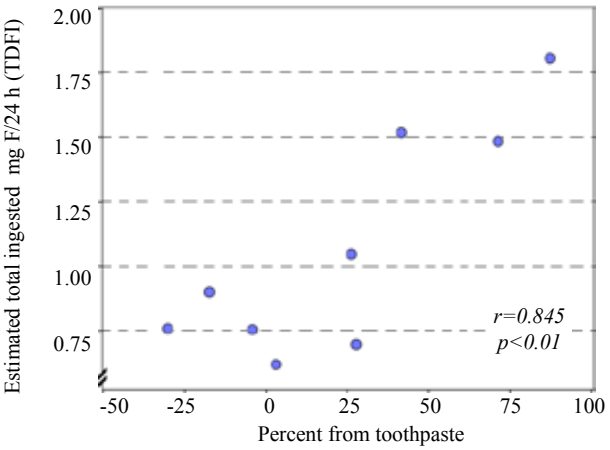

Figure 3. *Estimated total ingested fluoride in the placebo arm and the contribution of fluoride from toothpaste in nine children aged 2.5-4.5 years*

This study therefore determined urinary fluoride excretion in preschool children after intake of fluoridated milk. Urinary creatinine values were fairly stable on individual basis between the samplings indicating valid 24-hour collections (World Health Organization, 2014). The number of subjects was however limited as 24-hour urine collections in children are demanding and burdensome on families. The power was however sufficient to show statistically significant differences in urinary fluoride excretion after the different fluoride exposures. To ensure that we assessed stable fluoride levels, all intervention lasted for 7 days and there was a one-week wash-out period between the different interventions.

It was clear that the two active interventions added very little to the total fluoride excretion when fluoride toothpaste with 1,000 ppm was used twice daily against a background of 0.5 mg/L in the public drinking water; however the interventions resulted in a statistically significant increase in urinary fluoride excretion when non-fluoride toothpaste was used. In comparison with the WHO standards for urinary fluoride excretion, the placebo intervention and use of fluoride toothpaste indicated an optimal fluoride intake while the use of non-fluoride toothpaste indicated suboptimal intake. When fluoride toothpaste was used during the 0.375 mg F intervention, the urinary excretion was slightly above the upper margin for optimal intake in some individuals while it was below when non-fluoride toothpaste was used. Thus, our findings support the suggestion of a dose-related fluoride balance as shown by Ekstrand *et al*. (1994) and Zohoori *et al*. (2013).

According to previous data on the relationship between urinary fluoride excretion and total fluoride intake, an average of 35% of the ingested fluoride is excreted in the urine of children (Villa *et al*., 2010; Rugg-Gunn *et al*., 2011) and we based our calculation of the total fluoride intake on this figure. In the present study, the total ingested fluoride from diet, use of 1,000 ppm fluoride toothpaste twice daily and a background of 0.5 mg F/L in the piped drinking was calculated to be 1.065 mg/24 hour. For the present age group, this figure corresponds to an optimal fluoride intake of 0.05 to 0.07 mg/kg body weight which is in line with WHO recommendations (World Health Organization, 2014). In the 0.185 mg F intervention group with use of fluoride toothpaste the mean intake was 0.07 mg F/kg body weight while the corresponding value was 0.075 mg F/kg body weight in the 0.375 mg F intervention group. In the intervention groups, there was one child with a calculated 24 h fluoride intake slightly above the upper safe limit of 0.1 mg/kg body weight and this was the child with highest contribution of fluoride from toothpaste (87%). There were suboptimal intakes in all groups mainly when fluoride toothpaste was not used. The present data suggest that fluoride interventions with up to 0.185 mg F/day are within safe limits for children weighing 15-22 kg who use fluoride toothpaste according to recommendations for the age group and consume water with 0.5 mg F/L (0.5 ppm F). Milk fluoridation programmes with higher levels of fluoride for this age group should therefore primarily be directed to vulnerable children with non-regular use of fluoride toothpaste and low levels of fluoride in the drinking water.

All parents were given thorough instruction to use a pea-sized amount at each brushing but we were unable to measure the exact amount of toothpaste used. It is therefore possible that such individual variations may have influenced the results. Furthermore, the intake of water and food was not standardized during and between the different test periods in order to reflect the real life situation. This may explain why the contribution of fluoride from toothpaste was negative in some cases compared to the regimen with non-fluoride toothpaste. With these limitations, the contribution of fluoride from toothpaste gives an estimated mean value of 0.37 mg F/24 h. Our estimation of ingested fluoride from toothpaste was slightly higher than values of ingested fluoride from toothpaste reported in 4-5 year-old Malaysian children using duplicate technique (Siew Tan and Razak, 2005). That study found a high correlation between the amounts of toothpaste dispensed and ingested in this age group. In our setting, data indicate a close relationship between the total ingested fluoride and the amount of fluoride from toothpaste.

This finding of this study clearly illustrates the fact that all sources of fluoride must be considered when designing community programmes. It was concluded that with 0.5 mg F/L in the drinking water and use of fluoride toothpaste, most children had an intake of fluoride optimal for dental health. Additional intake of fluoride milk was safe up to 0.185 mg/day while conclusions about the safety of 0.375 mg/day were uncertain. Suboptimal fluoride levels following the fluoride milk interventions were found when non-fluoride toothpaste was used.

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