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Fluoride ingestion from toothpaste and diet in 1- to 3-year-old Brazilian children

Beatriz Simões de Almeida[1], Vanessa Eid da Silva Cardoso[2] and Marília Afonso Rabelo Buzalaf[3]

[1]Bauru Dental School, University of São Paulo, Bauru, Brazil, [2]Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, Brazil, [3]Area of Biochemistry, Department of Biological Sciences, Bauru Dental School, University of São Paulo, Bauru, Brazil

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Abstract – *Objective:* This study estimated the total daily fluoride intake of 1- to 3-year-old children from diet and dentifrice. The constituents of the diet were divided into solids, water, milk, and other beverages, which were analyzed separately. The correlation between fingernail fluoride concentrations and the total daily fluoride intake by children was also investigated. *Methods:* Thirty-three children, living in a fluoridated area, participated in the study. Fluoride intake from diet was monitored by the 'duplicate plate' method, investigating the different constituents of the diet. Fluoride ingested from dentifrice was determined by subtracting the amount of fluoride recovered after brushing from the amount originally placed onto the child's toothbrush. Fingernails were clipped and collected on three occasions. Fluoride was analyzed with the ion-specific electrode, after hexamethyldisiloxane-facilitated diffusion. Data were tested by ANOVA and Tukey's *post hoc* tests, Student's *t*-tests and linear regression ($P < 0.05$). *Results:* Mean (±SD) fluoride intake from diet and dentifrice was 0.025 ± 0.013 and 0.106 ± 0.085 mg/kg body weight/day, respectively, totaling 0.130 mg/kg body weight/day. A strong positive correlation ($r = 0.971$, $P < 0.0001$) was seen between the amount of dentifrice loaded onto the brush (0.49 ± 0.30 g) and the amount of fluoride ingested during each tooth brushing (0.59 ± 0.45 mg). Among the constituents of the diet, water and milk had a significantly higher contribution to the fluoride intake (0.18 ± 0.11 mg/day, $P < 0.0001$), when compared with solids (0.07 ± 0.05 mg/day) and other beverages (0.07 ± 0.04 mg/day). Mean (±SD) fingernail fluoride concentration on the three dates of collection was 3.11 ± 1.14, 2.22 ± 1.47 and 3.53 ± 1.40 μg F/g. There was no significant correlation between fingernail fluoride concentration and the total fluoride intake. *Conclusions:* Most of the children are exposed to a daily fluoride intake above the suggested threshold for dental fluorosis. The dentifrice alone is responsible for an average of 81.5% of the daily fluoride intake, while among the constituents of the diet, water and milk are the most important contributors. In addition, small variations in daily fluoride intake cannot be detected in fingernails.

Key words: dental fluorosis; dentifrice; diet; fluoride

Prof. Marília Afonso Rabelo Buzalaf, Faculdade de Odontologia de Bauru, Universidade de São Paulo, Al. Octávio Pinheiro Brisolla, 9-75, V. Universitária, Bauru, SP, Brazil, CEP: 17012-901
e-mail: mbuzalaf@fob.usp.br

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The prevalence and severity of dental fluorosis have increased in both optimally fluoridated and non-fluoridated areas in many countries, including Brazil (1, 2). Recent data show a prevalence of dental fluorosis of 8.56% in 12-year-old Brazilian children (3). However, previous Brazilian studies have shown that the prevalence of dental fluorosis differs across the communities, ranging from 0% (4) to 97.6% (5). In the few studies that reported the major cases of severity, they were attributed to endemic fluorosis and were due to high levels of fluoride in the naturally fluoridated drinking water.

Dental fluorosis is regarded as a systemic effect secondary to total fluoride intake and its

absorption. It depends on the quantity of fluoride and duration of exposure, the stage of tooth development at the time of exposure, and individual variation in susceptibility. Evans and Darvell (6) showed that the maxillary central incisor appears to be most at risk of fluorosis from fluoride ingestion between 15 and 24 months of age. Data are unavailable for the normal levels of plasma fluoride that can lead to avoidance of development of dental fluorosis (7). A fluoride intake of 0.05–0.07 mg/kg body weight/day is usually regarded as optimum, being established empirically (8). However, even though these values are most frequently cited as the optimum dose, investigations conducted in Kenya have found dental fluorosis with a mean fluoride intake as low as 0.04 mg/kg body weight/day (9). In addition, analytical results from several studies (10–14) support the concept that fluoride levels in the developing enamel are directly related to plasma fluoride levels. As plasma levels show small peaks because of the normal daily patterns of fluoride exposure from eating, drinking and brushing teeth (15), any substantial increase in plasma fluoride levels caused by the elevated dietary fluoride levels or fluoride intake from dentifrice should be avoided by children during the critical period of fluoride exposure for fluorosis development.

When assessing the safety of various levels of fluoride intake, it is important to take into consideration all potential sources. These sources might include drinking water, fluoride oral care products and the environment, as well as food and beverages (1, 8). The trio of diet, dentifrice and supplementation could lead to the exceeding of the optimal levels of fluoride intake. Some studies have been conducted to evaluate fluoride intake from diet and dentifrice (7, 16–21). Nevertheless, most of these studies analyzed total combined diet and not separate components.

The increase in the prevalence of dental fluorosis has intensified the search for biomarkers of exposure to fluoride that are easy to collect and analyze (22). Nail sampling is simple and non-invasive, and there are many reports suggesting the use of nails as biomarkers for fluoride exposure in humans (23–31).

The aim of this study was to estimate the total daily fluoride intake by 1- to 3-year-old Brazilian children from different constituents of the diet and dentifrice. In addition, fingernail samples were collected in order to evaluate the correlation between fluoride concentrations in nails and total daily fluoride intake by children.

Materials and methods

Study population

Thirty-three 1- to 3-year-old children who attended nine public full-time daycare centers in Bauru, state of São Paulo, Brazil, participated in the study. This age group was chosen considering the critical period for the development of dental fluorosis in permanent central maxillary incisors. Children who participated in this study were not randomly chosen; they were children for whom parental permission had been granted. Bauru has optimally adjusted fluoridated water (0.6–0.8 ppm). The protocol for the study was reviewed and approved by the IRB of the Bauru Dental School, University of São Paulo. The nature and purposes of the study were explained verbally and in writing to the subjects and their parents who signed an IRB-approved informed consent document.

Estimation of fluoride intake from diet and dentifrice

In order to estimate the total fluoride intake of the children, fluoride intake from diet was monitored by the 'duplicate plate' method, as described by Guha-Chowdhury et al. (7), and fluoride ingested from dentifrice was determined by subtracting the amount of fluoride recovered after brushing from the amount originally placed on the children's toothbrush.

Duplicate plate approach

Duplicate portions of all foods and drinks consumed by each child over 24 h were collected in two seasons (winter and summer) on two separate days over a 1-week period, once during the week and again on a weekend day. The constituents of the diet were divided into solids, water and milk, and other beverages, and were collected in three different plastic vials. Water and milk were grouped together because they are the most consumed beverages by children of this age range (32). Additionally, at the daycare centers, powdered milk is usually consumed by children, and water is used to reconstitute it (33). Samples were collected by teachers at the daycare centers and parents at home. They were instructed to stick to the usual dietary habits of the children and to duplicate the diet as precisely as possible by observing the actual amounts that the children had consumed. They were requested to remove parts of foods not normally eaten, such as seeds,

cores, skin, and bones, before placing the food in the container.

Solid samples were homogenized using deionized water and the total volume was measured. An aliquot sample of 50 ml of the homogenized solid foods, water, milk, and other beverages was taken individually and frozen (−20°C) until analysis. The use of fluoride supplements was assessed by a questionnaire.

Simulated tooth brushing test

As the children spent most of their hours awake at the daycare centers, the estimation of fluoride intake from the dentifrice was made at the daycare centers. Attempts were made to simulate real-life conditions by replicating whether the teacher or the child performed the brushing, the size of the toothbrush used, how much dentifrice was loaded onto the brush as well as its brand, whether the adults or the children applied the dentifrice to the brush, whether the children expectorated after brushing and the length of time spent brushing. The toothbrush was weighed (±0.01 g). Then the adult or child spread dentifrice onto the toothbrush, according to normal practice, and the final weight of the toothbrush plus dentifrice was recorded. This provided information on the amount of fluoride loaded onto the brush. Brushing was performed by the children with or without assistance and under the observation of the examiner. Depending on their habits, the children were allowed to expectorate or not and to rinse with deionized water or not. The expectorated material was collected in a weighed, plastic, wide-mouth vessel and analyzed for F. The toothbrush was thoroughly rinsed in 50 ml of deionized water, and the rinse was analyzed for fluoride to determine the amount of F left on the toothbrush. The amount of F left on the toothbrush and the amount expelled were added, to give the total amount of fluoride not swallowed. The amount of fluoride ingested was then indirectly derived, by subtracting the amount of fluoride expelled from the amount initially loaded onto the toothbrush. Information on frequency of brushing (in daycare centers and at home) was obtained through a questionnaire and was used to calculate the daily fluoride intake from dentifrice for each child. These procedures were carried out for two consecutive days.

In addition, samples of the usual dentifrice used by children were collected in order to analyze their fluoride concentration. Children were weighed in order that their fluoride intake could be expressed in terms of mg/kg body weight.

Tap water collections

As fluctuations in public water fluoride levels have been described in Bauru (34), two samples of tap water were collected at the houses of the children, on the same day of diet collection. Water samples, collected in 50-ml plastic vials, were frozen (−20°C) until fluoride analysis.

Nail sampling

Fingernails were clipped and collected three times. The first was in winter and the third in summer, on the same days when duplicate diet samples were collected. The second one was on an intermediate day, around 3 months after the first collection.

The parents were instructed to let the children's nails grow for 15 days before clipping. The researcher who clipped the nails (BSA) was trained to clip all the nails and to store them in marked vials.

Fluoride analysis

Fluoride analysis of water samples was made by the direct method using an ion-specific electrode (Model 9609: Orion Research, Cambridge, MA, USA), after sample buffering with an equal volume of total ionic strength adjustment buffer (TISAB). Standards were prepared by serial dilution of a 100 ppm NaF stock solution (Orion). The standard curve had a correlation coefficient ≥0.99. The mean repeatability of duplicate samples was 96.2%. In addition, fluoride analyses in 10% of samples were repeated, giving a mean reproducibility of 95.3%.

Diet, dentifrice and fingernail samples were analyzed for fluoride after overnight hexamethyldisiloxane (HMDS)-facilitated diffusion (35) as modified by Whitford (15), using the fluoride ion-specific electrode and a miniature calomel reference electrode (Accumet, no. 13-620-79: Fischer Scientific, Pittsburgh, PN, USA), coupled to a potentiometer (Orion Research, model EA 940). During the diffusion process, which was conducted at room temperature, the solutions in the non-wettable Petri dishes (Falcon, No. 1007: Becton Dickinson, Franklin Lakes, NJ, USA) were gently swirled on a rotatory shaker. Fluoride standards (0.0095, 0.019, 0.095, 0.190 and 0.950 μg F – for nails; 0.019, 0.095, 0.190, 0.950, 1.900, 4.750 and 7.500 μg F – for diet; 0.950, 1.900, 4.750 and 7.500 μg F – for tooth brushing and dentifrice) were prepared by serial dilution of a 0.1 M fluoride (Orion 940906) in triplicate and diffused in the

same manner as the samples. Comparison with identical non-diffused fluoride standards showed that recovery after diffusion was >99%. The standard curve had a correlation coefficient ≥0.99. All samples were analyzed in duplicate. The mean repeatability of duplicate samples was 94.4%. In addition, fluoride analysis in 10% of samples was repeated, giving a mean reproducibility of 95.9%.

Statistical analysis

Repeated-measures ANOVA was used to detect differences among the different dietary components. It was complemented by Tukey–Kramer tests for multiple comparisons. The same tests were used to detect differences among fluoride concentrations in fingernails, at the different collection times.

Pearson's correlation coefficient was used to assess the correlation between the total fluoride intake (mean of all collections for each child) and fingernail fluoride concentration (mean of three collections for each child), as well as between the amount of dentifrice loaded onto the toothbrush and the amount of fluoride ingested from each tooth brushing.

Differences between fluoride ingested from diet and dentifrice were evaluated through paired Student's *t*-test. The same test was used to evaluate differences between the amount of fluoride intake on the 2 days of collection and for the two seasons (winter and summer) of collection, as well as differences between fluoride concentrations in water collected in two periods. The significance level was set at 5%.

Results

The participants included in the study were from the deprived areas of the city. Children were aged 20–32 months (27 ± 3.3), of which 42.4% were female and 57.6% male. Their weight ranged from 10 to 16 kg (12.87 ± 1.46). The monthly per capita household income ranged from R$ 20 to 400 (143.52 ± 84.08, approximately 60 US$).

Table 1 shows the data regarding fluoride intake (mg F/day) from solids, water and milk, and other beverages, and the total diet of children ($n = 33$) during the two periods (winter and summer) of collection. There was no statistically significant difference among the parameters analyzed ($P > 0.05$).

Table 2 shows data regarding fluoride intake (mg F/day) from solids, water and milk, and other beverages, and in total by children ($n = 33$). The fluoride intake from water and milk was signifi-

Table 1. Mean, standard deviation, minimum, maximum and 95% CI of the fluoride intake by children (mg F/day, $n = 33$) from solids (S), water and milk (WM), other beverages (B) and the total diet (TD), during the two periods of collection: winter (1) and summer (2)

Ingested F	S1	S2	WM1	WM2	B1	B2	TD1	TD2
Mean	0.07	0.08	0.20	0.17	0.07	0.07	0.32	0.30
SD	0.06	0.06	0.16	0.09	0.05	0.05	0.21	0.14
Minimum	0.00	0.01	0.00	0.02	0.00	0.00	0.01	0.08
Maximum	0.22	0.29	0.63	0.38	0.24	0.25	0.71	0.72
95% CI	0.05–0.09	0.06–0.10	0.14–0.25	0.14–0.20	0.05–0.08	0.05–0.09	0.24–0.40	0.24–0.35

There were no statistically significant differences between summer and winter for any of the variables analyzed ($P > 0.05$). Values represent the mean of the collections made on two separate days over a 1-week period, once during the week and again on a weekend day.

Table 2. Mean, SD, minimum, maximum and 95% CI of fluoride intake (mg F/day) by children ($n = 33$) from solids, water and milk, and other beverages

	Components of the diet			
Ingested F (mg/day)	Solids	Water and milk	Other beverages	Total diet
Mean	0.08[a]	0.18[b]	0.07[a]	0.31
SD	0.05	0.11	0.04	0.16
Minimum	0.01	0.00	0.00	0.04
Maximum	0.19	0.48	0.17	0.70
95% CI	0.06–0.09	0.15–0.22	0.05–0.08	0.26–0.38

Mean values followed by different letters are statistically significant ($P < 0.0001$).

cantly higher than that from solids and other beverages ($P < 0.0001$). No children were reported to be using fluoride supplements.

Mean (±SD) fluoride concentration in water collected in two periods was 0.76 ± 0.28 and 0.57 ± 0.28 μg F/ml and this difference was statistically significant ($P < 0.01$). Mean (±SD) fluoride intake from diet in the weekday and in the weekend day was 0.34 ± 0.13 and 0.28 ± 0.20 mg F/day, respectively, and this difference was not statistically significant ($P > 0.05$). Table 3 shows the data regarding the amount of dentifrice used (g), total fluoride (mg) and ingested fluoride (mg), for each tooth brushing. Most of the children used 1500 ppm F dentifrices. The mean number of brushings per day was 2.3. Figure 1 shows the strong significant correlation between the amount of dentifrice loaded onto the toothbrush and fluoride ingested for each tooth brushing ($r = 0.971$; $P < 0.0001$).

Table 4 combines data from Tables 2 and 3 and converts them to per kg body weight. The data regarding fluoride intake (mg F/kg body weight/day) from diet, dentifrice and the total (diet + dentifrice), by children ($n = 33$), combined across seasons, are shown. The fluoride intake from dentifrice was significantly higher than that from diet ($P < 0.0001$). Dentifrice was responsible for on average 81.5% of total fluoride intake by the children ($n = 33$).

Mean (±SD) fingernail fluoride concentration in the three dates of collection was 3.11 ± 1.14, 2.22 ± 1.46 and 3.53 ± 1.40 μg F/g respectively. Mean fingernail fluoride concentration for samples collected in the second period was significantly lower than in the first and third collections ($P < 0.05$).

For children who ingested a dose above 0.07 mg F/kg body weight/day ($n = 24$), there was no significant correlation between fingernail fluoride concentration and total fluoride intake ($r = -0.052$, $P = 0.810$). There was no significant correlation between fluoride concentration in water and fingernail fluoride concentration ($r = 0.252$, $P = 0.235$), when all the children are considered ($n = 33$).

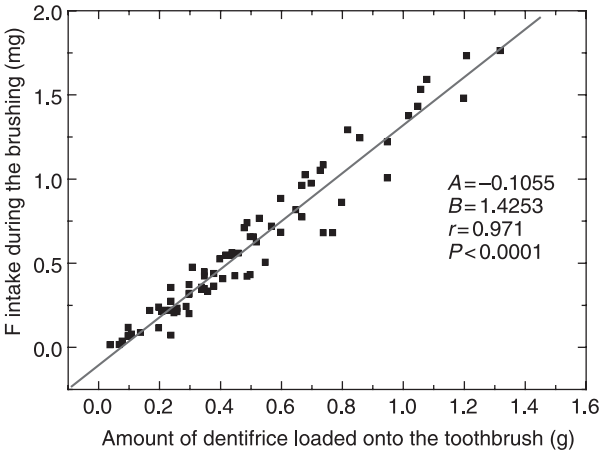

Fig. 1. Correlation between the amount of dentifrice loaded onto the toothbrush (g) and the amount of fluoride ingested (mg) for each toothbrushing.

Table 4. Mean, SD, minimum, maximum and 95% CI of the fluoride intake by children (mg F/kg body weight/day, $n = 33$) from diet, dentifrice and the total (diet + dentifrice), combined across seasons

Ingested F	Diet	Dentifrice	Total
Mean	0.025[a]	0.106[b]	0.130
SD	0.013	0.085	0.087
Minimum	0.003	0.004	0.027
Maximum	0.070	0.401	0.413
95% CI	0.021–0.029	0.076–0.137	0.104–0.165

Mean values followed by different letters are statistically significant ($P < 0.0001$).

Discussion

Fluorosis has been the subject of concern, as there has been a reported increase in its prevalence all over the world (2, 36–39). In Brazil, a critical review has shown that there are already more cases of

Table 3. Mean, SD, minimum, maximum and 95% CI of the amount of dentifrice used (g), total fluoride used (mg) and ingested fluoride (mg), per brushing and per day

	Dentifrice used per brushing	Total F used per brushing	Ingested F per brushing	Total F used per day	Ingested F per day
Mean	0.49	0.76	0.59	1.75	1.34
SD	0.30	0.50	0.44	1.24	1.08
Minimum	0.04	0.04	0.01	0.04	0.01
Maximum	1.32	2.20	1.76	6.06	5.19
95% CI	0.42–0.56	0.65–0.88	0.49–0.69	1.46–2.03	1.09–1.59

$n = 33$. Values correspond to the averages of four collections (2 days × 2 seasons).

fluorosis than would be expected, despite few studies reporting cases of major severity. Although dental fluorosis is not recognized as a public health problem in Brazil, measures are needed for its prevention and surveillance (2).

There are some studies regarding the fluoride intake of Brazilian children by diet and dentifrice. Nevertheless, these studies analyzed the total diet and not separate components (19–20, 40). In this study, diet was separated and analyzed in three distinct groups: solids, water and milk, and other beverages. This separation was based on the study of Clovis and Hargreaves (32), in which water and milk were the beverages most consumed by Canadian children of the same age as children in the present study. In the study by Rojas-Sanchez et al. (16), the children's diet was divided into two groups, separating foods and beverages. Mean ± SD (95% CI) of fluoride intake from diet was 0.025 ± 0.013 (0.003–0.700) mg F/kg body weight/day. These results are similar to the findings by Levy et al. (31), who showed a mean fluoride intake from diet of 0.029 mg F/kg body weight/day, by 2- to 6-year-old Brazilian children, living in Bauru. In the present study, among the diet constituents, the group of water and milk contributed significantly more to the dietary fluoride intake compared with the other groups. Fluoride intake from foods and beverages ingested by 16- to 40-month-old children, residing in fluoridated Indianapolis averaged 146 ± 17 and 396 ± 52 μg F/day, respectively (16). Although the children's age and the dose of fluoride intake are different in both studies, the percentage contribution of the beverages to the fluoride intake from diet was quite similar [73% in the study by Rojas-Sanchez et al. (16) and 77% in the present study, when all beverages are considered]. According to Rojas-Sanchez et al. (16), fluoride intake from beverages is directly related to the mean fluoride level in the drinking water. In Brazil, the study by Levy et al. (31) shows a fluoride intake from diet of 0.55 ± 0.61 and 0.09 ± 0.06 mg F/day for 2- to 6-year-old children, residing in a fluoridated and in a non-fluoridated community, respectively. These findings confirm the important role of water for fluoride intake from the diet. Regarding the fluoride concentration in the water samples collected in the houses of the children in this study, there was a statistically significant difference between the mean of fluoride concentration found in the first and second collections. In addition, the fluoride concentrations found spanned a wide range. These findings are in agreement with previous reports showing the occurrence of fluctuations in the water fluoride levels in Bauru (34, 41). The water fluoride concentrations at the daycare centers were not assayed. It is expected that they would vary similar to the pattern that was found for the houses, because the daycare centers are located close to the children's houses.

Table 1 shows that fluoride intake from diet during the winter was not significant different from that in the summer. The same was observed for each individual component of the diet. There are some controversies in the literature regarding this subject. Our results are not in concordance with those observed by Lima and Cury (42). These authors related that fluoride intake during summer and spring was significantly higher than the dose ingested during winter and fall. However, the authors made some calculations to get to this result, because the water fluoride concentration during winter and fall was statistically higher than that observed during the other seasons. In Bauru, as mentioned above, fluctuations in the public water fluoride levels are common (34, 41), but a recent study (MARB, unpubl. data) has shown no significant differences in water fluoride levels among the four different seasons of the year.

Moreover, there was no significant difference between fluoride intake from the diet collected in a weekday and that collected in a weekend day. These results are in agreement with the findings of Guha-Chowdhury et al. (7), who collected samples of duplicate diet for 1 year. On the other hand, Rojas-Sanchez et al. (16) found significant differences when duplicate diets were collected. The authors recommended that collections should be made for 3 days over 1 week. However, this procedure is more expensive and increases the chance of dropout of participants along the study. Another relevant fact is that, in this study, diet contributed only to 20% of total daily fluoride intake by the children. Thus, taking into account all these factors, a single duplicate plate collection seems to be enough when the estimation of total fluoride intake needs to be done for this age group. In addition, collecting only one duplicate plate sample is more ethical, especially for non-established market economy countries, where there could be a dilemma in collecting duplicate diets in places where there may be starvation.

In most of the studies that estimate total daily fluoride intake for young children, dentifrice was

found to be the main contributor to the total fluoride intake. Our findings showed a mean dose of 0.106 ± 0.085 mg F/kg body weight/day (0.046 mg F/kg body weight per brushing, as the mean number of brushings per day was 2.3) from dentifrice, which alone is above the recommended threshold of 0.07 mg F/kg body weight/day and corresponds to 81.5% of the total fluoride intake by the children. This percentage was 55% and 64% according to Lima and Cury (40) and Paiva et al. (19), respectively. One fact that may have contributed to the high amount of fluoride intake from dentifrice in the present study is the high fluoride concentration found in some dentifrices used by the children (data not shown). Values up to 1787 ppm were found, despite only being labeled 1500 ppm F. The lowest level found was 1093 ppm. A recent study investigated the fluoride intake from dentifrice in young children in seven European regions. The fluoride concentrations of the dentifrices used ranged between 289 and 1399 ppm F and the mean fluoride ingestion from dentifrice ranged between 0.009 and 0.023 mg F/kg body weight per brushing for 1.5- to 3.5-year-old children (43). The different contribution of dentifrices to the fluoride intake of both studies may be due to the lower fluoride concentrations of the European dentifrices, or to differences in the methods of estimating the fluoride intake from dentifrice. Another factor could be the different amounts of dentifrice used and/or the different percentage of dentifrice ingested in both studies. It should also be highlighted that the estimation of fluoride intake from dentifrice was only made at the daycare centers, where the children spend most of their hours awake. Some of the children brushed at home, as assessed by the questionnaire (data not shown). Thus, it is possible that the estimation of fluoride intake from dentifrice would be slightly different, if the tooth brushing performed at home were also considered.

However, if the bioavailability of fluoride were considered, the data on fluoride intake from dentifrice might be overestimated. A recent report from Pessan et al. (44), who monitored the urinary fluoride excretion of 4- to 7-year-old children using placebo or fluoridated dentifrices, speculated that this overestimation may be around 50%, and could be explained by a conjunction of factors: differences in absorption of NaF compared with MFP (45–49); reduced bioavailability if fluoride is swallowed soon after eating (50, 51); overestimation of brushing frequency and amount of dentifrice loaded onto the brush by mothers; and loss of dentifrice from the mouth.

Figure 1 shows the strong positive correlation between the amount of dentifrice loaded onto the toothbrush and the amount of fluoride ingested during tooth brushing. Children ingested an average of 77% of the total amount of dentifrice loaded onto the toothbrush, which is in agreement with the findings by Paiva et al. (19) and also with data obtained in a recent European study for 1.5- to 2.5-year-old children (43). Even considering that these data may be overestimated, this high fluoride intake from dentifrice is subject of concern. In addition, the cariostatic efficacy of the dentifrice is related to its topical effect and ingestion is not necessary. Considering that dentifrice is the main source of fluoride ingestion for 1- to 3-year-old children, who are the most susceptible for the occurrence of dental fluorosis in the permanent central maxillary incisors, it is important to instruct parents and teachers about the need to use small amounts of dentifrice during tooth brushing, as the amount of ingested dentifrice is directly related to the amount loaded onto the tooth brush. This is an important measure, but we cannot forget that nowadays it is common that both parents work and people who take care of the children do not always follow parents' instructions. In addition, the flavor of most children dentifrices encourages ingestion (52). Because of this, it has been proposed that dentifrices with lower fluoride concentrations should be developed and marketed for use by young children, as has been done in many countries (53, 54). In Brazil, although low-fluoride dentifrices (around 500 ppm F) are commercially available, they are much more expensive than regular dentifrices, containing around 1000–1500 ppm F. Thus, it is common that all the family members, including children, use the same regular dentifrice. A recent study showed that most of the dentifrices commercially available in Brazil have a fluoride concentration of 1500 ppm and are based on monofluorophosphate (54).

Considering 0.05–0.07 mg F/kg body weight/day (8) as the safe threshold of fluoride intake in terms of dental fluorosis, 24 of 33 children of the present study are possibly at elevated risk for dental fluorosis. Considering the mean of fluoride intake by these children, as most of the dentifrices used by the children contained 1500 ppm F, the use of a low-fluoride dentifrice (around 500 ppm F) would decrease the average dose of fluoride intake from dentifrice to 0.035 mg F/kg

body weight/day. Adding this to the dose of fluoride intake from the diet (0.025 mg F/kg body weight/day) would result in a mean dose of 0.060 mg F/kg body weight/day. If the possibility of overestimation of the data of fluoride intake from dentifrice is considered, due to the bioavailability of fluoride, the total amount of fluoride intake could be even lower. If a 1000–1100 ppm F dentifrice was used, the upper safe threshold of daily fluoride intake would be reached with the use of dentifrice only. In the present study, only eight of 33 children used a 1000–1500 ppm F dentifrice, while none of the children used a 500 ppm F dentifrice.

Therefore, an ideal dentifrice with low fluoride concentration should not only be able to reduce the fluoride intake, but also be as effective as the currently marketed formulations of 1000–1100 ppm fluoride in caries prevention. Some studies have been carried out to evaluate the efficacy of dentifrices with low fluoride concentration. Reed (55), Mitropoulos et al. (56) and Koch et al. (57) concluded that dentifrices with low fluoride concentration might be less effective than those with 1000 ppm. The only 'double-blind' study conducted with 2-year-old children that evaluated the efficacy of a dentifrice with 500–550 ppm F was done by Winter et al. (58). The group that used the 500–550 ppm F dentifrice presented caries increment a little higher (10%) after 3 years, but the difference was not statistically significant. The authors concluded that dentifrices with low fluoride concentration presented an anti-caries activity similar to control dentifrice, and so could be recommended to young children. However, this conclusion was based only on one study and additional longitudinal randomized clinical trials should be made on this topic.

In order to predict the risk of dental fluorosis, the possible use of biomarkers of fluoride exposure, such as fingernails, has been studied (23–31).

In a recent study (30), 2–3-year-old Brazilian children used a placebo dentifrice for a period, followed by a period using fluoridated dentifrice (1500 ppm). A threefold increase in nail fluoride concentration was observed after the use of fluoridated dentifrice. However, the authors did not estimate the dose of fluoride intake from diet and dentifrice. The results of the present study, showing a contribution of around 80% of fluoridated dentifrice to the total fluoride intake, could help to explain the increase in nail fluoride concentrations reported by Rodrigues et al. (30).

There are some reports in the literature showing that nails can be used as biomarkers to differentiate children exposed to different levels of fluoride from water (29, 31, 59). However, in our study, there was no significant correlation between the daily dose of fluoride intake and fingernail fluoride concentration. It must be taken into account that despite individual variations in the amount of daily fluoride intake, no treatments were applied and all the children were exposed to the same conditions. Thus, the question of sensitivity and specificity arises when nails are intended as biomarkers of fluoride exposure and predictors of dental fluorosis. It is possible that nails could only be used to differentiate levels of fluoride exposure, when there is a broad variation among them. In fact, Sampaio et al. (60), attempting to validate fingernail fluoride concentration as a biomarker of fluoride exposure, examined the severity of dental fluorosis in children residing in regions of the State of Paraíba, Brazil, where the natural levels of fluoride in the drinking water (private wells) are 0.1, 1.6 and 2.3 mg F/ml. These children had been previously examined with regard to fluoride concentration of fingernails (29). The mean fluoride concentration of fingernails of children who presented with TF 0 was significantly different from the mean fluoride concentration of children who presented with TF 5. Nevertheless, this significant difference was not observed in children with TF 1, 2, 3 and 4. Our results seem to be in agreement with the findings of Sampaio et al. (60), denoting that small variations in the daily dose of fluoride intake cannot be detected in fingernails. If the results of the present study are analyzed in conjunction with those of Sampaio et al. (60), it is suggested that fingernails give an indication of fluoride intake over the long term and are unlikely to be sufficiently sensitive to distinguish small day-to-day variations of fluoride intake.

Another important factor is a possible genetic susceptibility to dental fluorosis, already demonstrated in different mice strains by Everett et al. (61). Despite this not being demonstrated in humans so far, there are some reports on the occurrence of dental fluorosis with lower levels of fluoride intake (0.02 mg F/kg body weight/day) (9), which could indicate that this individual susceptibility to fluoride occurs in humans also.

A finding of this study that seems difficult to be explained is the reduction in fingernail fluoride concentrations in the second collection. In the

report by Levy et al. (31), these differences among samples collected on different dates were not observed. One fact that might have contributed to this reduction was that after the first estimation of fluoride intake, parents received instructions regarding the use of fluoridated dentifrices. These included the reduction of the amount of dentifrice loaded onto a child's toothbrush. This may have had an immediate impact on fluoride intake from dentifrice, causing a reduction in fingernail fluoride concentration. However, the impact of educative measures like this is higher in the short term and diminishes along time. This could help to explain why fingernail fluoride concentration increased in the third collection. More studies are still needed to evaluate the viability of fingernail use as biomarkers of chronic fluoride exposure, as well as the sensitivity and specificity of this method as a predictor of dental fluorosis.

In conclusion, most of the children are exposed to a daily fluoride intake above the suggested threshold for dental fluorosis. Dentifrice alone is responsible for about 80% of the daily fluoride intake, while among the constituents of the diet, the category water and milk combined was more important than solids or other beverages.

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