FLUORIDE

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Fluoride intake from food and beverages by 1-to-3-year-old toddlers attending a preventive dental program

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ABSTRACT

Purpose: To evaluate fluoride (F) intake by 1–3-year-old children from the diet, as well as F concentrations in fingernails and toenails.

Methods: Twelve-month-old Children (n=202), participants in a Preventive Dental Program for infants, had their F ingestion monitored by means of the application of a semi-quantitative food frequency questionnaire (FFQ), composed of 70 items, divided into 9 groups of foodstuffs. The FFQ was applied for 2 years, every 3 months, along with the collection of diet, nails and water samples used for drinking and food preparation. The concentration of F in the diet, nails, and water was determined with an ion-specific electrode, after hexamethyldisilane-facilitated microdiffusion or by the direct method. The data obtained were submitted to the 2-way analysis of variance, followed by the Student-Newman-Keuls' test, and by Spearman correlation coefficient ($p<0.05$).

Results: The average ingestion of F by diet and water was significantly higher from 12 to 24 months (0.015 mg F/Kg/day and 0.09 mg F/Kg/day) compared with values obtained after 27 months (0.011 mg F/Kg/day and 0.05 mg F/Kg/day) ($p<0.05$). At 36 months, a peak of F ingestion from the diet (0.013 mg F/Kg/day) ($p<0.05$) was observed. There was a continuous increase in F levels in finger- and toenails, with a significant difference in some periods of the study (18 to 27 months, $p<0.05$); higher F concentrations were observed for fingernails over toenails (3.7 µg F/g; 3.4 µg F/g, respectively, $p<0.05$). No significant correlation was observed between the estimate of ingestion of fluoride related to the weight of the child (mg F/kg/day) and the fluoride in fingernails (Spearman's r = -0.024; p=0.396) or toenails (Spearman's r = -0.002; p=0.957).

Conclusions: The ingestion of fluoride from the diet in 1–3-year-old children was shown to fall within safe limits, and the FFQ appears to be a satisfactory tool to estimate the ingestion of fluoride. Small variations of daily ingestion of F by diet were detected in the nails through the periods in the study (after 30 to 60 days).

Key-words: *Child; Fluoride; Nail; Enamel Fluorosis.*

INTRODUCTION

Dental caries is a multifactorial, biofilm-sugar-dependent disease commonly found in childhood.[1-3] Fluoride (F) has been regarded as the main agent used for caries control, with predominantly topical effect.[1,3-5] Thus, the excessive fluoride intake during tooth formation does not favor its anti-caries effect and can contribute to the development of dental fluorosis, as a reflection of F chronic toxicity.[5-7]

To date, the optimal daily fluoride intake (DFI; *i.e.*, a F dose effective in controlling de- and remineralization without triggering fluorosis) have not been precisely determined[1], as applying the duplicate-diet approach or the Food Frequency Questionnaire (FFQ) are methods difficult to perform. In addition to the diversity of F sources, duplicating foods consumed by children over a longer period using a double diet is an expensive method.[9,10] Furthermore, it is noteworthy that F ingested through the diet is not completely absorbed by the body.[1,7,8,11] Thus, the assessment of the optimum DFI necessitates careful consideration due to the diverse intrinsic factors influencing metabolism and, consequently, F absorption.[11] Despite the challenges in determining it, empirical estimates range from 0.05–0.07 mg/kg/day, although other studies report F levels ranging from 0.03 to over 0.1 mg/kg/day.[1,2]

In face of this challenge, various surveys have explored the use of biomarkers to assess the level of F exposure from daily consumption.[1,7-9,12,13] In brief, the most used biomarkers are plasma, bones, saliva, urine, nails and hair[1,7], among which, fingernails have been extensively investigated over the years[7,13-16] due to the non-invasive form of collection, as well as factors such as transportation and storage.[1,4,12,13,17] Within this context, nails samples have been used as predictors to evaluate acute[17], chronic[18] and subchronic[13,19] exposure to F, demonstrated a high positive predictive value indicating that F concentrations in the nail might be useful in public health research since it has the potential to identify around 80% of children at risk of developing dental fluorosis.[15,20]

Despite the widespread use of nail samples as a F biomarker, there are still few studies that analyze the intake of F from the usual diet of children in the age range of risk of developing fluorosis in permanent incisors (1-3 years old) and the concentration of F in their nails.[1,16,20] Among the studies available, most of them eighter used short collection periods, did not adopt a longitudinal study design, used a small number of individuals or did not cover the period of greatest susceptibility to dental fluorosis. Considering that the first 3 years of life are crucial regarding dental fluorosis,[1,8,13] longitudinal studies in this specific age group assume great clinical and epidemiological relevance.

Although important observations were noticed in previous studies, they are still not sufficient to accurately explain the variation rates of F in nails throughout the study.[11,18] The concern to evaluate this exposure to F over a long period of research and at specific ages[15,16,20,21] may favor an explanation more consistent with the reality of the facts exposed since there is no longitudinal study for the age considered the critical period for developing dental fluorosis.[1,10,16]

Based on the above, it would be interesting to conduct a study assessing the total fluoride intake (TFI) in the diet, as well as the concentrations of F absorbed in the nails of children, comprising the entire process of formation of permanent teeth, especially the incisors. Thus, this study aimed to evaluate dietary F intake using an FFQ and the concentration of F in nails in children aged 1 to 3 years.

MATERIAL AND METHODS

Study design

This is an observational, longitudinal, prospective cohort study, descriptive for questionnaires and experimental to analyze the concentration of fluoride in the diet and nails, lasting two years. The study was submitted and approved by the Research Ethics Committee of the São Paulo State University, Araçatuba, Brazil (protocol no. 50713715000005420). In the city where the research was performed, the average concentration of fluoride in the public water supply is 0.6–0.8 mg F/L.[22]

For this study, 312 babies were selected, duly registered and monitored in the preventive educational program of dental care for babies at the Faculty of Dentistry of Araçatuba - Unesp, São Paulo, Brazil. The nature and purposes of the study were explained verbally and in writing to the parents/caregivers who

signed an informed consent document. The inclusion criteria adopted were: babies aged 12 months and without dietary restrictions.

The babies' weight was measured and their nails and information on their diet intake, dentifrices and drinking water were collected during whole quarterly consultations until they reached 36 months of age. Thus, data were obtained at nine different times: 12, 15, 18, 21, 24, 27, 30, 33 and 36 months of age.

Estimation of dietary fluoride intake

To estimate fluoride intake, the FFQ developed by Collucci et al. (2004)[23], previously validated, with a reproducibility coefficient of 0.959, (KAPPA) to evaluate the usual diet of children aged 2 to 3 years old, adapted by Miziara et al. (2009)[24]. The FFQ was structured according to the food pyramid and each item with its previously established reference portion, composed of seven eating frequency alternatives, the same for all items in the diet.[23,25] The FFQ was applied to parents and/or caregivers who reported their children's usual dietary intake quarterly. The frequency of consumption of each food and beverage was classified as: never, less than once a month, 1-3 per month, once a week, 2-4 per week, once a day and 2 or more per day. Multiplier factors were defined to determine daily consumption. Table 1 shows the food groups included in the FFQ, which consisted of 70 food items subdivided into nine groups with respective reference portions.

To determine the F in food items, at the beginning of the study they were purchased from local supermarkets, homogenized in 100 mL of deionized water and 0.5 mL collected for measurements, only once during the study periods. Fluoride concentrations were determined by hexamethyldisiloxane (HMDS)-facilitated diffusion using the ion-specific electrode[26,27] (Orion 9409-BN, Orion Research, Inc. Beverly, MA, USA) and a miniature calomel electrode (Accumet, no. 13-620-79: Fischer Scientific, Pittsburgh, PA, USA), both coupled to a potentiometer (Orion 720 Aplus, Orion Research, Inc. Beverly, MA, USA). All samples were analyzed in duplicates and expressed in µg F. F values in each food were divided by the weight of its portion (g) resulting in the F concentration of the portion (µg F/g). These values were then multiplied by the frequency of each child's daily consumption and divided by the child's weight (mg F/kg of weight/day).

Estimation of fluoride intake from dentifrices

To estimate fluoride intake from dentifrices, the parents/guardians were questioned about the use of fluoridated dentifrices, brand, amount of dentifrice loaded onto the toothbrush (options: ¼ = 0.3 g; ½ = 0.6 g or completely covered = 1.2 g)[28] and the frequency of usage. The mean percentage of dentifrice ingested by the children was based on the study by Kobayashi et al. (2011)[28] and considered at 21 months as: 62.8%, 70.4%, and 66.1%, respectively the amount of dentifrice ingested in relation to the amount of dentifrice placed on the toothbrush; 24-27 months: 60.2%, 64.1% and 65.0%; 30 months: 51.6%, 55.0% and 54.8%; 33-36 months: 42.9%, 45.9% and 44.7%. The product of the amount placed on the toothbrush, F concentration in the dentifrice, frequency and percentage of intake were divided by the child's weight, obtaining an estimate of DFI from the dentifrices (mg F/kg/day).

Analysis of fluoride concentration in water and ingested fluoride

The water ingested or used in food preparation was collected by parents and/or caregivers in polystyrene bottles (10 mL), coded with the child's code, date of collection and origin of the water (home, daycare center, school and grandparents' house) and stored in a freezer (-20° C) for further analysis. F concentrations in water samples were determined by the direct method, buffered with TISAB II (1:1 ratio), using an ion-specific electrode and reference microelectrode coupled to a potentiometer (Orion 720 Aplus, Orion Research, Inc. Beverly, MA, USA).[26,27] All samples were analyzed in duplicate, and values were expressed in mg F/L.

To determine F ingested from drinking water, the average F values obtained from water samples from different places were multiplied by the portion volume (150 mL) and daily consumption frequency factor. These values were then divided by the children's weight (kg) obtaining the concentration of F ingested per kg per day (mg F/kg/day).

Determination of fluoride in nail samples

The nails of the babies were cut once a month by the parents. The first samples were collected a day before starting dentifrice use. Parents were not allowed to use nail varnish on their children throughout the entire experiment, as it may contain F.[29] Fingernails and toenails from all digits were cut and pooled separately in labeled vials, totaling 24 samples (12 from fingernails and 12 from toenails) for each child. Nail clippings were cleaned with deionized water using an interdental brush, sonicated (USC-1400, UNIQUE, São Paulo, Brazil) in deionized water for 10 minutes, dried at 37 °C, and weighed before F analysis. When the weight of the pooled samples was >20 mg, analyses were conducted in duplicate.

Statistical analysis

The data were analyzed considering age and gender for the variables estimating F ingested from the diet and dentifrice (mg F/kg/day), F ingested from water (mg F/kg/day), F in water drinking (mg F/L), and children's weight (kg). The interactions of age and gender and age and type of nails (hand and foot) were applied to the results of F in nails (µg F/g). Thus, even though the data presented a heterogeneous distribution (Shapiro-Wilk normality test), the results were submitted to 2-way analysis of variance (ANOVA) followed by the Student-Newman–Keuls's test. Also, Spearman correlation coefficient was performed to assess the correlation among the variables. Statistical analysis was performed using SigmaPlot 12.0 software (Systat Software Incorporation, San Jose, CA, USA) program, adopting $p<0.05$ significance level.

RESULTS

The final sample consisted of 202 participating children, 51% female (n = 104) and 49% male (n = 98). Throughout the study, 110 children were excluded, with greater losses at 12, 30, 33 and 36 months of age, however, maintaining gender balance. Weight increased due to the children's age (Table 2). The concentration of F in the water was higher at 18 months (0.53 mg/L; $p<0.05$), with no statistical difference ($p>0.05$) when the other periods were compared (Table 2).

The use of fluoridated dentifrices in daily hygiene was started at 21 months of age (8% of the sample of 165 children), gradually increasing throughout the study, and used by almost half of the children (47% of the sample of 92) at 36 months of age (Table 2). On average, 71% of children used fluoridated dentifrices containing 500-550 ppm F. Estimated intake of F from dentifrices was highest at 21 and 24 months ($p<0.05$) and gradually decreased as the children's age increased (Table 2).

F concentrations (in µg/g for foods or µg/mL for beverages) of the food items listed in the FFQ are described in table 1. Most foods had low concentrations of F, such as: carrots (1.4 µg F/g) and bananas (0.9 µg F/g) (Table 1). The highest values were found in cereal, Nescau® and black tea (62.3 µg F/g, 56.8 µg F/mL and 88.8 µg F/mL, respectively).

Fluoride concentration in fingernails and toenails increased between 12 and 30 months of age (2.6 and 3.6 µg F/g and 2.4 and 3.4 µg F/g, respectively), and decreased at 33 and 36 months (3.3 and 2.7 µg F/g and 2.7 and 2.4 µg F/g, respectively) (Figure 1A; $p<0.05$). Differences between F in fingernails and toenails were observed between 18 and 27 months ($p<0.05$), with higher values in fingernails (Figure 1).

F intake from water showed peaks at 15 and 36 months (0.0066 and 0.0073 mg/kg/day; $p = 0.317$), differing from the other periods ($p<0.05$), and these did not differ from each other ($p>0.05$). Estimated dietary F intake increased from 15 months of age, reaching a maximum value at 24 months (0.0095 mg/kg/day) and different from other periods ($p<0.05$). Afterward, dietary F intake values decreased to levels similar to 12 months (0.0061 mg/Kg/day; $p>0.05$). For F intake from the total diet (diet + water), higher values were observed at 15, 21, 24 and 36 months when compared to the other periods ($p<0.05$) and did not differ from each other ($p>0.05$). Furthermore, there was no difference between 12, 27, 30 and 33 months of age ($p>0.05$).

The following variables were not dependent on the children's gender: F concentration in nails ($p=0.116$) or drinking water ($p=0.704$); estimated amount of F ingested through diet + water ($p=0.357$), diet ($p=0.441$) and water ($p=0.573$), and children's

weight (p=0.058). There was a weak correlation between the concentration of F in fingernails and toenails (Sperman's r = 0.383; p<0.001). There was no significant correlation between the levels of F in the water consumed by children and the concentration of F in their fingernails (Sperman's r = -0.032; p=0.244), as well as between the intake of F from the diet and the concentration of F in their fingernails (Sperman's r = -0.024; p=0.396) or toenails (Sperman's r = -0.002; p=0.957).

Table 1. Food and drink groups included in the Food Frequency Questionnaire and the items evaluated in each group with the respective reference portions, portion weights and F concentration

Groups*	Food item	Portions of the FFQ	Portion Weight (g)	F (µg/g or mL)
Rice, bread, pasta and potato	Cooked rice	3 tablespoons	81.5	2.6
	Boiled mashed potatoes	1 serving spoon	51.0	1.7
	Boiled potato	1 slotted spoon	103.0	1.9
	Biscuit without Tucs filling	3 or 4 units	19.5	1.1
	Biscuit with filling	3 units	39.0	36.2
	Snowflakes® cereal	1 cup	39.0	62.3
	Cooked pasta	1 slo9tted spoon	72.0	3.9
	Miojo® pasta	1/3 of the package	25.0	4.6
	Bread roll	½ unit	25.0	16.8
	Thickeners	1 or 2 tablespoons	19.0	6.6
	Milk flour	1 or 2 tablespoons	19.0	5.2
	Mucilon	1 or 2 tablespoons	19.0	6.8
	Corn starch (Maisena®)	1 or 2 tablespoons	19.0	1.8
	Bean	½ ladle	39.0	5.8
Greens and legumes	Pumpkin	2 tablespoons	70.0	3.5
	Lettuce	2 leafs	15.5	0.6
	Cabbage	1 tablespoon	15.5	2.2
	Tomato	3 slices	50.0	0.6
	Tomato sauce	1 tablespoon	19.0	7.8
	Carrot	½ serving spoon	15.0	1.4
	Chayote	1 tablespoon	18.0	1.8
	Cassava	½ tablespoon	18.0	1.0
Fruits	Banana	1 unit	86.0	0.9
	Apple	1 unit	92.7	1.2
	Pear	1 unit	18.0	1.1
	Orange	1 unit	128.0	1.0
	Orange juice	½ cup	128.0	0.9
	Juice from other fruits	½ cup	116.0	1.3
	Papaya	½ cup	90.0	0.8
	Guava	1 slice	51.0	1.2
Meat and eggs	Beef	½ unit	76.0	2.7
	Cooked meat	1 unit	54.0	8.5
	Perdigão® Sausage	3 tablespoons	31.0	2.3

	Sadia® Ham	½ bud, 1 unit	31.0	1.5
	Liver	1 slice	95.0	1.4
	Chicken	1 piece, 1 unit	62.0	2.9
	Fish	½ piece, ½ fillet	31.0	7.1
	Egg	1 unit	41.0	1.5
Milk, Cheese, Yogurt	UHT whole milk**	1 cup	184.0	1.9
	NINHO FASES® whole powdered milk***	1 cup	29.0	4.6
	Aptamil whole milk powder**	1 cup	26.8	20.0
	NAN whole powdered milk**	1 cup	29.0	3.3
	Fruit yogurt	1 pot	138.0	15.0
	Yogurt/Chambinho®	½ pot	62.0	10.3
	Yogurt/ Danoninho®	1 pot	36.9	94.0
	Yakult® fermented milk	1 pot	40.0	15.8
	Margarine	1 teaspoon	7.0	1.3
	Butter	1 teaspoon	7.0	4.7
	Tirolez® mozzarella cheese	1 slice	14.0	2.2
Sugar, Sweets and Snacks	Cream cheese	1 dessert spoon	19.0	3.9
	Sugar	1 ½ dessert spoon	21.0	1.1
	Toddy®	1 ½ dessert spoon	13.0	1.4
	Nescau®	1 ½ dessert spoon	13.1	56.8
	Dona Benta® cake	1 slice	83.0	13.7
	Sonho valsa® candy	1 unit	29.0	2.2
	Chips® snack	1 small package	55.0	12.0
Savory and Preparations	Cooked Corn Meal	1 serving spoon, 1 slice	84.0	1.3
	Soup with meat	½ plate	120.0	3.5
	Soup without meat	½ plate	95.0	2.8
	Brazilian cheese bread	1 small unit	58.0	4.2
	Mozzarella pizza Perdigão®	½ slice	54.0	13.4
	Sandwich	½ unit	75.5	7.8
Beverages	Coffee with sugar	1 cup of coffee	40.0	61.4
	Coca-Cola® soda	½ cup	170.0	16.3
	Tang ® type artificial juice	½ cup	112.0	1.8
	Mate Industrialized Tea Sachet®	½ cup	12.0	22.1
	Mate® Industrialized Tea Bottle	½ cup	33.0	36.7
	Industrialized tea Chamomile	½ cup	96.0	12.7
	Black tea	½ cup	227.0	88.8
Others	Soy based milk powder**	1 cup	5.0	7.2
	Toddynho® Chocolate Milk	1 unit	200.0	9.9
	Isotonic drink Gatorade®	½ bottle	237.0	3.3
	Neston Cereal®	6 tablespoons	42.0	20.0
	Danix Biscuit®	3 units	39.0	0.3
	Chocolate bar	3 squares	15.0	3.2
	Chocolate M&M®	1 small bag	49.0	4.5

*Adapted: Colucci et al. (2004)[23].

** Milk homogenized with 100 mL deionized water.

Table 2. Mean values (SD) for weight, fluoride present in drinking water samples, percentage (%) of children who used fluoridated toothpastes, and estimated fluoride intake from toothpastes depending on age

Age	Weight[&] (kg)	F water[§] (mg/L)	Use of toothpaste	Dentifrice (mg F/kg/day)
12 months	9.24 (0.92)[a]	0.43 (0.50)[a] (0.01 – 1.94)*	0.0%	-
15 months	10.27 (0.97)[b]	0.45 (0.50)[a] (0.01 – 1.94)	0.0%	-
18 months	11.02 (0.94)[c]	0.53 (0.53)[b] (0.01 – 1.74)	0.0%	-
21 months	11.86 (1.11)[d]	0.39 (0.39)[a] (0.01 – 2.32)	8.0% (79[α]/21[β])	0.045 (0.027)[a] (0.014 – 0.104)*
24 months	11.41 (0.94)[e]	0.33 (0.28)[a] (0.03 – 1.22)	21.0% (67/33)	0.045 (0.021)[a] (0.016 – 0.106)
27 months	12.89 (1.48)[f]	0.30 (0.29)[a] (0.03 – 0.96)	28.0% (72/28)	0.038 (0.019)[a,b] (0.013 – 0.101)
30 months	13.64 (1.64)[g]	0.33 (0.30)[a] (0.03 – 1.45)	31.0% (71/29)	0.032 (0.018)[b,c] (0.010 – 0.087)
33 months	14.25 (1.73)[h]	0.35 (0.31)[a] (0.01 – 1.08)	33.0% (69/31)	0.025 (0.014)[c] (0.008 – 0.059)
36 months	14.26 (1.58)[h]	0.36 (0.33)[a] (0.04 – 1.28)	47.0% (67/33)	0.026 (0.012)[c] (0.006 – 0.057)

[&] Mean (SD), two-way ANOVA, Student-Newman-Keuls, p<0.05). (*) Variation range.
[α]Percentage of toothpaste containing 500-550 ppm F. [β]Percentage of toothpaste containing 1100-1500 ppm F.

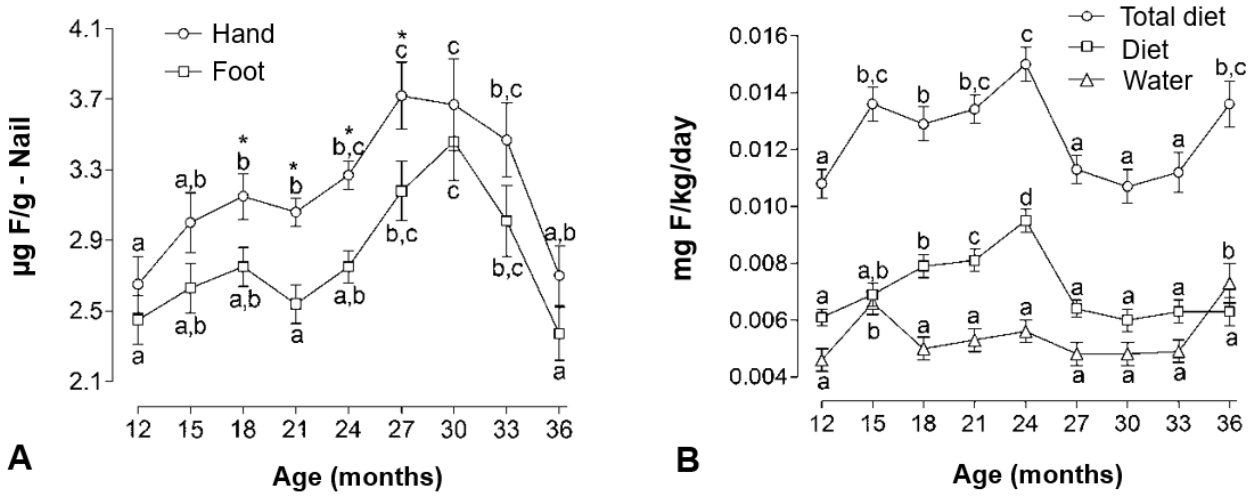

Figure 1. Mean values of: (A) F concentration in nails and (B) estimated F intake as a function of the child's weight (mg F/kg/day) as a function of age. Different letters indicate statistical differences between fluoride values at different ages. (*) Indicates statistical difference between fingernail and toenail values for each time. Vertical bars indicate standard error of the mean (Student-Newman-Keuls, p<0.05).

DISCUSSION

The literature is scarce regarding data on the F intake by children at risk for developing dental fluorosis, simultaneously with the estimation of F absorption (using biomarkers of exposure to F). Furthermore, there is difficulty in comparing longitudinal studies with others that evaluated specific age groups and with shorter collection periods than the present study. In this sense, the use of possible biomarkers of exposure to F, such as nails, has been the subject of investigations.[15,20,30,31] In the present study, all F concentration values in fingernails and toenails were greater than 2 µg F/g. Considering the only available study in which the direct relationship between F concentration in fingernails and dental fluorosis was evaluated, it was suggested that values greater than 2 µg F/g may present a substantial risk of development of dental fluorosis. Therefore, it is recommended to reduce or monitor TFI.[15] Few studies have been carried out in the age range considered the critical period for developing dental fluorosis in permanent incisors (*i.e.*, 1-3 years old), in which evaluated only chronic exposure (only 1 fingernail collection)[18] or subchronic (fortnightly monitoring for a few months)[19] to F. In none of these studies the intake from different sources was assessed throughout the period of incisor formation, as performed in the present one.[15,16,19-21,32]

The present study demonstrated that F concentration in toenails and fingernails over two years followed variations in F in the diet with a difference of 30 to 60 days. The results showed that in the study periods between 18 and 27 months of age F concentration values in the toenails significantly lower than in the fingernails (Figure 1A), similar to those found in the literature.[16,21,33] In fact, it was expected that this pattern could be observed due to the fact that fingernails may have greater contact with external "contaminants" and also reflect higher concentration of the ion in the bloodstream.[4,16,28] The literature reports that a fingernail can be used as a biomarker of different concentrations of F in water.[15] Nonetheless, there was no significant correlation between the F levels in the water consumed by children and the F concentration in their nails (r= -0.032; p=0.244), what might be justified by the absence of fluctuations in water F concentrations throughout the study.. Similar results were found in another study[24] in which the authors approached the same age group (1 to 3 years), with a lower number of collections (n = 3) carried out over 12 months. The authors suggested that fingernails can only be used to define different levels of F concentration in case of wide differences between them. In the present study, a pattern similar to that observed by Amaral et al. (2014)[16] was found, especially about the values of F intake from the diet and the variations in the concentration of F in the fingernails throughout the study. In this sense, it is worth highlighting that even at higher intake values[20], a similar variation in F in nails was detected at 12 and 36 months of age in the current study, suggesting that such variations are intrinsic characteristics of this biomarker.

Between 24-33 months of age (Figure 1A), F levels in nails increased (3.7 µgF/g), coinciding with the increase in TFI by the diet at 15-24 months of age (Figure 1). This pattern was similar to that observed for F concentration in nails concerning TFI in a previous study carried out with only one adult volunteer.[29] In that occasion, it was observed that a daily increase in F intake (3.0 mg) for 30 days led to a significant increase in nail F concentrations approximately 3.5 months after the beginning of the study. Considering a higher rate of metabolism and, consequently, greater speed of nail growth in children[27,34], it is possible that fluctuations in F intake from the diet are reflected earlier in the nails of this population (30 to 60 days), as observed in the current study. Furthermore, in the period from 27-33 months, dietary F intake declined and F levels in nails followed this behavior over the next 3 subsequent months of study (Figure 1A and 1B).

Although the chronic and subchronic assessment of F through nails is the most relevant factor considering the risk of fluorosis[7], factors such as concentration of F in water and toothpaste, age, sex, geographic area and degree of physical activity are cited as factors that can influence the concentration of F in nails.[1,11,33,35,36] Observational and longitudinal studies should be encouraged, as the clinical relevance of this model for preventable changes such as fluorosis provides strong evidence of the behavior of exposure to F. Therefore, the use of nails to estimate the risk of fluorosis in the first 3 years of life could provide insightful results for clinical practice, signaling the need for greater control of F intake at an age considered to be a window of susceptibility to dental fluorosis. For

this reason, studies with longer follow-up periods should be stimulated to evaluate the relationship between the amount of F ingested and the levels of F found in the nails, thus translating into a suitable tool for monitoring systemic exposure to F. It is also paramount to advance investigations on the validity of monitoring F systemic exposure using nails, what could be accomplished by assessing fingernails along with other biomarkers, (*e.g.*, urine and ductal saliva).

Of the more than 70 items included for dietary analysis, the majority had a low concentration of F, similar to the same foods analyzed in previous studies.[9,17,21] However, some foods (Table 1), such as filled biscuits and cereals, have a high concentration of F in their composition (36.2 and 62.3 µg/g, respectively).[9,17] Additionally, it was observed that teas had high concentrations of F (88.8 µg/g), higher than expected for a food considered suitable for children's diets[4], similar to the findings of Buzalaf et al. (2009)[9]. Despite the high concentration of F found in tea, in the current study, this product was not frequently used by children in that age group (data not shown). Chocolate milk Nescau® (56.8 µg/g) and Toddynho® (9.9 µg/100 mL) presented relatively high F concentration. In fact, previous data demonstrated substantially high F concentrations in Toddynho® (0.53 µg F/mL).[37]

Cake, cookies and chocolate contributed to increasing the F in the diet, as observed in a previous study[38], however, other carbohydrates had a low concentration of F (rice, bread, potatoes) (Table 1). Milk-based foods had a relatively high F level, in line with a previous study[39], such as soy-based milk powder (7.2 µg/mLg, Table 1). In a systematic review[40], the authors reported that the evidence that F in infant formula has caused enamel fluorosis is weak, as other mechanisms could explain the observed association.[40] In another study[41], the authors observed that the contribution of food items with low F values to the total F intake in the diet is not as significant, while those with higher F concentrations contributed significantly to the daily F intake, regardless of frequency of consumption.[41] Variations in the F concentration of food items in the FFQ[9,42] may be linked to different growing regions and medium, lots, irrigation with water containing F, such as in volcanic areas[33,43] and the variability between brands[44], which was not the subject of this study.

A low concentration of F was observed in the water collected in all study periods, even though care was taken to collect it in the different homes and/or daycare centers that the children attended, remaining at values considered below the permitted limits (0.33 to 0.45 mg/L). This fact did not represent a risk of developing dental fluorosis, although it may compromise the function of preventing dental caries (Table 2). Furthermore, the variation in the concentration of F in the water highlights the possibilities of finding different types of water, treated or not, mineral water and well water.[21,40] In the present study, the concentration of F in water, in winter or summer, remained similar in both periods (Table 2). Even though fluoridation of public water supplies is a comprehensive method of supplying F as well as low cost [45,46], care must be taken to evaluate the F levels in the water periodically, delimiting in studies the effects, separately, of the toothpaste and the diet. Brito et al. (2016)[47] observed benefit and risk values for municipalities that have average annual temperatures below 26.3 °C, concluding that the maximum benefit and low risk are at F levels between 0.65 and 0.94 mg/L. In some studies, it was observed that at an F level of 0.7 to 1.0 ppm in water, the prevalence of any fluorosis is about 40% to 48%.[47,48]

Although it has been empirically determined, it is still accepted that the optimal F intake for children is between 0.05 and 0.07 mg/kg.[12,49] In the current study, F intake from water alone (Figure 1B) was below limits within the study period (0.004 to 0.007 mg F/kg/day) reflecting the low levels of F found in water. Holg et al. (2006)[50] considered that the high daily intake of F (average of 0.059 mg/Kg) in the first 3 years of life leads to a greater risk of developing dental fluorosis. It is important to observe the various nuances of total water intake in the age group of the current study, whether through drinking water or routinely used in the preparation of other processed foods, infant formulas or natural juices. In this study, F intake below the lower limit may be related to the use of mineral water, as observed in another study[51], which explains the reduction in exposure to F in water.[52]

Dietary fluoride intake (Figure 1B) throughout the study showed wide fluctuations, with significant differences in some study periods (18, 24 and 36 months). Fluoride intake (IF) from water and diet had separate assessments to compare which of the two

sources were exceeding the limits of values considered optimal for TFI.[1] The diet contributed more than water to increase the total F intake from the diet (water and diet) (Figure 1B). ITF by the diet, within the current study, was variable and increasing until the 24-month period (Figure 1B) with peaks of dietary fluoride intake elevation from 12 to 24 months of age (0.012 mg F/Kg/day or 0.10 mgF/day; 0.015 mgF/kg/day or 0.17 mgF/day, respectively). This result was similar to those found by Levy et al. (2013)[21] who showed an average dietary F intake of 0.028 mg F/Kg body weight/day, by Brazilian children, aged 2 to 6 years, in Bauru-SP. Although the aforementioned study used sample of different age groups, the similarity in the TFI can be seen, although greater, in the case of fluoride intake, in which the study population was between 4 and 6 years of age. Values slightly above those found in the current study (Table 2) for TFI[20] were seen in a previous study in the same age group as the current study. The authors evaluated the intake of F through diet and toothpaste in children aged 1 to 3 years, in a fluoridated area, using the duplicated diet.[20] They observed that water and milk were the main dietary contributors to TFI by the diet (0.025 +/- 0.013 mg/kg body weight/day). Nonetheless, this TFI value increased when the authors evaluated the contribution of the toothpaste to the diet (0.130 mg/kg of body weight/day).[20]

Some alternatives have been proposed to minimize F intake by young children, without compromising the clinical efficacy of the product. According to the American Academy of Pediatric Dentistry[53], considering that the effects of F are dose dependent, the recommendation to reduce the F concentration of dentifrices has a direct impact on the availability of intraoral F, which could affect the clinical efficacy of the dentifrice.[53] In fact, previous data demonstrated that the amount of dentifrice and F concentration in the product considered together represent a more relevant parameter rather than considering one of these factors isolated.[54,55] Fluoride dentifrices can be considered the best method of using F to control tooth decay, as they have a wide range of applications.[56] However, with an increase in F intake from different sources, fluoridated toothpaste can contribute to increasing this F intake, especially when they are used by children under 5-6 years of age, favored by their sweet taste, especially when the products contain high concentration of fluoride (1100 ppm or above).[28,31]

In the current study, most children used fluoridated toothpastes containing 500-550 ppm F (Table 2). The decrease in F intake from toothpaste within the study period was expected, even with the use of toothpaste above 1100 ppm, as children develop expectoration reflexes with increasing age, as seen in other studies.[20,28] The highest amount of F ingested by children through toothpaste was observed at 21 (0.014 mg F/kg body weight/day) and 24 months (0.016 mg F/kg body weight/day) coinciding with the introduction of F into dental hygiene routine (Table 2). These values could be higher if the population evaluated comprised a sample that did not have regular and frequent access to information about the amount of toothpaste to be placed on the toothbrush and the F concentration of the toothpaste.

It is interesting to note that in the two initial periods in which fluoridated toothpaste was introduced into the oral hygiene routine, coincidentally, peaks of F elevation in nails were observed 30 to 60 days later (Figure 1A), corroborating the finding by Amaral et al. (2014)[16] and Correa-Rodrigues et al. (2004)[19] reporting peaks of F elevation in nails 16 weeks after the introduction of fluoride toothpaste into children's daily oral hygiene routine. Almeida et al. (2007)[20], observed an average F intake of 0.106 mg F / kg of body weight / day from toothpaste in children in the same age group, reporting that only the dentifrice reached values above recommended (0.07 mg F / kg of body weight/day) and corresponds to 81.5% of children's TFI. In the current study, the sample is part of a preventive program that strictly monitors the correct period that the child can introduce fluoridated toothpaste into their oral hygiene routine.

Despite the trends above, according to Almeida et al. (2007)[20], when considering the bioavailability of F, data on F intake from toothpaste may be overestimated. In another study with children aged 4 to 7 years, by monitoring F excretion through urine in defined groups of children using placebo and another group using fluoridated dentifrices, the authors report that this overestimation may be around 50% and could be explained by a combination of factors. Among them, reduced bioavailability if F is

swallowed right after eating, overestimation of brushing frequency, the composition of the toothpaste, as well as amount of toothpaste loaded onto the toothbrush bristles by guardians.[30] Regarding the latter, wide variations have been observed in the amount of dentifrice used by children as parents/caregivers' interpretation on verbal instructions regarding appropriate dentifrice quantities varied widely.[57] Also, social aspects influences such as parenteral schooling demonstrated to be inversely related to the amount of dentifrice used by the child.[58]

CONCLUSIONS

The results of the study showed that dietary fluoride intake from 12 to 36 months of age was below values considered ideal. Furthermore, the FFQ represents a good tool for estimating the F intake of children in the first 3 years of life and generates a list of the different food sources from the diet with the respective F concentration. Small variations in the daily dietary F intake could be detected in the nails within the study periods, and these increases and decreases in the F in nails could only be observed after 30 to 60 days. Furthermore, it is suggested that both toenail and fingernail samples can be used to monitor F intake. Measures to control F intake represent relevant strategies that should be implemented from a young age, especially when children do not participate in a preventive program. Ultimately, the levels of F ingested from diet could be higher if the foods were processed with seasonings and fluoridated water and if the F values assessed by ingesting toothpaste had been added to these findings.

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CONFLICT OF INTERESTS

None.

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