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Full Length Research Paper

The fluoride intake and urinary fluoride excretion in children attending a daycare center in Maracay, Aragua state, Venezuela

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The objectives of this study were to determine fluoride intake and urinary fluoride excretion levels in children exposed to fluoride from diet and dentifrice. Data were obtained from 31 children from a day care located in Maracay, Aragua State, Venezuela. Fluoride intake from diet was determined by using the "duplicate plate technique", and estimation of the ingested from dentifrice was made by the difference between fluoride in toothpaste taken for use and the fluoride in toothpaste used but not swallowed. Samples of foods, beverages and dentifrices were analyzed using the micro diffusion method. The results showed that children have a mean daily total fluoride intake for 15 - 35, 36 - 47 and 48 - 72 months-old of 0.07 ± 0.03; 0.09 ± 0.03 and 0.08 ± 0.02 mg/kg /day. Mean urinary fluoride concentrations values were 0.79 ± 0.51, 0.92 ± 0.30 and 0.83 ± 0.40 mg/L in 15 - 35, 36 - 47 and >48 months- old children and were not significantly different (p 0.05) when data from the three groups of children were compared. Age and gender did not affect urine total volume, urinary flow rate, urinary fluoride concentration and fluoride excretion rate (P > 0.05). The results from our study indicate that children have a mean fluoride intake and excretion within the expected value for optimally fluoridated areas.

Key words: Fluoride intake, dental fluorosis, rate fluoride excretion, children.

INTRODUCTION

The prevalence of dental caries in developed as well as in developing countries has declined over the past decades and this reduction has been attributed mainly to the use of fluoride (Narvai et al., 1999; Cisternas et al., 1994; Sampaio, 20001). In Venezuela, the government has sponsored two national dental caries studies. The first study carried out in 1972 (Cova Rey and Lozada, 1967-68), reported that the mean DMFT in children between 7-14 years of age was 3.14 with a caries prevalence of 95%.

The second national study was developed in 1997 by Rivera et al. (1998), and the main objective was to determine the prevalence of both dental caries and dental fluorosis. Results showed a reduction of 21% in caries prevalence compared with the first study. The DMFT index reported in the study at ages 12 and 15 years were 2.12 and 3.41, respectively. The prevalence of dental fluorosis was 15%, and was associated with water consumption from wells.

Due to the unsuccessful implementation of the water fluoridation program in Venezuela, in 1995 the govern-ment implemented the fluoridation of salt at 60-90 mgF/kg, which posteriously, has been increased to 180 - 220 mg F/kg of salt. This is considered to be the optimal fluoride level for the Latin-American population that would prevent dental caries development with a minimum risk of developing dental fluorosis (Salas, 1997). In Venezuela, about 80% of the table salt in the market is fluoridated.

Several studies have shown that fluoridated salt reduces caries prevalence in children, adolescents and adults (Cahen, 1993; Irrigoyen and Sanchez-Hinojosa, 2000).

Further studies have determined F intake at different

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ages (Rojas-Sanchez et al., 1999; Martinez Mier et al., 2003; Franco et al., 2005; Rodriguez et al., 2009) and the results demonstrated that the higher fluoride proportion came from the use of dentifrice.

Exposure to fluoride-containing dentifrice by young children, during amelogenesis could be an additional factor responsible for the prevalence of dental fluorosis, since young children tend to swallow some of the dentifrice while brushing their teeth (Rojas-Sanchez et al., 1999; Martinez Mier et al., 2003; Franco et al., 2005; Rodriguez et al., 2009). In Venezuela, dentifrice consumption per capita is around 1.7 g/day (National Data Colgate Palmolive, 2004), higher than in Brazil where dentifrice consumption of 1.4 g/day has been determined (Cury, 1990). No studies have ever been carried out to determine fluoride intake in Venezuelan children at the time of enamel formation.

Presently, there is a real concern about the increase in the prevalence of dental fluorosis (Ismail et al., 1990; Whelton, 2004). The optimal fluoride level that would not lead to the development of dental fluorosis has been determined based on an empirical calculation. Burt, (1992), has suggested that 0.05-0.07 mgF/kg/day as the upper limit for fluoride intake in children. Also, lower values such as those proposed by Hong et al (2006), have been found associated with dental fluorosis. On the other hand, the American Academy of Pediatric (2006), proposed a value of 0.1 mg/kg/day which is controversial.

The monitoring of urinary fluoride concentrations and excretion is a useful method to determine fluoride exposure in a human population; however, a large inter-individual variation has been reported (Zohouri et al., 2006). The best way to estimate fluoride intake is to use 24 h urinary fluoride output measurements (Marthaler et al., 1995; Lennon and Ketley, 2001), an approach that is independent of dietary habits, timing of meals and periods of maximal fluoride intake (Marthaler, 1999). Under steady fluoride ingestion conditions, several studies have estimated the percentage of fluoride excreted in urine by preschool children with percentages ranging between 51.5% and 85.0% (Villa et al., 2000; Haftenberger et al., 2001). On the other hand, some studies have reported fractional urinary fluoride excretions ranging between 0.30 - 0.33 (Villa et al., 1999; Lennon and Ketley, 2001; Franco et al., 2005).

The objectives of this study were to determine (i) the total fluoride intake (ii) the urinary fluoride excretion level in young children from a community with low fluoride levels in drinking water and consuming fluoridated salt (60 - 90 mgF/kg).

MATERIAL AND METHODS

Panelists

The study was approved by the Ethical Committee of the Faculty of Dentistry at the Universidad Central de Venezuela. A daycare center was selected in Maracay, Aragua State, Venezuela (water : 0.1 ± 0.02 mgF/L), since the research team reside in that area. The total number of children attending the day care was 250 and children who participated were not chosen randomly, since parent's permission had been granted. Data were obtained from 31 children (Table 1) who were split in three groups according to age range. In order to be included into the study the children had to be between 15 - 72 months of age; attend a certified and full time daycare center; and be in good health. At this time each child was also weighed and their body weight was recorded. Children's parents were requested to read and sign an informed consent and were asked to complete a questionnaire regarding the dental habits of their children.

Collection of foods and beverages

There was not a wash-out period prior to the study days. Duplicate meals and snacks were collected from each child on three separate days, two weekdays and one weekend day, using the duplicate plate method as described by Rojas-Sanchez et al. (1999). Duplicate plate samples of meals and snacks eaten at the daycare center and at home were collected by study personnel and parents, respectively. All foods collected at the day care on a given day were transported to the Faculty of Dentistry, pooled and processed as one sample. Beverages were collected using the same method and all beverages collected at the day care on a given day were processed, frozen and saved until the time of analysis.

When foods and beverages were collected at home, parents or relatives were instructed to collect duplicate meals, snacks and beverages following the same procedure. The importance of maintaining normal dietary habits at home and of visually duplicating each meal as carefully as possible by observing what the child actually ate was stressed. Parents were reminded to keep all collected samples refrigerated at 4ºC, and if any meals or snacks were eaten at restaurants, parents were reimbursed for purchasing an extra meal from which they could prepare a duplicate sample of what their child ate.

Measurement of fluoride intake from toothpaste

To determine the amount of fluoride ingested during dentifrice use, mothers were asked to brush their child's teeth within three different days at the daycare center using their child's own tooth paste and tooth brush. Mothers were asked to place the amount of dentifrice daily for the three days study period; she usually used on the pre-weighed toothbrush, and the toothbrush with the dentifrice was weighed. Then the child's teeth were brushed following their normal procedure. After brushing, the toothbrush was then thoroughly rinsed using deionized water (50 - 70 ml) to remove any toothpaste remaining on the toothbrush and the rinse water was added to the expectorate. Any expectorant from the child, the tissue used to wipe dentifrice slurry from the child face and any part of the child bid that contain dentifrice was also added to the rinse solution and saved for fluoride analysis. The difference between the fluoride placed on the toothbrush and the fluoride recovered was calculated to be the fluoride ingested. The amount of fluoride ingested was multiplied by the number of brushings reported by the parents in order to calculate the daily amount of fluoride ingested through toot brushing. Also a sample of the toothpaste used by each child was saved for fluoride analysis.

At the time of the study, a questionnaire was given to the children's parents in order to obtain data related to dental habits. Questions were asked regarding parents' education and annual income level, whether or not the parents had ever received instructions regarding the amount of toothpaste to use on their child's brush and/or the frequency with which they should brush their child's teeth. Parents were referred to a picture diagram when

Table 1. Age, gender, body weight and fluoride exposure in the study population.

Child No.	Gender	Age (months)	Body weight (kg)	Individual F exposure		Mean amount of toothpaste placed on the tooth brush (g)
				Use of F salt	Tooth paste F concentration (µgF/g)	
22	F	15	11.4	Yes	-	0.24 ± 0.09
27	F	21	13.1	Yes	1153	0.24 ± 0.09
29	M	27	11.4	Yes	1183	0.17 ± 0.03
9	M	30	15.8	Yes	1122	0.38 ± 0.07
8	M	31	14.2	Yes	1185	0.20 ± 0.01
6	M	32	14.8	Yes	1125	0.38 ± 0.05
16	M	36	14.4	Yes	1100	0.15 ± 0.05
13	M	39	15.0	Yes	-	0.24 ± 0.03
14	M	40	15.4	Yes	-	0.19 ± 0.03
1	M	44	15.6	Yes	1183	0.57 ± 0.21
4	M	44	12.8	Yes	1125	0.58 ± 0.22
7	F	44	15.6	Yes	1204	0.43 ± 0.25
21	M	44	13.6	Yes	1113	0.49 ± 0.07
23	F	44	16.1	Yes	-	0.24 ± 0.12
24	F	48	14.1	Yes	1168	0.47 ± 0.28
31	M	49	16.7	Yes	1095	0.53 ± 0.20
20	F	50	16.0	Yes	1118	0.19 ± 0.03
30	F	52	17.7	Yes	-	0.53 ± 0.21
19	M	53	15.4	Yes	1072	0.61 ± 0.30
5	F	56	16.9	Yes	1162	0.56 ± 0.10
12	F	57	15.8	Yes	1215	0.57 ± 0.17
11	F	63	18.3	Yes	1136	0.59 ± 0.18
15	M	63	17.4	Yes	965	0.47 ± 0.04
26	F	63	17.3	Yes	916	0.55 ± 0.14
18	M	66	22.0	Yes	-	0.46 ± 0.14
28	M	66	24.2	Yes	778	0.42 ± 0.08
10	M	68	19.5	Yes	1191	0.41 ± 0.12
3	M	69	20.1	Yes	1075	0.29 ± 0.05
17	M	69	20.1	Yes	1020	0.36 ± 0.10
25	F	69	18.5	Yes	846	0.47 ± 0.06
2	M	72	22.7	Yes	-	0.29 ± 0.12
		48.4 ± 15.2	16.3 ± 2.9		1095.0 ± 100.9	0.40 ± 0.15

answering a question about the amount of toothpaste usually placed on their child's brush.

Urine sample collection

All the children, even the youngest, were toilet trained and dry at night. After parents' consent, they received a 2 L plastic bottle and they were asked to collect their child's urine during 24 h. Urine sample collection was performed using the traditional method for collecting 24 h urine sample. The time for the first emptying bladder was recorded in the morning but discarded the sample, then collected and recorded times of subsequent samples, up to and including the first sample the next morning. The total volume collected and the collection period was recorded to give 24 h data. The research team held instruction sessions with the parents and these instructions were repeated by the investigators on the day before urine collection. On the day of the urine collection, parents were instructed to start collecting the child's second emptied bladder and continue the collection until the first emptied bladder the next morning; they were also asked to maintain the urine samples refrigerated during the time they were collecting it. The urine samples were returned to the daycare the following day and kept refrigerated immediately after receipt. The refrigerated bottles were subsequently transported to the Faculty of Dentistry, Central University of Venezuela, Caracas DC, using polystyrene cooling boxes. Two samples from each container were placed in 10 ml bottles and frozen until further analyses. For each child, the measured concentration of fluoride in the 24 h urine sample was multiplied by the 24 h urine volume to calculate the 24 h urinary excretion of fluoride in milligrams.

Drinking water samples were also collected from the daycare center and from each child's home to document the water fluoride level in the city.

The water samples were directly analyzed for fluoride using a fluoride ion-specific electrode (Orion #96-909-00) and an Orion 710A pH/ion meter (Orion Research Inc, Cambridge, Mass., USA). Samples were mixed 1:1 with Total Ionic Strength Buffer (TISAB II) and placed under the electrode. The fluoride concentration of each sample (mgF/L) was determined from a standard curve prepared by analyses of a series of fluoride standard solutions conducted at the same time.

Analyses of all food homogenates, beverages, dentifrice slurries and dentifrice recovery samples were conducted using the hexamethyldisiloxane (HMDS: Sigma Chemical Co., St Louis, MO, USA), microdifussion method of Taves, 1968, modified by Rojas-Sanchez et al., 1999. To validate these analyses, all samples were analyzed in duplicate at the same time and duplicate analyses of a subset of samples (10%) (Food homogenates, beverage mixtures, dentifrice recovery solutions, dentifrice slurries) were conducted on different days with newly diffused standards.

Direct urinary fluoride analyses were conducted using the fluoride ion specific electrode. The urine samples were analyzed by duplicate, for fluoride concentration "blind" in haphazard order, after thawing to room temperature. Before analyses, samples were centrifuged in a bench centrifuge, at 3000 rpm during 10 min.

Statistical analyses

The age groups were compared for differences in the survey results using chi-square tests. The three groups were compared for differences in weight, amount of toothpaste used (g/brushing), food fluoride intake (µg/day), drink fluoride intake (µg/day), toothpaste fluoride intake (µg/day), total fluoride intake (mg/day), total fluoride intake (mg/kg/day), urine fluoride concentration (mg/L), urinary fluoride excretion rate (mg/day or µg/hour), fluoride retention (%), and urine volume (ml) using Kruskas-Wallis test. Similar analyses were performed to compare gender. Pairwise comparisons between groups were made using the Mann-Whitney test. Comparisons were considered statistically significant if the p-value was less than 0.05. Intraclass correlation coefficients (ICCs) were also computed from random effect models.

RESULTS

The present study measured total fluoride intake from diet and dentifrice from 31 children. It also determined fluoride concentration and excretion from 24-h urine samples in the same group of children in order to determine the percentage of fluoride excreted. Intraclass correlation coefficient values for each type of samples were higher than 0.95.

Table 1 illustrates data regarding age, gender, body weight and fluoride exposure in the study population. Statistical analysis showed that children over 48 months of age had significantly higher weight than the children between 15 - 35 months ($P = 0.01$), but there was no significant difference in weight when comparing children between 36 - 47 months with the groups between 15 - 35 months and over 48 months ($P > 0.05$). The oldest group had significantly higher amount of toothpaste used than the youngest group ($p = 0.01$), but the middle group did not have significantly different amount of toothpaste used than the youngest group ($P = 0.35$) or the oldest group ($P = 0.25$).

Data for the fluoride intake from diet and dentifrices according to age, and expressed in µg/day are illustrates in Table 2. Mean total fluoride intake derived from diet was significantly higher in the group >48 months old children when compared with the group of children ages 15 - 35 months**.** The same pattern was observed when the combine source (diet+dentifrice) were compared ($P < 0.05$).

Table 3 summarizes mean fluid intake, urine volume, urinary flow rate, urinary fluoride concentration, amount of fluoride excreted, mean fluoride excretion rate and fractional urinary fluoride excretion and retention for the whole study population according to age. When data among children were compared, no significant differen-ces were detected for urine volume, urine excretion rate, fluoride concentration and excretion rate and per-centage of fluoride excretion and retention ($P > 0.05$). In all the children a positive fluoride balance was observed, the retention of fluoride ranged from 5 - 85% of fluoride ingested. It is important to point out that the amount of fluoride retained was calculated after taking into account the 10% of fluoride not absorbed and excreted by feces, as reported by Ekstrand et al. (1984).

Table 4 illustrated the pattern of children dental habits and parents educational level. Eighty-seven percent of the parents reported a tooth brushing frequency in their children of two times per day and, 17 out of 31 mothers reported that they brushed their kid teeth. Only 3 out of 31 children never expectorated after tooth brushing and the 35.4% of the parents reported that they place an amount of toothpaste equivalent to the whole toothbrush. The mean (±SD) amount of toothpaste used was 0.40 ± 0.15 g per brushing, and according to age, children 15 - 35 months used an amount of toothpaste significantly lower than that used by children over 48 months; however, children 36 - 47 months did not use significantly different amounts of toothpaste than the youngest or oldest group.

The number and percentage of children below and above the fluoride threshold dose are presented in Table 5. As it can be seen, 42.0% of the children had a daily mean fluoride intake within the threshold range, 45% are lower the limit of 0.05 mgF/kg/day, meanwhile, only 13% exceed the upper limit of 0.07 mg/kg/day. In addition, we observed a significant direct relationship (Pearson's correlation coefficient 0.549; $p < 0.0001$) between amount of toothpaste placed on the toothbrush and fluoride ingested from dentifrice (Figure 1).

DISCUSSION

Fewer studies in Latin-Americans have analyzed fluoride intake from different sources and urinary fluoride excre-tion (Franco et al., 2005; Martines Mier et al., 2003; Rodriguez et al., 2009; Villa et al., 1999, 2000; Franco et al., 2005; Acevedo et al., 2007). One of the advantages

Table 2. Mean fluoride intake from food, beverages and dentifrice according to age (Data collected from three days).

Months	Mean total fluoride intake from diet (µgF/day)	Mean fluoride intake from dentifrice (g/day)*	Mean total fluoride intake (g/day)
15 - 35 N = 6	424.1 ± 284.4[a]	550.1± 287.5	974.2 ± 439.9 [a]
Mean % intake	43.5	56.5	100.0
mgF/kg bw/day	0.03± 0.02	0.04± 0.02	0.07 ± 0.03
Age 36 - 47 N = 8	572.4± 158.6	822.3± 472.4	1394.7 ± 576.6
Mean % intake	41.0	59.0	100.0
mgF/kg bw/day	0.04± 0.01	0.05± 0.03	0.09 ± 0.03
Age >48 N =17	679.3 ± 246.2[b]	706.4± 343.0	1385.6 ± 404.6[b]
Mean % intake	48.6	51.0	100.0
mgF/kg bw/day	0.04± 0.01	0.03± 0.02	0.08 ± 0.02
Mean total F intake	602.3± 232.0	706.0± 370.2	1308.3 ± 474.2
mgF/kg bw/day (N	44.4%	55.5%	100.0%
= 31)	0.04± 0.01	0.04± 0.02	0.08 ± 0.03

*Mean of F ingested from toothpaste was calculated after taking into account frequency of tooth brushing reported by the parents.
Note: Values with different letters are significantly different (P < 0.05) as determined by Mann-Whiney test.

Table 3. Total fluoride excretion. Mean fluid intake, urinary flow, urinary F concentration, urinary F excretion rate, fractional urinary F excretion and retention by preschool children from Maracay, Edo. Aragua, Venezuela.

	Mean	15 - 35	36 - 47	>48
Volume of fluid intake (ml)	675.3 ± 194.4	733.1 ± 66.6	522.2 ± 71.3	815.0 ± 61.6
Urine volume (ml/24h)	439.5±104.1	400.2± 146.0	440.7± 113.2	453.2 ± 115.8
Urinary F concentration (mg/L)	0.80 ± 0.4	0.79± 0.51	0.92± 0.30	0.83 ± 0.40
Mean amount F excreted (µg/day)	368.2 ± 183.4	329.9± 214.9	398.5± 162.7	367.6 ± 189.9
Urinary fluoride excretion rate (µgF/h)	14.6 ± 1.8	12.8 ± 1.5	15.3 ± 1.7	15.8 ± 1.4
Fractional urinary F excretion	0.30 ± 0.17	0.36± 0.20	0.37± 0.20	0.26 ± 0.12
Fluoride retention (%)	69.7 ± 17.0	63.9± 20.0	62.7± 20.5	73.9 ± 12.6

of the massive salt fluoridated program is that the salt can reach the consumer through several channels including domestic salt, meals at school, cheese factory and in bread. In Venezuela most, if not all, of these channels are used. Another attraction of the program of salt fluoridation is that it can be sold along side with a non fluoridated alternative. When most salt for human consumption is fluoridated the effectiveness of salt fluoridation approximates that of water fluoridation.

Table salt has been shown to be an effective vehicle for providing fluoride in preventive programs and more preferable (Cisterna et al., 1994; Narvai, 1999; Sampaio, 2000) than water fluoridation in Venezuela. In October 1993, fluoridated domestic salt (FS), at a concentration of 60 - 90 mgF/kg became available in Venezuela and provides children in this study with a daily fluoride dose of 0.04 mgF/kg bw/day, a similar amount than that reported by Franco et al. (2005), in four Colombian cities (0.03 mg F/kg body weight/day in high social economic status; and 0.02 mg F/kg bw/ day for the lower social economic status) using a higher amount of fluoride in table salt (180 - 220 mgF/kg salt). This difference of fluoride ingestion from diet could be due to a higher salt intake by children involved in this study. We have to consider that the increment of fluoride in salt will contribute to increase the fluoride ingestion through the diet, which is expected.

When a fluoridation program is implemented, by water or salt, we expect that the major fluoride ingestion should come from diet and the lower percentage from dentifrice. After this study was done, in 2005, the Venezuelan government increased the fluoride in salt to 180-220 mgF/kg salt and in that situation a higher fluoride intake from diet will be expected; therefore, a permanent education program should be implemented to the population in order to control the amount of toothpaste placed on the toothbrush. The parents should be also recommended to supervise their children while tooth brushing in order to avoid dental fluorosis.

This study revealed that fluoridated dentifrice was the main source of fluoride (55.5%) . In this situation it will be necessary to control the use of toothpaste by children at this age. Otherwise, the percentage of children ingesting an optimal dose of fluoride (Table 5) could be skewed toward the upper range increasing the risk of developing

Table 4. Pattern of children dental habits and parents educational level.

Times of daily tooth brushing	
<1 time/day	1 (3.2%)
2 times/day	27 (87.1%)
> 3 times/day	2 (6.5%)
5 times/day	1 (3.2%)
Expectoration after brushing	
Yes	23 (74.2%)
No	3 (9.7%)
Sometimes	5 (16.1%)
Who brush children teeth	
Parents	10 (32.3%)
Mother	17 (54.8%)
Parents/Teacher	2 (6.5%)
Mother/Child	1 (3.2%)
Child	1 (3.2%)
Size Toothbrush	
Adult	0 (0%)
Child	31(100%)
Amount of Toothpaste	
Pea size	2 (6.5%)
1/4	6 (19.4%)
2/4	9 (29.0%)
3/4	3 (9.7%)
Whole brush	11(35.4%)
Educational level	
University	Mother 28(90%) Father 28(90%)
High school	Mother 1(3.2%) Father 2(6.5%)
Other	Mother 2(6.5%) Father 1(3.2%)

Table 5. Number of children above and below the threshold dose of fluoride of 0.05 - 0.07 mg/kg/day.

Age (months)	0.05 - 0.07 N (%)	<0.05 N (%)	>0.07 N (%)
15 - 35	2 (6.5%)	3 (12.9%)	1 (3.2%)
36 - 47	2 (6.5%)	4 (12.9%)	2 (6.5%)
>48	9 (29.0%)	7 (22.6%)	1 (3.2%)
Total	13(42.0%)	14 (45.0%)	4 (13.0%)

dental fluorosis.

Total fluoride intake from diet

The average daily diet fluoride dose of the children studied was 0.04 mg F/kg bw/day. Children older than 48 months ingested a significantly higher amount of fluoride from diet than those with ages between 15-35 months old. This difference might be due to the fact that as children grow up, the amount of food ingestion is bigger resulting in an increase of the total amount of fluoride intake.

Several studies in different countries have assessed the fluoride intake from diet in children. Murakami et al. (2002) and Kimura et al. (2001), reported a mean daily fluoride dose from diet of 0.017 and 0.019 mg/kg bw/day, respectively, lower than that reported in this study (0.04 mg F/kg bw/day). These differences could be explained by the fact that in our study the amount of food ingested was greater as well as, cultural diet particularities. However, Zohouri and Rugg- Gunn (2000), studied a population of Iranian children from a non fluoridated region and obtained similar results to those reported here which suggests that studies that estimate fluoride intake from diet diaries might over-estimate the amount of fluoride intake.

Lower concentration of fluoride was obtained from beverages by the children, and these lower amounts could be related to the negligible fluoride level in the drinking water (0.1 ± 0.02 mgF/L).

When the results from individual sources were analyzed, it was seen that the main source of fluoride from diet was food (81%), rather than beverages (19%), a result that was expected in this study, since table salt is fluoridated at the level of 60-90 mg/kg; while the fluoride concentration in the drinking water in Maracay city is negligible. These percentages are very similar to those reported in Colombia (Franco et al., 2005) and those reported for children residing in Veracruz and Mexico cities (Martinez Mier et al., 2003), which represent similar dietary patterns.

An important amount of the total daily fluoride intake in all three groups of children came from dentifrice, and this was the highest of all the studies reporting this parameter (Rojas-Sanchez et al., 1999; Tan and Razak, 2005) with the exception of the data reported by Simard et al. (1989) by children ages 2 - 5 years.

In Venezuela, there are not studies quantifying fluoride ingested from toothpaste. All of the children used fluoridated toothpaste, and the questionnaire data indicated that 93.5% (29 out of 31) of the children used more than a pea-size amount each time they brushed their teeth. The mean (±SD) amount of toothpaste used was 0.40 ± 0.15 g for brushing, and according to age, children with ages comprised between 15 - 35 months used an amount of toothpaste significantly lower than that used by children over 48 months; however, children 36 - 47 months did not use significantly different amounts of toothpaste than the youngest or oldest group. The difference in the quantity of toothpaste used by the children might be because the children at the youngest age do not spit out therefore, the mother place less amount of toothpaste.

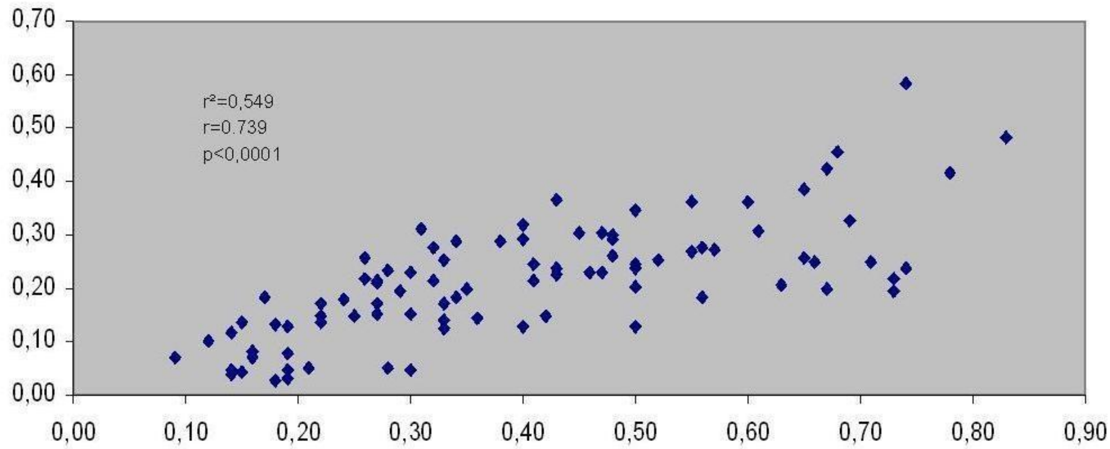

Figure 1. Correlation between the mean amount of dentifrice placed on the toothbrush (g) and the mean amount of fluoride ingested (mg) for each tooth brushing.

In this study, 24 different toothpaste samples were analyzed with an average fluoride concentration of 1100 ppm (1095.0 ± 100.9 ppm (Table 1), lower than the concentration reported from the Colombian study (Franco et al., 2005). This means that if children place similar amounts of dentifrice, the percentage of fluoride that is ingested from a tooth paste probably will be higher.

The total daily fluoride intake and the mean total daily fluoride dose for the children in this study were 1.2 ± 0.4 mg/day and 0.082 ± 0.03 mgF/kg bw/day, respectively. The total daily fluoride dose was higher than the estimated threshold dose (Burt, 1992) . However, most children in the community ingested an amount of fluoride from diet that was within the safe threshold for fluoride dose of 0.05 - 0.07 mg F/kg bw/day (Zohouri and Rugg Gunn, 2000).

When comparing the total daily fluoride intake among the three age study groups, children over 48 months ingested a significantly higher amount of fluoride than younger children ($p < 0.05$) . This could be directly related to the significantly higher amount of fluoride ingested from diet by older children. In these children the amount and variety of food increases, the feeding patterns change and children have a more complex diet with more content of salt (Table 3), as seen in results reported for Brazilian children (Paiva et al., 2003; de Almeida et al., 2007).

We have to consider that the daily fluoride dose ingested by the young children in this study could be increasing the risk of developing dental fluorosis even though Ekstrand et al. (1983), proposed a lower threshold for the developing of dental fluorosis (0.03 mgF/kg bw/day).

Urine output and fluoride excretion

Several studies have reported 24-h urinary output in 3 to 5 years-old children in developing countries (Villa et al., 1999; Franco et al., 2005) and developed countries (Obry-Musset et al., 1992; Lennon and Ketley, 2001; Zohouri and Rugg-Gunn, 2000) . The values reported in those studies were fairly close to our mean figures of 400.0 ± 18.62; 440.0 ± 39.9 and 452.7 ± 27.9 ml/day for children ages 15 - 35; 36 - 47 and older than 48 months respectively and they were consistent with values previously reported for healthy children (Villa et al., 2000; Franco et al., 2005) for the same ages. Baez et al. (2000), reported higher values (633 ml/daily) than those reported in our work, possibly due to the fact that children were older, therefore, the fluid intake could be higher.

A proportional relationship between fluid intake and urine output has been shown (Crosby and Sheppard, 1957) . In this study, children had a mean fluid intake of 675.3 ± 194.4 ml/day which is considered relatively low compared to the estimated amount of fluid in children of the same ages in USA, ranging from 2030.2 - 2337.3 ml/day (Sohonn et al., 2001). The low urinary volume could not directly be explained by the total fluid intake and that other factors as frequency and quantity could be involved. However, similar fluid intake was also observed in Mexican children suggesting common cultural patterns between the two Latin American populations studied.

Fluoride concentration and excretion

From studies reported in the literature, there is no doubt that higher fluoride intake results in higher excretion. Although analysis based on our data of 24 h urine do not support this statement, no difference in the amount of fluoride excreted in the urine was observed in children with the high and low fluoride intake (Tables 2, 3). A possible explanation for this fact is the fluoride bioavailability from the different sources.

Data from our study indicated that children have a mean fluoride excretion rate of 14.6 ± 1.8 gF/h. Since the cost-benefit of measuring total fluoride intake is quite demanding in terms of costs, measuring the fraction of fluoride eliminated could be a simple procedure to estimate total fluoride intake. However, it should be taken into account that the fraction of fluoride excreted in urine could be affected in a given group of children by urinary pH, growth period as well as other factors. In this investigation the fraction of fluoride was 30.3 ± 17.0%, a result reported for Chilean and Colombian children (Villa et al., 1999; Villa et al., 2000; Franco et al., 2005).

Based on the analyses, the fraction of fluoride excreted as a procedure to estimate total fluoride intake should be considered carefully, since large variety of factors affect the mechanisms of fluoride excretion, however, more research including larger population should be performed.

In conclusion, the data from this study indicate:

(a) that the total daily fluoride intake is slightly above the threshold dose suggested; being the fluoridated toothpaste the major source; and
(b) the urinary fluoride excretion was within the range suggested for this age group.

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