ABSTRACT

The aim of the present work was to obtain information on the total urinary fluoride concentration and excretion of Venezuelan children at the age of 3 to 5 years, as part of a program to monitor fluoride ingestion. A 24 hour urine sample was collected from each of 63 children, between 3 and 5 years of age and analyzed for (i) total urinary volume (ml), (ii) urinary flow rate (ml/h), (iii) fluoride concentration (ppm) and (iv) fluoride excretion rate (µg/h). The group comprised 32 boys and 31 girls who resided in two different communities, 32 in Caracas, DC and 31 in San Juan de los Morros, Guarico state. Fluoride analyses were done with an ion-specific electrode.

Age and gender did not affect total urine volume, urinary flow rate, or urinary fluoride concentration and excretion rate. The children from San Juan de los Morros had a significantly higher total urinary volume (426.45±36.31ml) and flow rate (17.09±1.57ml/h) than children from Caracas (297.06±23.59 ml and 12.40±0.98, respectively) (p=0.0039). Significant differences were also observed when the urinary fluoride excretion rate of the two communities was compared. Mean urinary fluoride concentrations did not differ significantly (p<0.05) between the two communities. These values were 0.67±0.40, 0.57±0.34, 0.76±0.27 ppmF in the 3, 4 and 5 year-old children of Caracas and 0.69±0.33, 0.65±0.20, 0.63±0.36 mgF in San Juan de los Morros children.

The results showed low urinary fluoride concentration and excretion, indicating that children residing in the evaluated communities are receiving fluoride below the recommended optimal range.

Key words: urinary fluoride, excretion, concentration, children.

EXCRECIÓN DE FLUORURO EN ORINA EN NIÑOS DE 3 A 5 AÑOS QUE CONSUMEN SAL FLUORURADA 60 A 90 mgF/Kg EN DOS CIUDADES VENEZOLANAS. ESTUDIO PILOTO

RESUMEN

El objetivo de este estudio fue determinar la concentración y excreción de fluoruro en orina de niños venezolanos en edades entre 3 y 5 años, como parte del monitoreo del Programa Nacional de Fluoruración de la sal. Se recolectaron muestras de orina de 24 horas de cada uno de los 63 niños que participaron en el estudio, en edades entre 3 y 5 años. En cada una de las muestras se analizó (i) volumen total de orina (ml) (ii) concentración de fluoruro (ppm) y (iv) tasa de excreción de fluoruro (ml/h). La muestra se conformó por 32 niños y 31 niñas, quienes residían en dos comunidades diferentes, Caracas DC y San Juan de los Morros, Estado Guarico. Los análisis de fluoruro se realizaron utilizando un electrodo específico para el íon fluoruro conectado a un potenciómetro marca Orion modelo 710 A.

El volumen total de orina, la tasa de flujo urinario, y la concentración y excreción de fluoruro en orina no varió significativamente cuando los datos se compararon de acuerdo a la edad y el género. Los niños procedentes de San Juan de los Morros mostraron un volumen total de orina (426.45±35.31 ml) y tasa de excreción urinaria (17.09±1.57 ml/h) significativamente mayor que los niños residentes en Caracas (297.06±23.59 ml y 12.40±0.98 ml/h) respectivamente (p=0.0039). Adicionalmente, se observó una diferencia estadísticamente significativa cuando se compararon los valores de la excreción de fluoruro en orina entre los niños de las dos comunidades. Sin embargo la diferencia obtenida en la concentración de fluoruro en orina no fue estadísticamente significativa. (p>0.05). Los valores de la concentración de fluoruro urinario para los niños de Caracas en edades de 3, 4 y 5 años fueron 0.67±0.40; 0.57±0.34 y 0.76±0.27 ppm. En los niños de San Juan de los Morros los resultados fueron 0.69±0.33; 0.65±0.20 y 0.63±0.36 ppm respectivamente.

Los resultados demostraron una baja concentración y excreción de fluoruro en orina, lo que indica que los niños evaluados están expuestos a una dosis de fluoruro por debajo de los niveles recomendados.

Palabras clave: fluoruro urinario, excreción, concentración, niños.
INTRODUCTION
Table salt has been shown to be an effective vehicle for providing fluoride in preventive programs and is preferable (1-4) to water fluoridation in Venezuela. In October 1993, fluoridated domestic salt (FS), at a concentration of 60-90 mgF/kg in the form of potassium fluoride (KF), was authorized by the Venezuelan government for dietary use as a part of its dental caries national preventive program. This concentration was selected because at the time that the salt program was implemented, there was no knowledge of exposure to fluoride from different sources such as food, beverages and dentifrices in the Venezuelan population. This decision was made although the Venezuelan authorities were aware that a study carried out in Switzerland using fluoridated salt with a concentration of 90 mgF/kg showed a limited cariostatic effect. Fluoridated salt became available on the market in January 1995, in packages of 1.0 kg in all shops and was also delivered to restaurants, hospitals, cafeterias, and other large kitchens through the food distribution system. Recently, studies carried out in Venezuela to determine fluoride concentration in soft drinks, food and bottled water, indicated a relatively low fluoride concentration (5-8). Furthermore, the specified fluoride concentration in domestic salt does not necessarily reflect fluoride exposure, since the industries that process salt in Venezuela are reported not to maintain a constant fluoride level (9), which could lead to an excessive or suboptimal fluoride intake level.

Approximately 80% of the Venezuelan population is now supplied with fluoridated salt; therefore, it has become necessary to monitor the total fluoride excreted by children susceptible to the development of dental fluorosis. A clinical relationship between fluoride ingestion and occurrence of dental fluorosis has been established during the susceptible period of tooth development (10, 11). A urinary fluoride concentration of 1.0 mg/L is regarded as indicative of an optimal fluoride intake from table salt (12, 13, 14). Obry-Musset et al. (15) compared the fluoride excretion level in children consuming FS in Strasbourg (France), with children taking fluoride tablets. The study showed that children ingesting fluoridated salt (FS) (250 mgF/kg) for at least three years, excreted a significantly higher amount (26.34±8.73 µgF/h). In addition, few studies have measured the amount and excretion rate of fluoride in 24-h urine samples, some of which consider morning, afternoon and night sample collections (16-22). Marthaler et al. (17) evaluated the urinary fluoride excretion in children who had either a lower fluoride intake or had received supplemental fluoride through salt or water. Children with a history of low fluoride intake excreted a mean of 10 µgF/h during the night and the morning, and 13-16 µgF/h after the main meal. However, after the intake of meals prepared with fluoridated salt (250 ppm) the mean fluoride excretion ranged between 31 and 49 µgF/h.

In the available studies using 24-h urine samples, the urinary fluoride excretion rate in optimal fluoridated communities ranges from 11.6 to 19.8 µgF/h. (22-26). On the other hand, Báez et al. (20) concluded that an excretion rate of 16.7 to 28.3 µg/h in children between 3 to 6 years could be considered as an indicator of “optimal fluoride intake”.

The objective of this study was to collect and measure the fluoride levels in 24 h urine samples in order to evaluate fluoride excretion rates in children 3 to 5 years of age consuming fluoridated salt (60-90 ppmF). The children lived in the cities of Caracas and San Juan de los Morros and participated in the national program to monitor fluoride ingestion.

MATERIAL AND METHODS
Subjects and sites
A cross-sectional study was carried out to evaluate urinary fluoride excretion in Venezuelan preschool children. Prior to study initiation, human use approval was granted by the Technical Council of the Institute of Dental Research and permission from school authorities and parents to collect urinary samples was obtained. Parents and school staff cooperated extensively in the collection of samples. A convenience sample of 63 healthy children between 3 and 5 years of age was evaluated; 32 were from a certified daycare center in Caracas and 31 were from a similar center in San Juan de Los Morros. The geographical, climatic and socio-economic status differences between the two cities could have an impact on dental hygiene habits. Inclusion criteria were age between 3 and 5 years, good health, permanent residence for at least the preceding two years, and consumption of fluoridat-
ed salt. All of the children that participated in the study used 1100 ppm fluoridated toothpaste that was introduced into the Venezuelan market in 1968. The fluoride concentrations in the drinking water supply in San Juan de Los Morros and Caracas were 0.34±0.15 ppm and 0.12±0.1 ppm, respectively.

**Collection of 24-hour urine samples**
Each subject was given a 2-liter plastic bottle and asked to collect urine during a period of 24 hours. The research team held instruction sessions both with the parents and the children prior to the date of collection. Each parent was instructed to have his/her child empty his/her bladder at the start of the 24 h collection, collect the child’s urine after emptying the bladder on the day of the collection, and continue collection until the bladder was emptied on the following morning. Instructions were given to keep the urine refrigerated between times of collection. The same method of urine preservation and storage was used at both study sites. The urine samples collected by the parents were returned to the daycare center the next day and subsequently transported to the Faculty of Dentistry, in Caracas, in polystyrene cooling boxes where total volume was recorded for each child. Two samples from each container were placed in 10-mL bottles and stored in freezers until further analysis. A defined number of bottles were taken out of the freezer and analyzed.

**Fluoride analysis**
After thawing at room temperature, urine samples were centrifuged at 1740 g for 10 minutes and samples were removed for fluoride analysis using a fluoride specific electrode (Orion Research Inc, Cambridge, Mass., USA) and a digital pH meter after the addition of TISAB II at a ratio of 1:1. The fluoride concentration of each sample was calculated against a fluoride standard curve prepared daily. Recovery experiments using standard fluoride solutions were performed for approximately 5% of the samples in order to assess the reproducibility of the analytical procedure for fluoride. In all cases, recovery was 90 to 98%. Following the study, a questionnaire was mailed to each of the children’s parents, to obtain data concerning the fluid intake habits in the two communities. It included questions regarding the time, in years, that the child drank from baby bottles and the time of day he/she drank beverages thereafter.

**Data analysis**
The normal distribution of the samples was examined by ANOVA. Because of the possible effects of gender and age on the measurements, age (as categorical variable) and gender were included as covariates in the ANOVA. The differences in total urine volume, urinary flow rate, and urinary fluoride concentration and excretion rates between the two cities were analyzed by analysis of variance (ANOVA). The data derived from questionnaire scores regarding liquid intake were compared by the Mantel-Haenszel Chi-square test. The Chi-square test was used to compare the groups and determine the differences in beverage intake.

**RESULTS**
Parental response to the questionnaire was 45% and 47% in Caracas and San Juan de los Morros, respectively. This level of response was similar to what we experienced in other field studies. In the present investigation urinary parameters were determined in sixty-three pre-school children, 31 female (49%) and 32 male (51%). No significant differences by age and gender (p>0.05) were observed in the number of children residing in the two cities. The data by gender was combined for further analysis. Table 1 summarizes the mean (±SEM) total urine volume and urinary flow rate in 3 to 5 year old children from Caracas and San Juan de los Morros, expressed as mL and as mL/h, respectively. Children from San Juan de los Morros had a significantly higher mean total urine volume and flow rate than children from Caracas (p=0.004 and p=0.017, respectively). Age did not significantly affect total urine volume. The statistical analysis of urinary flow rate data according to age, revealed that the 3 year-old children from San Juan de los Morros had a significantly higher mean total urine volume and flow rate than children from Caracas (p=0.004 and p=0.017, respectively). Age did not significantly affect total urine volume. The statistical analysis of urinary flow rate data according to age, revealed that the 3 year-old children from San Juan de los Morros had a significantly higher urinary flow rate than the 4 year-olds (p=0.02) but the values of 5 year-old children were not significantly different from those of the 3 year-old (p=0.57) or the 4 year-old (p=0.08) children. However, no significant difference by age was observed in the children from Caracas.

Data on the urinary fluoride concentration and excretion rate of children by age are summarized in Table 2. There was no significant difference in urinary fluoride concentration among the three age groups in each city. Age (p=0.7), gender (p=0.98) and location (p=0.97) did not significantly affect
Urinary fluoride concentrations. Urinary fluoride excretion showed a similar pattern in the two cities; the four year-old children consistently had a lower urinary fluoride excretion when compared with the 3 and 5 year-old children at both study sites. The mean values of urinary fluoride excretion rate determined in children from Caracas (7.29±3.24 µgF/h) were significantly lower (p=0.0039) than those in children from San Juan de los Morros (10.65±6.18 µgF/h).

The analysis of the results by age, showed that the mean values of urinary fluoride excretion rate in children at ages 3, 4 and 5 years were lower in Caracas but not significantly different from the values obtained in San Juan de los Morros (Table 2). The analysis of the data by age (p=0.24) and gender (p=0.74) within each city, revealed that these variables did not significantly affect the urinary fluoride excretion rate.

Table 3 illustrates beverage consumption habits reported by the children’s parents in each study site. Significant differences between the two cities were found when comparing these parameters. Children from San Juan de los Morros drank from baby bottles for a longer period of time (p=0.0409) but consumed fewer drinks per day than the children from Caracas (p=0.0401).
DISCUSSION

To the best of our knowledge, no previous studies on fluoride excretion have been reported in Venezuela. Thus, this investigation constitutes the first on urinary fluoride excretion in children in this age group. Consequently, these results greatly contribute to increase the data on regional fluoride excretion within the context of a preventive caries program based on salt fluoridation.

The evaluation of fluoride excretion employing 24-h urine samples is more reliable than spot or single samples (27). Fluoride is rapidly excreted after intake reaching a peak within 2-h (28); after 6 hours, 30% is excreted and 60% is excreted within 24-h. This illustrates the importance of analyzing 24-h urine samples (29). Therefore, spot or single samples may not reflect the average concentration and even less allow reliable estimates of total fluoride intake. The fluoride concentration for single samples varies the most in 24-h samples. However, the 24-h urine samples used in this study may more readily reflect the average fluoride concentration ingested by the children.

Evidence from research suggests that the critical time for development of dental fluorosis in anterior permanent teeth is during the second or third year of life (30-33). However, the children included in this study were older due to the limited number of 2 and 3 year-olds in day care, and the difficulties in collecting urine samples in 2 year-old children.

When the urine volumes and urinary flow rates were measured for Caracas and San Juan de los Morros, significant differences between these communities were observed. Children from Caracas showed a significantly lower mean total urine volume than children from San Juan de los Morros (p<0.05). In an attempt to explain the difference, beverage consumption habits reported by the parents were reviewed. It was found that children from San Juan de los Morros drank from baby bottles for a longer period of time (p=0.0409) but consumed fewer drinks per day than the children from Caracas (p=0.0401). This observation leads to the assumption that both the total urine volume and urinary flow rate do not directly depend on the frequency of fluid ingested in these children and that the amount of fluid rather than the frequency of intake could be the determinant factor. Rugg-Gunn et al. (34) reported that there is a direct proportional relationship between fluid intake and urine output. Also, the difference in the mean moderate temperature between the two cities (Caracas 22°C; 72°F and San Juan de los Morros, 27°C; 81°F) does not seem to be directly associated with fluid consumption or urinary output, in agreement with results obtained by Sohnn et al. (35).

The data on 24-h urine output in 3 to 5 year-old children in Latin America (16, 22, 36-39) and developed countries (22, 24, 25, 34, 40, 41) is sparse. Nonetheless, the values reported are in good agreement with the figures obtained in San Juan de los Morros (426.8±201.9 mL) but higher than those found for Caracas. Rugg-Gunn et al. (42) reported 24-h urine flow values of 225, 282 and 303 mL in Saudi Ara-

| TABLE 3. Beverage consumption habits in children from Caracas and San Juan de los Morros, Venezuela (expressed as a percentage). |
| Cities | San Juan de los Morros | Caracas |
| Total number of children | 14 | 15 |
| 1) Number of years drinking from baby bottles | | |
| 2 years | 36a | 7c |
| 3 years | 21 | 14 |
| >4 years | 43 | 79 |
| 2) Beverage drinking: Times per day | | |
| 2 times | 7a | 0c |
| 3 times | 21 | 0 |
| 3+ times | 71 | 100 |

Note: Values with different letters are significantly different as determined by Chi-square analysis and Mantel-Haenszel test (p<0.05). Number of years drinking from baby bottles SJ>C p=0.041. Beverage drinking: Times per day C>SJ p=0.041
bian children ages 2, 3 and 6 years, respectively. These are similar to the values found in Caracas (297.0±23.59 ml). These values have been considered by Marthaler et al. (19) to be low and the result of an incomplete sample collection. In our study, this can be ruled out since results from a national study carried out (246 children 3 and 4 years-old) in ten Venezuelan Cities showed that these urine volumes are constant in the study population. This supports the assumption that low urine output values could be related more to a low amount of fluid intake than to sample collection errors (43).

Mean fluoride urinary concentrations in children from San Juan de los Morros and Caracas were similar to the values reported by Warpeha and Marthaler (16) before the implementation of the fluoridated salt program in Jamaica. These fluoride values could indicate that the children are not receiving the “optimal” amount of fluoride from salt and are therefore considered at risk of dental caries. Another way to monitor fluoride exposure is to assess its relation to the urinary fluoride excretion. However, there are no comparative studies dealing with 24-h urine collections from children living in communities with 60-90 mgF in the dietary salt supply. Compared to communities with optimal fluoridation levels (22-26), our fluoride excretion rates are low.

Children from San Juan de los Morros showed a significantly higher urinary fluoride excretion rate than children from Caracas (p<0.05). A reason for this difference could be that children residing in Caracas have a high socio-economic status and ingest smaller amounts of fluoride from all sources, especially from toothpaste (44). These authors reported that children with a high socio-economic status ingested significantly lower amounts of fluoride from toothpaste than children with a low socio-economic status. The researcher also emphasized that the low fluoride ingestion might be due to substantially smaller amounts of toothpaste being dispensed on average onto the toothbrush. This finding could be attributed to a better educational level of parents who instruct their children in appropriate oral hygiene techniques taught by the dentist. Another possible explanation could be the effect produced by a low mean total urine volume, as a variable considered in the calculation of the fluoride excretion rate. Mean urine volume in San Juan de los Morros children was 1.4 times higher than in Caracas children. This difference in volume would result in a 1.4-fold increase in fluoride in the urine excreted by children from San Juan de los Morros.

Based on our findings, we can assume that the children from the two cities under study are not receiving the optimal fluoride concentration necessary to achieve a cariostatic effect. The amount of fluoride in the salt was too low to provide the optimal fluoride dose to the population. Therefore, the Venezuelan Health Authorities were advised to increase the fluoride concentration in domestic salt to provide the population with the optimal amount of fluoride for effective protection against dental caries.

It is important to mention that two major decisions had to be made by the Venezuelan government recently. The first was to increase the fluoride concentration in domestic salt from 60-90 to 180±20 mg/kg and concomitantly require companies that market toothpaste with fluoride to reduce the concentration of fluoride (<500 ppm).

- This paper adds relevant data regarding urinary fluoride excretion in preschool children that can be used to monitor fluoride intake in countries with different sources of fluoride intake.
- The paper is important to the paediatric dentists since:
  1) They can administer safe amounts of fluoride in different dental products such as dentifrices, gels, mouth rinses and supplements.
  2) Excessively high levels of urinary fluoride excretion could indicate a risk of development of dental fluorosis.

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REFERENCES