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Is the fluoride/creatinine ratio of a spot urine sample indicative of 24-h urinary fluoride?

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Abstract – *Objective:* The main aim of this study was to determine how representative the fluoride/creatinine (F/Cr) ratio of a spot urinary sample is of the fluoride content of a 24-h urine sample in young children aged 12–36 months. Subsidiary objectives were to: (a) evaluate the use of 24-h urine samples in monitoring fluoride exposure and (b) estimate the retention of fluoride in very young children. *Methods:* Seven healthy children residing in a fluoridated area completed the study. Dietary information was obtained using a 3-day estimated food diary followed by an interview on the fourth day. Samples of foods and drinks were analysed for fluoride content. Ingestion of fluoride from toothpaste was also measured. A 24-h urine sample and a morning spot urine sample were collected from each child. *Results:* The mean age of the children was 32 months (range: 16–36 months). The mean total daily intake of fluoride was 0.71 (±0.41) mg or 0.05 (±0.02) mg/kg bodyweight, of which 37% came from the diet, the remainder being from toothpaste ingestion. None of the children used any other sources of fluoride such as fluoride tablets or fluoridated salt. The mean F/Cr ratio was 1.49 (±0.63) mg F/g creatinine. A positive correlation (Pearson's correlation $= 0.76$, $P = 0.05$) between fluoride excretion estimated using the F/Cr ratio in a morning spot urine sample and fluoride excretion in a 24-h urine sample was found. There was also a positive correlation (Pearson's correlation $= 0.83$, $P = 0.02$) between total daily fluoride intake and 24-h urinary fluoride excretion. Less than half (43%) of the total daily fluoride intake was retained in the body. *Conclusions:* The F/Cr ratio of a morning spot urine sample may be used to estimate mean 24-h urinary excretion of fluoride and therefore has potential application for larger-scale epidemiological studies where 24-h samples are impractical. Estimates of 24-h urinary fluoride excretion can be used to gauge fluoride exposure.

Key words: dietary fluoride, fluoride, fluoride/creatinine ratio, 24-h urine

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The role of fluoride in preventing dental caries has been well recognized. However, excessive consumption of fluoride during the pre-eruptive stages of enamel formation (i.e. during infancy) can put permanent teeth at risk of development of dental fluorosis (1). There has been an increase in the prevalence and severity of dental fluorosis (2) because of increased fluoride exposure from a number of sources (1).

The World Health Organization has recommended that fluoride exposure, and therefore retention, should be monitored regularly as part of any fluoride supplementation programme in order to ensure that exposure to fluoride in a population is at an appropriate level (3). The most sensitive period for dental fluorosis in the permanent central incisors, from ingested fluoride, appears to be between age 15 and 24 months for boys and between 21 and 30 months for girls (4), and for the eight permanent incisor teeth is the period from birth to 5 years of age (5). However, there is little information on fluoride intake, excretion and retention by children under 3 years of age, possibly because of the considerable practical difficulties in

obtaining data from very young children, in particular, collecting 24-h urine samples from infants and young children who are not toilet-trained.

As spot urine samples are easier to collect, they have now superseded 24-h urine collection in clinical investigations and diagnosis or screening for some diseases (6). However, urinary concentration of many analytes such as fluoride may vary throughout the day, and therefore measuring their concentration in a spot urine sample may not be representative of 24-h urinary fluoride excretion. Urinary creatinine output can be used to estimate the excretion rates of certain analytes by calculating their respective ratios to creatinine. As total urinary creatinine excretion is relatively constant throughout the day in healthy subjects (7), it can be used to help correct for daily variation in urinary dilution, and standards for 24-h urinary creatinine by different age groups are available. The mean 24-h urinary creatinine value of 15 mg/kg bodyweight (bw)/day with 5th and 95th percentiles of 8 and 22 mg/kg bw/day has been reported as the standard urinary excretion of creatinine for young children (8, 9). Measured ratios of analytes such as iodine (10), albumin (11), porphyrin (12), mercury (12) and calcium (6, 13) to urinary creatinine have been used successfully in adults, to estimate 24-h excretion of the analytes by multiplying these ratios with creatinine reference values. However, despite a positive correlation between urinary clearance of fluoride and creatinine being found in 16–79-year olds (14), its use to assess 24-h urinary fluoride excretion in infants and very young children has not been investigated. It has been suggested that the fluoride/creatinine ratio of a spot urine sample can be used as an index of 24-h urinary fluoride excretion in populations (7) in the absence of dietary fluoride supplementation such as fluoride tablets or fluoridated milk.

To provide useful information on the most appropriate method of determining 24-h fluoride excretion in infants and very young children for any future fluoride-exposure studies, the main aim of this study was to determine how representative the F/Cr ratio in a spot urinary sample is of the fluoride content of a 24-h urine sample in children aged 12–36 months living in a fluoridated area. As a result of the limited fluoride data on this age group, subsidiary aims of the study were to: (i) evaluate the use of a 24-h urine sample for monitoring fluoride exposure in this age group, and (ii) estimate the retention of fluoride in very young children.

Materials and methods

The study was conducted in spring 2002, in an area of north-east of England where the drinking water supply is fluoridated at 0.81 (±0.09) mg F/l. Approval for this study was obtained from the relevant Local Research Ethics Committee and written consent was obtained from the parents of all children. Seven healthy children aged between 12 and 36 months were enrolled from the staff of the University of Newcastle and Royal Victoria Infirmary Hospital. All the children had been residing in the same area since birth, and were not on any therapeutic diet or taking any dietary fluoride supplements.

Assessment of fluoride intake

A 3-day estimated food diary was used to collect dietary information. The diary was given to a parent of each child with instructions for its completion, emphasizing the importance of recording all food, and especially drink, consumption over the 3-day period: two weekdays and one weekend day. An interview was held in the family home with the parent on the fourth day, to ensure that all food and drink items had been accurately recorded. In order to determine the fluoride content of the items consumed, samples of milk, food and other drinks consumed by the children were collected. Home-made food and drink samples were collected from parents/households and stored in the refrigerator at 4°C until analysis. Other identified food and drink products were purchased from local shops as appropriate, prior to fluoride analysis.

Measurement of fluoride content of food and drinks

All the food and drink samples were analysed, at most 24 h after collection, in triplicate. The fluoride content of non-milk drinks and water was determined directly using a fluoride ion selective electrode (fluoride-ISE) (model 96-09, Orion: Analytical Technology, Inc., Boston, MA, USA) in conjunction with an ISE Meter (Model 720A, Orion) after adding TISAB II. The modified Venkateswarlu's silicon-facilitated diffusion method (15) was used to measure the concentrations of fluoride in foods and milk-based drinks samples.

Measurement of fluoride ingestion from toothpaste

Information on toothbrushing habits, including frequency of toothbrushing and the brand of

toothpaste used was obtained. Six children used childrens'-formulation toothpaste containing 600 ppm F as sodium fluoride (NaF) or sodium monofluorophosphate (MFP) and one child used adult toothpaste containing 1350 ppm F as MFP. The amount of fluoride ingested through toothbrushing was calculated by weighing each child's toothbrush before and after dispensing toothpaste onto the toothbrush and collecting all expectorated saliva, liquids and toothpaste during toothbrushing and rinsing. The toothbrush was then thoroughly rinsed to remove any toothpaste remaining on the toothbrush and the rinsing added to the expectorate (16). The concentration of fluoride in expectorated saliva and samples of toothpaste was measured using a fluoride-ISE, after enzymatic (acid phosphatase) hydrolysis of MFP (16). The amount of fluoride not recovered from the mouth per brushing was multiplied by the number of brushings per day to estimate the daily amount of fluoride ingested through toothbrushing. Toothbrushing was performed once and the manufacturers' labelled fluoride concentration on the toothpaste was used for the calculations.

Measurement of fluoride and creatinine excretion

Urine samples were collected on the third day of food diary recording. For toilet-trained children, urine samples were collected using two screw top urine bottles – one for a 24-h sample, and the other for the first voided urine in the morning. Urine volumes were recorded for each sample and an aliquot taken for subsequent analyses. Urine samples were analysed for fluoride content directly using fluoride-ISE after adding TISAB II to the samples. For children wearing nappies, specially designed pads (Ontex Ltd, Northamptonshire, UK) were used to collect urine samples (17). The pads were fluoride-free (<0.02 μg F/g of dry pad) which was confirmed during pilot testing; i.e. they neither added nor removed fluoride from urine. All nappies and pads used were pre-weighed. The nappy was worn inside out and the pad stuck to the inside of the nappy. Using the 5-ml syringe provided, parents were asked to take a 3–5-ml urine sample from the wet pad and transfer the sample to a sterile container. Each wet pad was then placed in a separate plastic bag. At the end of 24 h, all used pads were collected and weighed to measure the weight of urine excreted. The fluoride concentration of urine samples was measured directly by fluoride-ISE, after adding TISAB II. Urinary creatinine of each 24-h and spot urine sample was measured by the Jaffe method (18).

Calculation of 24-h urinary excretion of fluoride (mg)

Based on 24-h urine sample collection: 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.

Based on the F/Cr ratio of the spot urine sample: The 24-h urinary excretion of creatinine (mg/day) was estimated for each child by multiplying the body weight bw (kg) of each child by the standard creatinine value of 15 mg/kg bw/day (9). A standard creatinine value rather than the individually measured creatinine values was used, since the aim of the study was to investigate how useful this ratio would be when 24-h urine samples are not available. The estimated 24-h urinary excretion of creatinine was multiplied by the F/Cr ratio of the spot urine sample to estimate the 24-h urinary excretion of fluoride for each child.

Validation of the study methods

Dietary intake data were validated by comparing the reported energy intake (EI) with predicted basal metabolic rate (BMR) to estimate the number of subjects' potential under-reporting their energy intake. Predicted BMR was calculated using the following equation (19): 'BMR (kcal/day) = [61 × weight (kg)] – 53'. The physical activity level (PAL) value which is the ratio of estimated EI to predicted BMR was then compared with the reference ranges for this age group (20).

Completeness of 24-h urine collection was validated by measuring the urinary excretion of creatinine in each 24-h urine sample and comparing this with the standard reference value for creatinine excretion (19). The validity of the analytical method for fluoride analysis of foods and milk-based drinks was evaluated by adding 0.10 μg fluoride as sodium fluoride to 20% of samples before the percentage of fluoride recovery was measured, using the diffusion method (15).

Statistical analysis

Following descriptive analysis to report the mean (±SD) of the variables, Pearson's correlation was used to investigate the relationship between: (i) fluoride excretion estimated using the F/Cr ratio in a morning spot urine sample and fluoride excretion measured in a 24-h urine sample, and (ii) fluoride intake and 24-h urinary excretion of fluoride.

Results

Subjects and validity of data collection

The mean (±SD) age, weight and height of the children were 32 (±7) months, 15 (±2) kg and 95 (±9) cm, respectively. The mean (±SD) daily energy intake and PAL value of the children were 1704 (±348) kcal and 2.01 (±0.45), respectively (Table 1). Mean 24-h urinary creatinine excretion was 13.2 (±3.1) mg/kg bw/day (Table 1). One of seven children wore nappies.

Fluoride analysis of foods and drinks

The recovery of fluoride added to food samples, ranged from 90% to 109%, with a mean of 96%. The range of fluoride concentration in the food items consumed by the children ranged from <0.02 μg/g (in breakfast cereals) to 0.44 μg/g (in peas).

Fluoride intake

Diet and toothpaste ingestion were the main sources of fluoride exposure for the children studied. Overall, the mean fluoride intake from toothpaste ingestion (0.45 ± 0.26 mg F/day) was higher than that from the diet (0.26 ± 0.22 mg F/day). In only one child was the intake of fluoride from diet higher than fluoride intake from toothpaste ingestion (Table 2). Most children (five of seven) ingested 100% of the toothpaste used, while for the remainder the ingested proportion of toothpaste used was 91% and 85%. There was a wide between-subject variation in total daily fluoride intake with a mean of 0.71 (±0.41) mg, of which 37% came from the diet. The mean daily intakes of fluoride from diet and toothpaste ingestion were 0.02 (±0.01) and 0.03 (±0.01) mg F/kg bw/day respectively (Table 2).

Urinary excretion of fluoride and creatinine

Table 3 presents urine volumes, urinary fluoride concentrations and weights of fluoride excreted in the 24-h and the spot urine sample for each child. The variation in the volumes of spot and 24-h urine samples between subjects was wide, with mean values of 102 (±54) and 523 (±263) ml respectively.

Table 1. Age, anthropometric parameters, energy intake, physical activity level (PAL), and measured 24-h urinary creatinine excretion (24-h U_{Cr}) of seven children

Subject ID	Age (months)	Gender	Body weight (kg)	Height (cm)	Energy intake (kcal)	Physical activity level (PAL)	Measured 24-h U_{Cr} (mg/kg bw/day)
1	36	Male	15.9	94	1874	2.05	18.5
2	33	Female	15.1	95	1249	1.50	14.1
3	35	Male	17.5	110	2280	2.25	15.7
4	34	Male	15.1	94	1333	1.54	9.1
5	33	Male	12.7	92	1842	2.55	11.9
6[a]	16	Male	11.8	80	1672	2.52	12.6
7	35	Female	16.8	97	1679	1.73	10.8
Mean	32		15.0	95	1704	2.01	13.2
SD	7		2.1	9	348	0.45	3.1

[a]Was wearing nappies.

Table 2. Daily fluoride intake in mg and in mg/kg bw/day from diet and toothpaste and the per cent of total fluoride intake from diet for each subject ($n = 7$)

Subject ID	Fluoride intake						
	Diet		Toothpaste ingestion		Total		% from diet
	mg/day	mg/kg bw/day	mg/day	mg/kg bw/day	mg/day	mg/kg bw/day	
1	0.10	0.01	0.23	0.01	0.33	0.02	30
2	0.50	0.03	0.32	0.02	0.82	0.05	61
3	0.61	0.04	0.93	0.05	1.54	0.09	40
4	0.08	0.01	0.67	0.04	0.75	0.05	11
5	0.26	0.02	0.41	0.03	0.67	0.05	39
6	0.19	0.02	0.24	0.02	0.43	0.04	44
7	0.07	0.00	0.35	0.02	0.42	0.03	17
Mean	0.26	0.02	0.45	0.03	0.71	0.05	37
SD	0.22	0.01	0.26	0.01	0.41	0.02	

Table 3. Mean and standard deviation (SD) of volume of urine (ml), urinary fluoride concentration (mg/l), weight of fluoride excreted (mg) in spot and 24-h urine samples, per cent weight of spot to 24-h sample and fluoride/creatinine (F/Cr) ratio (mg F/g creatinine) in spot morning urine samples of children

	Volume of urine (ml)		Fluoride concentration (mg/l)		Weight of fluoride excreted (mg)		Ratio of weight of fluoride in spot/24-h[a] (%)	F/Cr ratio (mg F/g creatinine)
Subject ID	Spot	24-h	Spot	24-h	Spot	24-h		
1	135	510	0.36	0.41	0.05	0.21	24	0.70
2	99	780	1.41	0.62	0.14	0.48	29	1.99
3	110	935	0.65	0.56	0.07	0.52	13	1.22
4	180	545	1.01	0.82	0.18	0.45	41	2.14
5	34	205	1.50	1.57	0.05	0.32	16	2.04
6	125	425	0.47	0.44	0.06	0.19	32	1.69
7	32	264	0.60	0.44	0.02	0.12	17	0.67
Mean	102	523	0.86	0.70	0.08	0.33	24	1.49
SD	54	263	0.45	0.41	0.06	0.16		0.63

[a]Ratio of urinary excetion of fluoride (mg) in spot urine sample to 24-h urine sample.

On average, 24% of the 24-h urinary fluoride was excreted in the spot morning sample. The mean weight of 24-h urinary fluoride excretion when calculated from 24-h urinary excretion was 0.33 (±0.16) mg/day. The mean F/Cr ratio was 1.49 (±0.63 mg F/g creatinine). When the F/Cr ratio for each spot urine sample was used to estimate weight of 24-h urinary fluoride excretion, the mean weight of fluoride excreted was 0.33 (±0.13) mg/day.

A statistically significant positive correlation between the measured weight (mg/day) of urinary F excretion in the 24-h urine samples and estimated weight of urinary F excretion using the F/Cr ratio in the spot morning urine sample was obtained (Pearson's correlation = 0.76, $P = 0.05$) (Fig. 1).

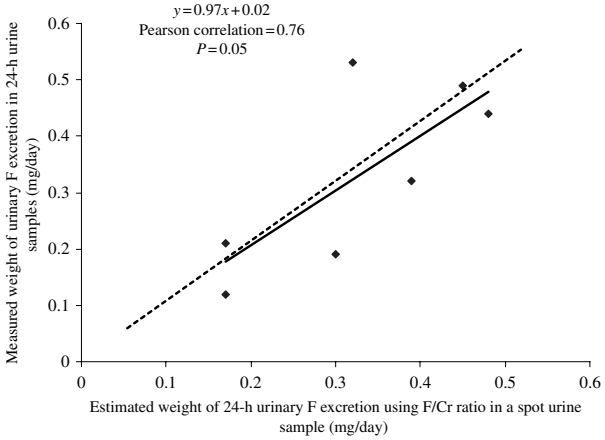

Fig. 1. Relationship between the measured weights of fluoride (mg) excreted via urine using 24-h urine samples and estimated weight of urinary fluoride excretion using fluoride/creatinine (F/Cr) ratio in spot morning samples: The solid line shows the best-fit straight lines for the relationships. The dotted line gives the hypothetical 1:1 ratio.

Relationship between fluoride intake and excretion

A significant positive correlation between total daily fluoride intake and measured daily urinary fluoride excretion was found (Pearson's correlation = 0.84, $P = 0.02$) (Fig. 2).

Fluoride retention

There was a large variation in the proportion of fluoride retained by the young children in this study, with the mean weight of fluoride retained being 0.35 (±0.28) mg/day (Table 4), indicating that a mean proportion of 43% of the total daily fluoride intake was retained in the body.

Discussion

The mean weight (15 kg) and height (95 cm) of the children in the present study was similar to the weight and height values reported for British children in the same age group: 14.7 kg and 95.6 cm, for boys and 13.9 kg and 94.7 cm for girls (21). The mean daily energy intake of the children (1704 kcal) was higher than the estimated average requirements of 1230 kcal for males and 1165 kcal for females aged 1–3 years (22). The PAL value for young children should range between 1.7 and 2.5, however, for less active children the level can be reduced to 1.5. A value of <1.5 might indicate under-reporting of food intake. In the present study, the PAL value (mean 2.01 ± 0.45) for all children indicated valid dietary information. The 24-h urinary excretion of creatinine ranged from 9.1 to 18.5 mg/kg bw which was within the reference

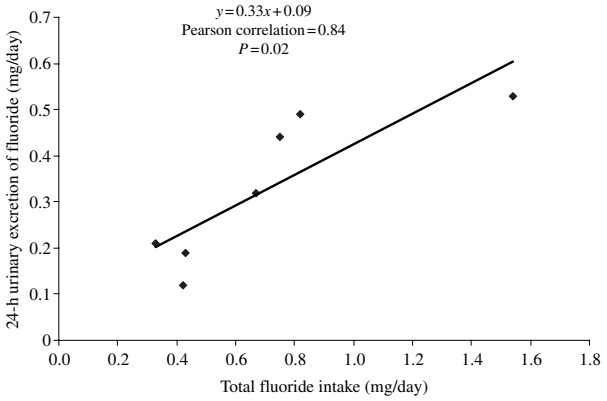

Fig. 2. Relationship between the 24-h urinary fluoride excretion (mg/day) and daily fluoride intake (mg/day).

Table 4. Fluoride intake, excretion and retention (mg/day) and the percent of ingested fluoride retained by seven subjects

Subject ID	Fluoride intake (mg/day)	Fluoride excretion[a] (mg/day)	Fluoride retention mg/day	%
1	0.33	0.23	0.10	29
2	0.82	0.54	0.28	34
3	1.54	0.58	0.96	62
4	0.75	0.48	0.27	36
5	0.67	0.35	0.32	48
6	0.43	0.21	0.22	51
7	0.42	0.13	0.29	69
Mean	0.82	0.36	0.35	43
SD	0.68	0.18	0.28	

[a]Fluoride in faeces was estimated as 10% of total excretion.

range of 5th and 95th percentiles of 8 and 22 mg/kg bw/day, indicating complete urine collection.

The results showed large standard deviations for most variables as a consequence of the small sample size and the between-subject differences in this study and therefore a larger study would be useful.

Total daily intake of fluoride from diet and toothpaste ingestion

Most of the children did not use fluoridated tap water as a main drink, instead they drank non-fluoridated bottled water (<0.08 mg F/L) and pre-packed drinks (<0.3 mg F/l) (Zohouri FV, Swinbank CM, Maguire A, Moynihan PJ, unpublished observation). The mean intake of fluoride from the diet of 0.02 (±0.01) mg/kg bw/day, was similar to the reported average intake of 0.018 mg/kg bw/day for 3–6-year-old German children residing in nonfluoridated areas but consuming fluoridated salt and taking fluoride tablets (23). Other studies have also reported similar fluoride intakes e.g. 0.019 mg/kg bw/day for 1–6-year-old Japanese children living in non-fluoridated areas (24), 0.019 mg/kg bw/day for 3–4-year-old New Zealand children in a non-fluoridated community (25), 0.021 mg/kg bw/day for Chilean children aged 3–5 years residing in an area with a fluoride concentration of 0.5–0.6 mg/l in the drinking water (26) and 0.023 mg/kg bw/day for 4-year-old Iranian children receiving supply water containing 0.32 mg F/l (16). In contrast, the results of the present study were lower than the 0.056 mg F/day reported for 15–36-month-old Mexican children consuming fluoridated salt and receiving non-fluoridated water (27), while 19–38-month-old Brazilian children living in two communities, both fluoridated at 0.7 mg F/l have been reported as consuming 0.027 and 0.040 mg F/kg bw/day (28).

These differences in mean dietary fluoride intake between studies might be explained by differences in the fluoride content of foods and drinks in different areas studied, as well as variation in the quantities of items consumed containing higher concentration of fluoride. Additional factors contributing to the differences would include the age group of children investigated and the different methods of dietary data collection.

All the children in the present study used fluoridated dentifrices and ingested an average weight of 0.45 mg F/day when brushing and rinsing their teeth. Five children ingested almost all the toothpaste dispensed onto their toothbrush as habitually they did not rinse or spit out after brushing. By increasing the number of performances of toohbrushing – matching with the number of days which food diaries collected – more accurate information on fluoride intake from toothpaste ingestion may have been obtained, although this may be too labour-intensive and burdensome to subjects in a large-scale study. This toothbrushing habit was in agreement with a study on children aged 1–4 years in Iowa (29) which reported that 95% of children younger than 2.5 years old did not rinse their mouth after toothbrusing; and 27% of all children with a rinsing habit swallowed all of the rinse. A study on fluoride ingestion from toothpaste in seven European countries (30) showed that between 36% (in Athens) and 70% (in Reykjavik) of the 1.5–2.5-year olds swallowed between 80 and 100% of the toothpaste. The intake of fluoride from toothpaste ingestion for the children, in the present study, was more than the intake of fluoride from their diet and represented

63% of total daily fluoride intake with large between-individual variation. Similar contributions to fluoride intake from toothpaste have been reported for Brazilian children (58–69%) aged 19–38 months (28) and also 15–36-month-old Mexican children (64%) (27). A higher contribution of 70% has been reported for Colombian children aged 22–35 months (31). In contrast, lower contributions to total fluoride intake from toothpaste ingestion have also been reported: 29% for German 3–6-year olds (23), 47% for 3–4-year olds from New Zealand (25) and 44% for the US children aged 16–40 months (32). The lower contributions to total fluoride intake from toothpaste ingestion by children in these studies were probably due to the subjects being older and therefore more likely to expectorate, differences in the fluoride concentration of toothpaste used, or higher total fluoride intake from dietary sources and fluoride supplements.

The total fluoride intake of the children in the present study varied from 0.02 to 0.09 mg/kg bw/day, and only three children ingested fluoride in the so-called 'optimum' range of 0.05–0.07 mg/kg bw/day (33). The fluoride intake of one child was below the lower limit of 0.03 mg/kg bw/day (34), and for another child the estimated fluoride intake of 0.13 mg/kg bw/day was in excess of the upper limit of 0.10 mg F/kg bw/day (35) which was sufficient to put the child at risk of dental fluorosis.

Urinary excretion of fluoride

The mean urinary fluoride excretion in the present study of 0.33 mg/day was similar to the mean value of 0.36 mg/day reported for Irish children aged 1.8–5.2 years (36), and to the value of 0.34 mg/day reported for 4-year-old Iranian children (16) living in areas with fluoridated water, but was lower than the 0.48 mg/day reported for German children aged 3–6 years living in a non-fluoridated community and consuming fluoridated salt (23).

Urinary fluoride excretion has been suggested as a useful biomarker for short-term fluoride exposure in a population (7). The fraction of the total daily fluoride intake that is excreted through urine [fractional urinary excretion of fluoride (FUEF)] has been estimated in a few studies (26, 37). It has been suggested that approximately 50% of the ingested fluoride in adults and 30–40% in pre-school children is excreted through urine (38). However, recent investigations have shown a wide range in mean FUEF from 30% to 52% for children aged 3–6-year olds (23, 26, 36). The mean FUEF for the children in the present study was 48% with a range from 29% to 64%. A large inter-individual variation between 37% and 80% was also reported for German children aged 3–6 years (23). There are no comparative data in the literature on the FUEF of children in the age group investigated in the present study.

Retention of fluoride

By assuming 10% of total fluoride excretion through faeces (39), the mean fluoride retention was estimated to be 43%, (although the range was 29% to 69%) which is much lower than a previous estimate of 70% published by the World Health Organization (3). The lower estimated mean fluoride retention in the present study may be due to the study's small sample size. In addition, the convention of assuming a constant mean value of 10% of fluoride intake for faecal fluoride excretion would benefit from further investigation with more subjects. Ekstrand et al. (40) reported a lower fluoride retention of 12.5% in formula-fed infants under the age of 1 year, with a mean fluoride intake of 0.02 mg/kg bw/day. However, when the mean daily fluoride intake of these infants increased to 0.05 mg/kg bw/day (similar to the mean fluoride intake of the children in the present study) by consumption of dietary fluoride supplements, the retention increased to 47%; closer to the value obtained in the present study. In contrast, studies of 4-year-old Iranian and American children have shown average fluoride retentions of 20% (16) and 15% (41) respectively. Retention of fluoride by bone is influenced by the stage of bone maturity (42) and the most efficient uptake of fluoride into bones and teeth occurs during periods of rapid growth development (43). The differences seen in the levels of fluoride retention in young children may therefore be attributed to the status of a child's bone maturity and uptake of fluoride into developing teeth and bone. Another explanation might be the type of diet consumed. Urinary pH is an important factor in urinary fluoride excretion (44). Many fruit and vegetables increase the pH of urine (19), resulting in a reduction in the proportion of fluoride intake retained in the body through increased in urinary excretion of fluoride.

Relationship between total intake and 24-h urinary excretion of fluoride

A statistically significant correlation between total fluoride intake and 24-h urinary excretion was found, which is in broad agreement with the strong association between total fluoride intake and

urinary fluoride excretion previously reported in 4-year-old (16) and 8–9-year-old children (45).

Relationship between the estimated excretion of fluoride using F/Cr ratio in the morning spot urine sample and measured in a 24-h urine collection in young children

There are few studies on F/Cr ratio published in the literature, and none have investigated infants and very young children. The ratio of 1.49 mg F/g creatinine which was obtained in the present study is comparable with the ratio of 1.51 reported for 8–13-year-old Hungarian children (46), but higher than the ratio reported for Romanian children aged 3–7 years of 1.00 (47). In the present study, the mean weight of 24-h urinary fluoride excretion, estimated using the F/Cr ratio in a spot urine sample of 0.33 ± 0.13 mg/day was in excellent agreement with that measured in the 24-h urine samples (0.33 ± 0.16 mg/day). However, the agreement found on an individual basis was not as high as that found on a group basis. Similar results have been reported for 3–7-year-old Romanian children in a recent abstract which calculated urinary fluoride excretion as 0.34 ± 0.19 mg/day when based on supervised 16-h time-controlled urine sampling compared with 0.32 ± 0.05 mg/day when estimated from the F/Cr ratio (47).

Evidence suggests that in order to prevent enamel fluorosis, fluoride exposure should be monitored during the first 5 years of life. Urine is a generally accepted indicator of fluoride exposure. While most children achieve day-time urine control by 3–4 years of age, it may take months or years to achieve night-time dryness. Therefore, collecting 24-h urine samples from young children might not be practical. The positive correlation obtained in this study, between the measured weight of 24-h urinary fluoride excretion and estimated weight of 24-h urinary fluoride excretion based on F/Cr ratio in spot morning urine samples, when no dietary fluoride supplements such as fluoride tablets or fluoridated milk were taken, suggest that this ratio might be used for estimating 24-h urinary fluoride excretion in populations; and therefore, be of use in much needed larger-scale studies of infants and children under 3 years of age.

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