Urinary fluoride excretion by children and elderly individuals in Romania (Timisoara and Bucarest)

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Abstract:

Objective: **Estimate fluoride exposure via renal excretion in institutionalized children 2-16 years of age and adults 26 to 97 years-old.** ***Methods:*** **Procedures followed WHO guidelines. Daytime and nocturnal urine samples planned to cover 24 hours were collected from 50 children in Timisoara and 40 from Bucharest, and from forty-eight elderly adults from Timisoara. Subjects' weights, times of initial bladder voiding and end of collection period, as well as volume for each micturition were recorded. Samples were analyzed using potentiometer and specific fluoride electrodes Samples were tested in duplicate on site and later at the University of Zurich.** ***Results:***

	Mean Fluoride Concentration mg/l (Standard Deviation)		Excretion ì g/F/24h (SD)	Excretion/body weight ì g/F/24h/kg (SD)
	Nocturnal	Daytime		
Children Timisoara	0.366 (0.246)	0.621 (0.269)	260 (142)	8.4 (4.5)
Children Bucharest	0.603 (1.081)	0.401 (0.260)	173 (109)	6.2 (2.9)
Adults Timisoara	0.299 (0.127)	0.259 (0.101)	159 (121)	2.7 (1.7)

Conclusions: **The low fluoride excretion findings by children and adults in two Romanian cities are important and indicate the need for increasing fluoride supply by systemic fluoride for dental caries prevention. Nationwide salt fluoridation could be implemented in Romania, thereby improving substantially dental health. Since most of the cariostatic effect is due to topical fluoride protection mechanisms, adults would also benefit. Simultaneously use of fluoridated salt and dentifrices containing fluoride, rapid improvements of dental health in Romania would be obtained. It would be necessary to demonstrate existence of isolated water supplies or entire regions where natural content of fluoride would be > 0.5 or 0.7 mg/l.**

Keywords: **Fluoride exposure; renal fluoride excretion; urinary fluoride excretion; children fluoride exposure; adult fluoride exposure; salt fluoridation, fluoride excretion by body weight.**

Introduction

Remarkable progress has been attained over the last six decades in improving oral health and controlling dental disease in populations around the world. Unfortunately, dental caries continues to affect individuals of all ages regardless of ethnicity and socio economic status, although it is recognized that persons from disadvantaged groups can be more severely afflicted. Several countries

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in the European region have reached the World Health Organization' (WHO) goal of less than 3 DMFT at 12 years of age. Dental caries remains a serious problem in Romania where in 1998 the mean DMFT in children at 12 years of age was 7.3 being one of highest in the European region indicating the urgency for implementing community prevention programmes. In Romania, the use of fluoridated salt has been considered on the university and government levels.

An essential prerequisite for developing and implementing a fluoridation programme is the establishment of baseline information on the population's exposure to fluoride. This paper examines fluoride excretion in order to gauge whether systemic fluoride is indicated in Romania.

Materials and Methods

In late 1998, urines were collected from institutionalized children and elderly adults to estimate fluoride exposure in Timisoara in Western Romania. The working procedures as described in the respective WHO-manual (WHO 1999) were closely followed. From each subject, nocturnal urine was collected in the morning at the first micturition and a daytime collection from morning to late afternoon followed. Times at initial voiding of the bladder and at the end of the collection periods were noted. In this way, two collections of time controlled urine covering roughly 24 hours were obtained. The same methods and procedures were used to assess fluoride excretion of children in a home in Bucharest. The fluoride concentration in the drinking waters used in the homes and schools were below 0.3 mg F/l

The volumes of the urinary collections were determined in the laboratory of the University of Timisoara, faculty of preventive dentistry. Approximately 30ml were deposited into a tube containing a few crystals of Thymol; 5 ml were taken and analysed for fluoride using specific ion electrodes and portable potentiometers (Orion, Beverly MA) within one day. Personal data, the urinary volume and the time at the beginning and end of each collection periods were entered in a Table (adapted from Figure A.2 in WHO 1999). The tables as well as the remaining aliquots of same 2 ml urine were transported to Zurich where the fluoride concentrations were again determined according to the methods described elsewhere (Marthaler 1999).[4] These repeated analyses gave results very similar to those from Timisoara. All data as determined in Timisoara were evaluated (in Zurich and San Antonio) using the automated computing tables described in the WHO manual.

Results

Table 1 summarizes basic data of the samples and presents statistics on fluoride concentrations and urinary flow. Cooperation was very good in all three studies. From the Bucharest children, seven did not hand in their nocturnal urine and four of the adult subjects did not provide the day time collection (see top of Tables)

Medians of nocturnal fluoride concentrations were low, ranging from 0.265 to 0.392. One of the Bucharest children had a very high fluoride concentration in the nocturnal urine, 6.53 ppm. This cannot be due to normal circumstances and renders fluoride results from this child (no. 37 from Bucharest, Table 2) inappropriate for inclusion in the statistical evaluation; the standard deviation was reduced from 1.08 to 0.26. Day time fluoride concentrations were almost twice as high in Timisoara children but not in the other two groups. The lowest part of Table 1 summarizes urinary flow statistics.

The lowest flow figures, of 0.1, 0.6 and 1.0 ml/h as evident from the ranges indicate grossly incomplete recovery of urine during the 8 to 12 hour collections periods. On the other hand, the highest urinary flow of 150 ml/h (subject 38, 78 years, 86 kg) was not unduly high (Table 4 in WHO 1999 allows for up to 300ml/h). For this subject, it may

Table 1. Urinary fluoride data from Romania, summary of data obtained in 1998

	Timisoara children	**Bucharest children**		**Timisoara adults**
Number of subjects, age, weight				
Subjects (females)	50 (24)	40 (17)		48[a] (11)
Subjects with both collections	50	33		44
Number of successful collections [b]	100	73		92
Median age, years (range)	12 (2-16)	9 (5-14)		72 (26-97)
Median weight, kg (range)				69 (41-104)
Duration of collection, hours, within one 24 -hour cycle (only subjects with both collections)				
Average (range)	22.6 (20-24)	22.9 (21-25)		20.1(15-22)
Fluoride concentration, ppm				
			Excl37[c]	
Nocturnal median (N)	0.28 (50)	0.392(33)	0.389(32)	0.265(48)
Range	0.08-1.40	0.15-6.53	0.15-0.89	0.13-0.72
Average	0.366	0.603	0.418	0.299
Standard deviation	0.246	1.081	0.189	0.127
Daytime median (N)	0.650(50)	0.370(40)	0.366(39)	0.240(44)
Range	0.12-1.200	0.07-1.52	0.07-0.89	0.010-0.56
Average	**0.621**	**0.401**	**0.372**	**0.259**
Standard deviation	0.269	0.260	0.187	0.101
Urinary flow, ml/h*				
Nocturnal median (N)	19.9(50)	14.6(33)		23.7(48)
Range	0.6-58.3	5.6-36.0		1.0-150
Average	**23.4**	**16.3**		**34.9**
Standard deviation	14.9	8.5		32.8
Daytime median (N)	21.1(50)	15.7(40)		17.0(44)
Range	0.1-63.4	2.5-83.5		2.6-54.2
Average	**22.5**	**21.7**		**18.8**
Standard deviation	13.6	16.8		10.7

a: Of the original number of 51 adults, 3 provided no urine at all
b: With all data: time at initial voiding of the bladder, time at last micturitions included in collection, volume of urine and fluoride concentration
c: case No. 37 excluded
N: Number of participants

be assumed that the collection period was probably incorrectly recorded, from 0300 to 0600. Since the urinary volume (450 ml) and amount of fluoride in it (72µg) were by no means exceptional among these adults, and the 24-hour results regarding fluoride concentration and excretion would not be affected by incorrect duration of the collection period (by e.g. assuming a collection period from 2200 [the group median] to 0600 instead of from 0300 to 0600).

Results regarding fluoride excretion in the 50, 33 and 44 subjects with both collections, "complete subjects", are shown in the upper part of Table 2. By disregarding child No. 37 in Bucharest who had the excessive urinary fluoride concentration of 6.53 (cases over 5 ppm to be excluded, see Table 4 in WHO 1999), the median and mean of fluoride excretion were moderately lowered but both measures of variability, the standard deviations and ranges, were strongly reduced and similar to the statistics for the other two groups. Regarding flow, application of the lower limit of 200 ml/24h led to the rejection of three children from Timisoara (including the only child who was only two years old, otherwise the minimum age was 4), four Bucharest children (including No. 37 who would have been rejected

Table 2. Urinary fluoride excretion in Romania

	Timisoara, Children (Tc)	Bucarest, Children (Bc)	Timisoara, Adults (Ta)
Fluoride excretion, µgF/24 h			
All complete subjects, number	50	33	44
Median	228	154	129
Range	19-520	69-673	21-702
Average	260	173	159
Standard deviation	142	109	121
Confidence limits (p=0.95)	220-301	135-212	122-196
Mean fluoride concentration	0.490	0.497	0.267
Mean urinary flow, ml/24h	535	442	606
Subject No.37 with excessive fluoride concentration eliminated			
Remaining subjects, number		32	
Median		150	
Range		69-315	
Average		**158**	
Standard deviation		64	
Confidence limits (p=0.95)		135-181	
Mean fluoride concentration		0.386	
Mean urinary flow, ml/24h		451	
Definitive samples after exclusions, fluoride excretion, µgF/24 h			
Subjects with low/high urinary flow eliminated (3 from Tc, 4 from Bc, 6 from Ta)			
Remaining subjects, number	47	29	38
Median	247	154	147
Range	40-520	78-315	47-702
Average	**271**	**165**	**178**
Standard deviation	138	62	120
Confidence limits (p=0.95)	142-189	139-218	47-702
Mean fluoride c oncentration	0.487	0.375	0.271
Mean urinary flow, ml/24h	560	479	684
Definitive samples, excretion per bodyweight, µg/24h/kg			
Only subject with 6.53 ppm F excluded (child No. 37)	47	29	37*
Median	7.8	6.2	2.2
Average	**8.4**	**6.2**	**2.7**
Standard deviation	4.5	2.9	1.7
Range	2.1-22.5	1.4-12.1	0.7-9.5
Confidence limits (p=0.95)	5.1-7.2	2.1-3.3	

* body weight not recorded in one adult

anyway because of 6.53 ppm F) and six adults subjects.

The definitive samples appropriate for further statistical evaluation were 47 children in Timisoara, 29 in Bucharest and 38 adults in Timisoara. The changes in the fluoride parameters due to the exclusion were approximately 10% or less or even zero.

Among the subjects included in the definitive statistics, the Timisoara children had the

Table 3 Average Fluoride excretion when a selected number of subjects with the lowest urinary flow are excluded from the sample

	Number of subjects included	Percent excluded	Average excretion	% increase due to exclusion
Timisoara children				
All subjects	50	0	260	
WHO-excluded	47 (3)	6.0	271	4.1
20% excluded	40 (10)	20.0	297	14.2
Bucharest children *				
All subjects	32	0	158	
WHO-excluded	29 (3)	9.4	165	4.2
20% excluded	26 (6)	18.8	173	9.5
Timisoara adults				
All subjects	44	0	159	
WHO-excluded	38 (6)	13.6	178	10.7
20% excluded	35 (9)	20.5	185	16.4

WHO-excluded, as done in Table 2, flow <200 ml/24h
20 % excluded: the 20% with the lowest flow
*Case 37 with 6.53 ppm F disregarded

highest excretion, 271 µgF/24h on average and the lowest 165 µgF/24h was in the Bucharest children (lower part of Table 2). This difference was obviously statistically significantly as the confidence intervals do not overlap. When fluoride excretion was expressed per kg of bodyweight, the adults had less than half the excretion of the children.

Discussion

Exclusion of data must be done carefully. In the case of subject no. 37 of the Bucharest children, accidental or purposeful swallowing of a fluoride preparation, most probably of toothpaste.

Incomplete urinary collections are difficult to avoid, particularly when 24 hour urine should be obtained. The exclusion of the few individuals with less than 200ml urine in 24 hours did in fact result in higher excretion parameters, but the changes were not substantial. Table 3 shows that the WHO-based exclusions as used in order to obtain the definitive sample based on the 200ml/24h-minimum, raised the average fluoride excretion of children by less than five percent only. The table shows in addition that exclusion of the 20 %, or one/fifth, of the subjects with the lowest urinary flow would result in an average increase of fluoride excreting by 8.5 to 16.4% (Table 3). It must be assumed than even in the individuals handing in 200ml to for instance, 400 ml urine/24h, some micturitions were not placed in the bottles. To some extent, unjustified exclusion of individuals with less than 200 ml/24h tends to compensate urines lost in the definitive sample of collections. All subsequent statistics are based on the "definitive sample" remaining after the exclusions described above.

Statistics for subsamples restricted to age four to nine for the children in both cities, were reevaluated in Table 4. Fluoride excretion in µg/24h and per body weight (µgF/24h) were similar in Timisoara and in Bucharest. In the resulting pool of 29 children, 182 µgF/24h and 8.1 µgF/24h/kf were excreted on average. In these youngest children the average excretion of 182 µgF/24h (Table 4, N=29) was about as low as those from German children not taking fluoride

Table 4 Urinary fluoride excretion in Romania
Children aged 4-9 years, definitive samples

	Timisoara, Children (Tc)	Bucarest, Children (Bc)	Children pooled
All complete subjects	11	18	29
Average age	6.4	8.0	7.4
Average weight	18.4	24.7	22.3
Fluoride excretion, µgF/24 h			
Median	179	178	179
Range	40-427	98-315	40-427
Average	**180**	**183**	**182**
Standard deviation	101	59	76
Confidence limits (p=0.95)	112-247	154-212	153-211
Mean fluoride concentration	0.451	0.404	0.422
Mean urinary flow, ml/24h	417	502	473
Fluoride excretion, µgF/h			
Average	**7.5**	**7.5**	**7.6**
Confidence limits (p=0.95)	4.7-10.3	6.4-8.9	6.4-8.8
Fluoride excretion, µgF/24h/kg body weight			
Median	10.0	7.4	7.5
Range	2.7-22.5	3.9-12.1	2.2-22.5
Average	**10.0**	**7.5**	**8.1**
Standard deviation	5.6	2.4	4.1
Confidence limits (p=0.95)	6.2-13.7	6.3-8.7	6.8-79.3

t-test comparing the Timisoara and Bucharest children
µgF/24h: t=1.01, p>0.1
µgF/24h/kg: t=1.67. p>0.1

tablets (204 µgF/24h or 8.5µgF/h, age 3-6, Schulte et al 1995)[10]. Bulgarian children aged 5 and 6 excreted 5.8, 8.2 and 5.6 µgF/h in morning, afternoon and night collections, respectively (Marthaler and Phillips 1994)[6], and their averages were thus similar to the Romanian children (7.6 µgF/h, N=29). Excretion in Swiss children aged 3 and 4 years who had been consuming fluoridated domestic salt was 245 µgF/24h (Marthaler et al 2000, N=15), [8] which was significantly higher than the present results ($p<0.05$). The Swiss children were younger (3 and 4) and when the excretion per body weight (14.7µgF/24h/kg) was used for the t-test, the difference turned out to be highly significant ($p<0.001$). English children, (mean age 5.8) receiving a daily supplement of 0.5 mg fluoride in milk excreted 330 µgF/24h (0.33 mgF/24h, Ketley and Lennon, 2000).[2] Another group of 12 children living under the same condition excreted on average 300 µgF/24h (0.30 mgF/24h, Ketley and Lennon 2001) [3] ; that paper cites additional data available from published papers. In the hot climate of South Texas (USA) where drinking water contained 1.2 ppm fluoride, the average 24 hour excretion by children four to six years of age amounted to 749 micrograms; children in this community had a modest level of fluorosis (Baez et al 2000).[1]

Tables 2,4 and 5 present the data on fluoride excretion per kg body weight. Most often, the “optimal” excretion - meaning that the possibility of fluoride usage is approaching the exposure levels leading to more than slight enamel fluorosis common in affluent countries - is accepted to be at 0.05 to 0.07 mgF/24h/kg body weight. T his would correspond to an excretion between approximately 0.02 to 0.025 mg

Table 5 Urinary fluoride excretion of children aged 10-14 years in Romania (definitive samples)

Romania	Timisoara children	Bucharest children, Bc	Pooled data
Complete subjects, number	32	11	43
Average age, years	12.2	11.6	12.1
Average weight, kg	37.3	37.2	37.3
Fluoride excretion, ì gF/24 h			
Median	275	122	220
Range	64-520	78-263	64-520
Average	282	136	245
Standard deviation	134	57	135
Confidence limits (p=0.95)	234-331	98-175	203-287
Mean fluoride concentration	0.493	0.329	0.451
Mean urinary flow, ml/24h	575	442	541
Fluoride excretion, ì gF/h			
Average	11.8	5.7	10.2
Confidence limits (p=0. 95)	9.7-13.8	4.1-7.3	8.5-11.9
Fluoride excretion, ì g/24h/kg body weight			
Median	7.5	3.6	6.1
Range	2.1-19.6	1.4-8.2	1.4-19.6
Average	**7.9**	4.0	6.9
Standard deviation	4.3	2.2	4.2
Confidence limits (p=0.95)	6.4-9.5	2.5-5.5	5.6-8.2

t-test comparing the Timisoara and Bucharest children
µgF/24 h: t=3.48, p<0.0025
µg/24h/kg: t=2.87, p<0/.005

F/24h/kg body weight. In the children of this study, the averages of fluoride excretion (per 24 hours per kg body weight) were between 0.010 and 0.004. That means that fluoride intake may be raised by a factor 2 to 2.5 without risk of objectionable enamel fluorosis.

Results from the children age 10 to 14 are compared with results from other European countries in Table 5. In the 11 children from Bucharest, the average excretion of 136 µgF/24h was significantly lower than the 282 µgF/24h in the 32 Timisoara children. Apparently, these children had ingested unusual amounts of fluoride during daytime as suggested by both their high median (0.650 ppm F) and average (0.621 ppm F) urinary fluoride concentrations; the corresponding nocturnal was 0.37 ppm F, similar to the 0.418 ppm and 0.372 ppm in the Bucharest children (Table 1, case 37 excluded). When related to body weight, excretions in the children 10 and older were lower than in the younger group.

On the basis of fluoride excretion per hour, µgF/h, Romanian children excreted similar or even lower amounts of fluoride than Swiss and French children using neither fluoridated water nor fluoridated salt. The two averages of 631 and 516 µgF from 24h are approximately twice as high as the Romanian data. Fluorosis in children of these Swiss regions is negligible (Steiner et al 1995, Marthaler 1995). [13,7] Nevertheless, it must be taken into account that the vast majority of children below school age use low-fluoride toothpastes (mostly with 500 ppm F) in Western Europe.

In the adults (median age 72) comparatively low fluoride excretion were met with in all evaluations. When their excretion was related to body weight, it was lower than all other results. This may be explained to

Table 6 Urinary fluoride excretion in micrograms by children aged 10-14 in Romania (definitive samples), Switzerland, France and WHO standards

	Morning µg/h	Afternoon µg/h	Night µg/h	µg/24 hours
Romania, present results				
Timisoara	(daytime : 15.0)		7.4	282
Bucharest	(daytime : 5.9)		5.7	136
Switzerland from Marthaler et al (1995)				
Geneva				
Prior to salt fluoridation, 1984	10.5	14.0	8.8	
Domestic salt with 250 ppmF, 1988	16.2	43.3	16.5	
Lausanne, F-salt for household and bread	25.3	47.8	24.6	
Glarus, F-salt for household and bread, **ì g/24h**				**516**
Binningen, water from Basel, 0.6-1.0 ppmF	23.5	33.3	19.0	
France (Strasbourg) from Obry-Musset et al (1992).				
Control group	7.3	20.0	12.9	**310**
1 mg fluoride tablets	28.3	23.6	27.0	**631**
WHO Provisional Standards*				
Low F intake	Low limit	12	8	220
	High limit	18	12	340
Optimal f usage	Low limit	30	19	600
	High limit	48	30	820

*Extracted from Table 5, Provisional standards for urinary fluoride excretion and concentration. Page 38 (WHO 1999)

lower intake of food in these relatively old people whose physical activity was minimal.

It is evident that in the two homes studied, both the intake and excretion of fluoride in Romanian children are among the lowest when compared to the available European data. Likewise, when compared to WHO provisional standards for urinary fluoride excretion (see Table 6), the Timisoara results were in the range corresponding to low fluoride intake; the excretion in the Bucharest children was even below the lower limit for children with low fluoride exposure. That means that there is room for increasing fluoride supply by so-called systemic fluoride for prevention of dental caries.

Water fluoridation has been used for 3 to 5 decades in several countries where central water supplies are available. Salt fluoridation has been used successfully as an alternative choice in several countries {Marthaler, 2001}[5]. Studies conducted in several countries have confirmed salt fluoridation as an efficient, safe and inexpensive method for prevention of dental caries. With fifty years of experience and current technology available salt fluoridation programmes can satisfy the needs of large or

small countries. At the 50th Anniversary Conference on Salt Fluoridation in 2005 in Zurich, Switzerland, 16 papers were presented on the state of salt fluoridation (all published in the Schweizer Monatsschrift für Zahnmedizin 2005 and April 2006).

Nationwide salt fluoridation could be easier to implement in Romania, thereby improving substantially the health of the teeth, a benefit that could reach all socioeconomic strata (Marthaler 2005)[9]. In accordance with other Western continental countries, widespread use of fluoridated salt would be suitable and is also by far the cheapest method .

Conclusions

Toothbrushing with dentifrice containing fluoride has also been recognized as main contributor for dental caries reduction, most obtained in some 20 affluent industrialized countries. This and other methods for topical application of fluoride can and should be used in children in combination in order to reduce dental caries levels substantially. The significant role of fluoride exposure in the crown completion phase of pre-eruption has been recently reconfirmed (Singh, et al 2003 and Singh/Spencer/Brennan 2007).[11,12] Through implementation of nationwide fluoridation of salt and promotion of the use of fluoridated dentifrices which are expected to be increasingly used by younger people, rapid improvements of dental health in Romania would be obtained. It remains to be demonstrated whether there are isolated water supplies or regions where the natural content of the drinking waters are high, for example above 0.5 or 0.7 ppm. In Switzerland overlap of domestic fluoridated salt, used by over 80% of the population, with water containing up to 0.7 ppm fluoride has not resulted in objectionable fluorosis levels. Cases with slight fluorosis, seldom visible to the general population, are the trade-off from using the indispensable fluoride for prevention of the destruction of teeth by caries. Fluorosis is purely cosmetic, very rarely unsightly and remains unchanged or even wears off after years whereas dental caries destroys the teeth progressively and can lead to dissemination of pathogenic bacteria through circulating blood.

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