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The fractional urinary fluoride excretion of adults consuming naturally and artificially fluoridated water and the influence of water hardness: A randomized trial.

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Aims To assess whether there was any significant difference in the average fractional urinary fluoride excretion (FUFE) values among adults consuming (NaF) fluoridated Ca-free water (reference water), naturally fluoridated hard water and an artificially (H_2SiF_6) fluoridated soft water. ***Design*** Sixty adult females (N=20 for each treatment) participated in this randomized, double-blind trial. The experimental design of this study provided an indirect estimation of the fluoride absorption in different types of water through the assessment of the fractional urinary fluoride excretion of volunteers. ***Results*** Average daily FUFE values (daily amount of fluoride excreted in urine/daily total fluoride intake) were not significantly different between the three treatments (Kruskal-Wallis; p = 0.62). The average 24-hour FUFE value (n=60) was 0.69; 95% C.I. 0.65-0.73. ***Conclusions*** The results of this study suggest that the absorption of fluoride is not affected by water hardness.

Key words: Fluoridated water, fluoride availability; fluoride urinary excretion; naturally and artificially fluoridated water.

Introduction

Fluoride in drinking water may be present naturally or added using one of several chemical compounds, usually hydrofluorosilicic acid or its sodium salt. The addition of fluoride to low-fluoride drinking water in order to increase its concentration to values in the range 0.6-1.0 mg F/l is carried out as a means of reducing the prevalence of dental caries in a population.

A recent systematic review on the health effects of fluoride, by the University of York Centre for Reviews and Dissemination (McDonagh *et al*., 2000) concluded that "the assessment of natural versus artificial water fluoridation effects is greatly limited due to the lack of studies making a comparison". A subsequent Medical Research Council Working Group established to determine what further research was required to improve knowledge on fluoride and health, identified absorption of fluoride from naturally fluoridated water versus artificially fluoridated water, and of hard versus soft water, as important areas of uncertainty (Medical Research Council, 2002). The latter documents also stated that "If the bioavailability of fluoride from artificially and naturally fluoridated water is the same, then studies of fluoride accumulation in people who have lived in naturally high fluoride areas could be informative" (Medical Research Council, 2002). Of the two issues – hard water versus soft water and naturally fluoridated versus artificially fluoridated water – the latter comparison is of less importance. This is because; at concentrations relevant to water fluoridation (0.5 to 1.0 mgF/L) complete dissociation of the fluoride compound occurs, as argued and discussed by Jackson *et al*. (2002).

The issue of water composition and fluoride metabolism has been studied intermittently over the last 50 years: for example McClure (1950), Zipkin and McClure (1951) and Zipkin *et al*. (1956). These authors showed that the absorption, accumulation in calcified tissues and caries prevention in animal experiments, and urinary fluoride excretion in human subjects, was similar for sodium fluoride and sodium fluosilicate, when given in physiological doses (up to 50ppm in animals and 1ppm in human subjects). Very recently, Maguire *et al*. (2005) reported on the fluoride pharmacokinetics and bioavailability of fluoride from naturally and artificially fluoridated drinking waters with different degrees of hardness. Their results suggested that any differences between waters for these variables are small. Second, Whitford and co-workers (2006) compared the pharmacokinetics of fluoride after ingestion of naturally fluoridated water, or water fluoridated with sodium fluoride or disodium fluorosilicate: they concluded (in a published abstract) that the chemical compound used to fluoridate water does not affect the pharmacokinetic parameters of fluoride.

The conclusions obtained in these recent studies (Maguire *et al*., 2005; Whitford *et al*., 2006) were based on a classical pharmacokinetic experimental design, i.e., measuring the area under the time-plasma-F-concentration curve (AUC) after the participating volunteers, who were young healthy adults in both studies, ingested a single dose of fluoride under fasting conditions. As pointed out by Maguire *et al*. (2005), there were relatively wide

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variations in AUCs for plasma F among the volunteers: these relatively high standard deviations make it more difficult to detect differences statistically.

An alternative method for comparing fluoride absorption from naturally and artificially fluoridated waters with different degrees of hardness could be to assess the fractional urinary fluoride excretion (FUFE) of young adult subjects under standardised fluoride intake conditions over 24-hour periods, during which different waters would be consumed. The results obtained might provide additional support to the initial conclusions previously obtained (Maguire *et al*., 2005; Whitford *et al*., 2006). Moreover, a recent publication (Villa *et al*., 2004) showed that the fractional urinary fluoride excretion of young adults under customary fluoride intake conditions in a community with 0.6 mg F/L in its drinking water had a relatively small variability around the average value. This would allow the detection of smaller differences in absorption between naturally or artificially fluoridated waters with different degrees of hardness. It can be assumed that under standardised, controlled fluoride ingestion conditions, coming essentially from the different types of water consumed by adult volunteers on separate occasions, any significant difference in the average FUFE values would reflect different proportions of absorbed fluoride. Thus, the study design could provide a reliable indirect estimation of the fluoride absorption in different types of water through the assessment of the fractional urinary fluoride excretion of adults.

This study aimed to assess whether there was any significant difference in the average fractional urinary fluoride excretion (FUFE) values in adults consuming (NaF) fluoridated Ca-free water (reference water), naturally fluoridated hard water and an artificially (H_2SiF_6) fluoridated soft water.

Subjects and methods

Subjects

The Ethics Committee for Human Research of the Institute of Nutrition and Food Technology (INTA), University of Chile, approved the study protocol. The nature and purposes of the study were explained verbally and in writing to the participating subjects, who signed an informed consent document. Volunteers received a financial reward for their collaboration. Participating subjects were permanent residents of Santiago, the Chilean capital city, located at 500-700 m above sea level. The volunteers were administrative employees and graduate students of INTA who have participated in previous clinical trials. Thus, their adherence to the established protocol was assured. Healthy female volunteers aged 20-40 years with no history of metabolic disease or acid-base disturbances, and who were not receiving a therapeutic diet, were recruited.

Experimental design

The study was a human experimental, parallel-group, randomised, double blind, clinical trial. Volunteers who met the inclusion criteria were assigned randomly to each of the three study groups. They (total n = 60; n = 20 for each treatment) were blind to the type of water they were going to drink during the 24-hour period of each study, as were the laboratory assistants and the project leader. The statistician participating in this study was in charge of the enrolment and random allocation, using random number tables, of volunteers to each treatment group, and group allocation was not disclosed until analyses were complete. Each separate study was carried out at the INTA facilities in September to December, 2005. The volunteers, 20 in each of the three groups, ingested the same standardised, low-F solid food diet during each study day and used non-fluoridated toothpaste during the test 24-hours. Thus, a high proportion of the 24-hour fluoride intake came from water.

The three different types of fluoridated water (approximately 80 litres for each study) were prepared in a separate institution: the Chilean Centre for Chemical Metrology (CMQ). Details of the origin, fluoride concentration adjustment, and fractionation and water-hardness assessment are described below.

The fluoride concentrations of the three different drinking waters were adjusted upwards to 0.8 mg F/L in order to match the customary daily total fluoride intake of the volunteers (Villa *et al*., 2004) since, in this study, they would consume solid food containing a low amount of fluoride and not use fluoride dentifrices during each study day.

Sample size

Using the average FUFE and standard deviation values found in young adults in a recent study carried out in our laboratory (Villa *et al*., 2004), we estimated the number of subjects in each of the three groups that would be necessary to identify a difference of 0.08 between groups with the lowest and highest FUFE values. The estimation was performed following Cohen's criteria for the size effect when using a one-way ANOVA analysis for three or more groups: twenty subjects in each group would be enough to identify the above-mentioned difference with $\alpha = 0.05$ and $1\text{-}\beta = 0.8$.

Food and beverage intake

Volunteers arrived early in the morning to the Institute of Nutrition and Food Technology (INTA) facilities, after an overnight fast. Each subject ingested a standard breakfast consisting of 200 ml of herbal tea prepared with double-distilled water and four standardized cookies. Lunch was skinless baked chicken breast with green salad, a small piece of white bread and an apple. At about 4 p.m., volunteers drank another 200 ml of herbal tea prepared with fluoride-free water and four standardized cookies. Volunteers received a standardised dinner prepared at INTA's cafeteria, and were instructed to eat it not later than 8 p.m. Their dinner included sliced ham, hard-boiled eggs, fresh green vegetables and a banana. In addition to herbal tea, the only liquid intake allowed during the study day was one of the three different F-adjusted drinking waters prepared as described below. Handling and storage procedures of food samples were reported previously (Villa *et al*., 2004). Each volunteer was provided with a plastic 2 L bottle freshly filled with the different types of water prepared when they arrived at INTA early in the morning, and another one when they went back home. Volunteers were carefully instructed to drink water only from the bottle provided

and to avoid drinking water or other beverage, from any other source. These bottles were returned and the quantity consumed was measured for each participant. Each participant consumed approximately 1.2 litres of water during the diurnal period (8 a.m. - 6 p.m.) and approximately 0.8 litres of water during the nocturnal period. Volunteers performed their usual activities during the study days.

Urine collection

Volunteers recorded the time when their first urine of the study day was passed, but did not collect it. Just before leaving INTA (at approximately 6 p.m.), they returned the plastic 2 L flask (F-1) containing all the urine passed until that moment. They received another plastic flask (F-2) to take home, where they continued collecting their urine, this time including the first one passed the following morning, recording the time at which this last sample was collected. Flasks for urine collection contained thymol crystals. Volunteers used a non-fluoridated toothpaste which was supplied for use, during the 24 hours.

Drinking waters used by the three groups

The chemical characteristics of the three different fluoridated waters used in this study were the following:

Group 1 consumed a 'reference water' prepared using double-distilled water and a NaF solution certified reference material (NIST SRM3183; lot 991510; 1,000 mg/L). This was calcium-free water with a hardness value practically equal to zero. Its fluoride certified concentration and (95% expanded uncertainty) were 0.804 (0.005) mg F/L.

Group 2: Naturally fluoridated water (originally containing 0.58 mg F/L) was refrigerated and transported from Ovalle (Chilean Fourth Region) and served as the matrix for the adjustment, with NaF, to 0.8 mg/L. The calculated volume of the certified NaF (NIST SRM3183; lot 991510; 1,000 mg/L) was added to eighty litres of the former water and homogenised by overnight continuous circulation with a peristaltic pump. The certified fluoride concentration and (95% expanded uncertainty) were 0.801 (0.005) mg F/L. The average (n = 5) calcium and magnesium concentrations were 151 and 32.9 mg/L, respectively. The concentrations of Calcium amd Magnesium in the drinking waters used in this study were determined by Flame (Air –Acetylene) Atomic Absorption Spectrometry following standard rechniques (Greenberg *et al*., 1992a), using a Perkin-Elmer AAnalyst 100 instrument. Thus, the water hardness expressed as equivalent milligrams of $CaCO_3$/L (Greenberg *et al*., 1992b) was 513 mg/L, a value that is considered to define very hard water. The fluoride concentration in the waters consumed by Groups 1 and 2 was adjusted upwards with NaF, rather than with calcium fluoride for example, as NaF is readily obtained and dissociates easily.

Group 3 consumed artificially fluoridated water. This originally contained 0.63 mg F/L and was refrigerated and transported from Talca (Chilean Seventh Region) and served as the matrix for the (H_2SiF_6) adjustment to 0.8 mg/L. After measuring its original fluoride concentration, a calculated volume of a 23% H_2SiF_6 solution - which is the fluoridating compound usually employed for artificially water fluoridation in Chile - was added to eighty litres of the original drinking water. The certified fluoride concentration and (95% expanded uncertainty) was 0.798 (0.006) mg F/L. The average (n = 5) calcium and magnesium concentrations were 24.9 and 4.1 mg/L, respectively. Thus, the water hardness expressed as equivalent milligrams of $CaCO_3$/L was 78.9 mg/L, a value which is considered to define a moderate soft water. The fluoride compound used to adjust upward the concentration of fluoride was the same as used in the water fluoridation process.

After distribution of the three types of water into 2 litre plastic flasks, homogeneity tests were performed following internationally accepted procedures (Taylor, 1993). In all cases, the fluoride concentration of the individual 2-litre flasks was considered to be homogeneous.

Chemical analyses

Beverages

Fluoride concentrations and hardness values of the three types of water that were used in this study were determined at the Chilean Centre of Chemical Metrology as described previously. The (negligible) fluoride concentration of herbal tea samples was checked at our laboratory, using a previously described, blank-corrected technique for low fluoride concentration samples (Villa, 1988).

Urinary assessments

The urine volume and pH of each specimen were measured within minutes of receiving flasks 1 and 2 from each subject. The assessment of the pH value for each urine specimen was carried out with a portable ISE/pH meter Model 290 A and a Model 91-55 pH electrode, both from ORION Research Inc. (Beverly, MA, USA). The F concentrations of urinary samples from each of the subjects were determined in duplicate by a blank-corrected, direct calibration technique (Villa, 1988) using a combination fluoride-ion-selective electrode (Orion, model 9609, Beverly, MA, USA) connected to an Orion model 940 digital pH/mV meter. This latter procedure has a reproducibility coefficient of variation of 3-4%.

Measurement of fluoride in foods

On each study day, five duplicate-plate samples of the study days' diet were separately taken and their F concentrations were assessed by means of the hexamethyldisiloxane micro diffusion method (Whitford, 1996). The different foods consumed over the study day were pooled and a single homogenate with double distilled water was prepared (1:1 mass ratio). About one gram of the homogenate was diffused overnight at room temperature in sealed Petri dishes with 2 mL of 4 N $HClO_4$ solution saturated with hexadimethyldisiloxane. Petri dishes were covered with lids prepared with 50 microliters (in about 5 drops) of 0.5 N NaOH . After the sample was diffused, the lids were dried in a dessicator. The dried layer was dissolved with 50 microliters of 0.5 N HCl and 1 mL of a solution (pH 5.2) containing deionized water and TISAB III (10:1 v/v). Fluoride concentration was measured with a combination fluoride electrode using a (blank corrected) calibration curve separately prepared

(Villa, 1988). The average fluoride concentration of the five replicates (on each occasion) was used as a constant value in order to assess the total fluoride ingestion of each volunteer. However, the amount of fluoride ingested from water was individually determined. The amount of fluoride excreted with the urine was individually measured. The coefficient of variation of the average value of weight of food was 10.5%, while that corresponding to the average fluoride content of the 5 plates was ≤ 5.1% in the three study days.

Calculations

The total volume of water ingested during each period was determined by subtracting the volume left in the bottle from the initial volume, measured individually. The total fluoride intake from liquids for each subject was calculated by multiplying the corresponding volumes of water and herbal tea ingested by their fluoride concentrations. The mean fluoride intake from foods was calculated by multiplying the average fluoride concentration of each of the five homogenates (mg F/kg of food) by the weight (kg) of food (5 replicates) eaten. For each individual the total 24-hour fluoride intake comes from the addition of the average value for food intake and the individually measured amount of fluoride from beverages.

Urinary fluoride excretion was calculated by multiplying urine volumes by the corresponding fluoride concentrations. The rate of urinary F excretion was calculated by dividing the amount of fluoride excreted over the (approximately) 24-hour period by the corresponding individual period of collection. Individual fractional urinary F excretion (FUFE) was calculated dividing the amount of fluoride excreted during the individually adjusted 24-hour period by the amount of fluoride ingested in that period.

Statistics

Data were analysed with an IBM-compatible computer using standard descriptive statistics (Excel 97 and Analyse-it™ for Microsoft Excel). One-way ANOVA, and Kruskall-Wallis tests were conducted with the STATA 8.2 version software. A retrospective power calculation was made using Cohen's criteria for the size effect using the results obtained in this study.

Results

Fluoride concentrations of herbal teas prepared with double-distilled water were less than 0.02 mg F/L. Thus, the contribution of this fluoride source to the 24-hour fluoride intake was almost negligible.

The average fluoride concentrations of the drinking water used in the three treatments were not significantly different (ANOVA; $p = 0.31$): These average values were 0.804; 0.801 and 0.798 mg F/L, for groups 1, 2 and 3, respectively. Thus, the calculations of ingested fluoride from drinking water for each of the three test days were made using 0.801 as the average value.

The average values, standard deviations and 95% confidence limits for age, body mass index and urinary pH according to the three treatment groups are shown in Table 1. The average anthropometric values as well as the urinary pH values for the three groups were not significantly different (one-way ANOVA, $p > 0.05$, for all comparisons).

Table 2 presents the 24-hour averages, standard deviations and 95% Confidence Intervals, for fluoride intake, fluoride excretion, rate of fluoride excretion, and fractional urinary fluoride excretion (FUFE) according to the three treatment groups. There were no significant differences in the average 24-hour fluoride intake from water, solid foods and total intake (ANOVA; $p > 0.67$, for all comparisons) between the three groups. Neither the amount nor the rate of fluoride excretion was significantly different between the three groups (Kruskal-Wallis; $p > 0.14$, for all comparisons). The 24-hour average values for the fractional urinary fluoride excretion were not significantly different between the three treatments (Kruskal-Wallis; $p = 0.62$).

Discussion

The current study only included a naturally fluoridated hard water study group and an artificially fluoridated soft water group. This was because: (a) the natural versus artificial fluoride issue is of less relevance in view of the publication of Jackson *et al*. (2002) showing dissociation of fluoride compounds at concentrations relevant to water fluoridation; (b) under Chilean geochemical conditions it was not possible to find communities with either naturally fluoridated soft water or artificially fluoridated hard water; and (c) the two groups included were considered to be the 'extreme groups' and the two excluded groups (naturally fluoridated soft water and artificially fluoridated hard water) would be likely to fall in intermediate positions relative to the other two groups.

Results obtained for the 24-hour average FUFE values measured in female volunteers ingesting either a calcium-free (NaF) fluoridated water, or a very hard naturally fluoridated water, or a moderately soft artificially (H_2SiF_6) fluoridated water, indicate that there are no significant differences between them in the parameters under discussion. Under the experimental conditions of this study, it can be assumed that any significant difference in the volunteers' 24-hour FUFE values between the three types of water would have been caused by a different degree of fluoride absorption. The latter assumption is supported by the following arguments:

1. The volunteers' gender was the same, their daily work, their age range and body mass indices were similar across the three groups under study. Food intake and toothpaste were the same for the three groups, and assignment of volunteers to groups was randomised. Under the present experimental conditions, it is improbable that there were differences between groups in physical activity or in the metabolism of fluoride between its absorption in the gut and excretion in the kidney.
2. A very high proportion (approximately 90%) of the 24-hour total fluoride intake came from the three different types of water under study. In addition, fluoride intake from solid foods was essentially the same for all of the volunteers. Thus, slight differences in fluoride absorption from foods on an individual basis, would be compensated on average terms.
3. The volunteers in the three groups had a fluoride total daily intake (1.5-2.2 mg F/day) that was similar to

Table 1. Age, body mass index and average urinary pH according to the three treatment groups

	*Group 1**	*Group 2**	*Group 3**
n	20	20	20
Age (average) y	29.0	26.5	29.3
Standard deviation	5.4	3.8	4.8
95% Confidence Int.	26.7-31.4	24.8-28.2	27.2-31.4
BMI♦ (average)	23.6	22.2	23.6
Standard deviation	2.4	2.3	2.7
95% Confidence Int.	22.5-24.7	21.2-23.2	22.4-24.8
Average 24-hour urinary pH	6.06	6.11	6.14
Standard deviation	0.26	0.39	0.29
95% Confidence Int.	5.95-6.17	5.94-6.28	6.01-6.27

* Treatments corresponding to the different groups are described in the text

♦ Body mass index (body weight (kg)/ (height, [metres])^2) Data for age, BMI and average 24-hour urinary pH are not significantly different ($p > 0.05$) as determined by one-way ANOVA

Table 2. Twenty-four-hour average, standard deviation (SD) and 95% Confidence Interval values [C.I.] for F-intake, F-excretion, rate of F-excretion, and fractional urinary fluoride excretion (FUFE) according to the three treatment groups

	F-intake			*Urinary F-excretion*		*FUFE*
	Water	*Food*	*Total*	*Amount*	*Rate*	
	(mg/24h)	*(mg/24h)*	*(mg/24h)*	*(mg/24h)*	*(µg/h)*	
*Group 1**	1.67	0.201	1.87	1.27	52.1	0.693
	(0.76)	(0.022)	(0.76)	(0.47)	(20.0)	(0.104)
			[1.54-2.20]	[1.06-1.48]	[43.3-72.1]	[0.647-0.734]
*Group 2**	1.51	0.216	1.73	1.15	45.5	0.666
	(0.63)	(0.018)	(0.63)	(0.41)	(16.0)	(0.161)
			[1.45-2.01]	[0.98-1.32]	[38.5-52.5]	[0.598-0.734]
*Group 3**	1.66	0.191	1.85	1.26	50.4	0.702
	(0.42)	(0.015)	(0.42)	(0.23)	(9.6)	(0.190)
			[1.66-2.03]	[1.16-1.36]	[46.2-54.6]	[0.612-0.780]

* Treatments corresponding to the different groups are described in the text

Data given in the columns are not significantly different ($p > 0.05$) as determined either by one-way ANOVA or Kruskal-Wallis test.

their customary total daily fluoride intake (Villa et. al., 2004) since, although their fluoride intake from solid foods and non-fluoridated toothpaste were lower than in the previous study, this was compensated by the slight upwards adjustment of the fluoride concentration in the three types of drinking waters. Thus, a 24-hour study as the present one neither increases nor decreases their usual total fluoride intake.

4. The 24-hour average urinary pH values for the three groups were not significantly different. Since it is well known that urinary pH affects the fractional urinary excretion of fluoride (Whitford, 1996), the similarity in pH also minimises this possible source of bias.

In the light of the above-mentioned facts it seems reasonable to consider that the outcome variable (FUFE) is adequate for identifying differences in fluoride absorption from very hard naturally fluoridated water and moderately soft artificially fluoridated water.

Some caution is necessary when examining the data on the pH of urine since it is well known that pH changes during storage (Whitford, 1996). The choice of female only volunteers was made to reduce variation between subjects (*vide supra*) strengthening the power of the study, but this should be remembered when extrapolating the findings. Nevertheless, there is no reason to suppose that the observed effect would be different in male subjects. The standardised meals were made as attractive as possible so that all the food was consumed by all subjects, thus minimising variation in food intake between subjects.

As can be seen in the last column of Table 2, average FUFE values for the reference water and the naturally and artificially fluoridated waters are not significantly different. In absolute terms, the FUFE for Group 2 (hard water) was 5% less than the FUFE for Group 3 (soft water). This allows formulating a preliminary conclusion: fluoride absorption (and retention) from naturally fluoridated hard and artificially fluoridated soft water is not significantly different. Thus, within the power of this study, the argument that different proportions of fluoride are absorbed from water depending on its hardness and its "natural" or "artificial" fluoridation status that has been traditionally advocated by fluoridation opponents appears to be incorrect.

From the results obtained in the current study (Table 2), a retrospective statistical power calculation was made. The minimum difference in FUFE that the present study was capable of detecting with 20 subjects in each group, a pooled standard deviation of 0.16 and $\alpha = 0.05$ and $1 - \beta = 0.8$, is estimated as 0.14.

A difference of 0.14 from the "reference" average FUFE value of 0.693 would mean a difference of approximately 15% in fluoride absorption. Thus, if there was a real difference in fluoride absorption between hard, naturally fluoridated water and soft, artificially fluoridated water lower than 15%, the present experimental design would have not been able to detect it. However, when observing the rather high variability (standard deviations) associated with the average FUFE values in Table 2, which amounts to relative standard deviations in the range of 15-27%, it can be concluded that this variability, caused by inter-individual differences within the same study group is substantially higher than a "true" difference in fluoride absorption. Then, on a community basis, it can be stated that from a practical point of view there is no significant difference in fluoride absorption from both types of water.

The latter conclusion is coincident with the conclusions obtained very recently by Maguire *et al*. (2006) who studied fluoride availability from different types of artificially and naturally fluoridated waters by means of pharmacokinetics methods.

The FUFE values recorded in the study are worthy of comment. Taking into account that the 24-hour average FUFE values (Table 2, last column) were not significantly different, pooling the 60 individual results would provide a reliable estimation of the average FUFE value for females aged 20-40 years. This average 24-hour FUFE value (S.D.) of 0.69 (0.15) and 95% confidence interval 0.65-0.73 is remarkably similar to the one previously obtained (average 0.70; S.D. 0.10) by Villa *et al*. (2004) for young male and female adults under stable fluoride intake conditions. Accepting, as it is usually done, that approximately 10% of fluoride intake is not absorbed (Ekstrand, 1996), a simple arithmetical calculation yields that the proportion of the absorbed fluoride that is excreted in the urine on a **24-hour** basis is approximately 0.70/0.9 = 0.78, under customary fluoride intake conditions for subjects living in an area with 0.6 mg F/L in their drinking water. Then, the proportion of ingested fluoride that is retained daily under these conditions would be approximately 22%. This value, which is based on the current and recent experimental findings and on the reasonable assumption of 90% absorption, is substantially lower than the usually mentioned value of approximately 50% (Whitford, 1996). This preliminary conclusion needs further experimental support as it might have relevant implications when the problem of fluoride accumulation in bone is considered. This is why the phrase "24-hour basis" was previously highlighted: twenty-four hours is the natural time unit that repeats itself along the whole life.

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