Urinary Fluoride Excretion after Milk and Tea Consumption in Young Adults

Melinda Székely[1], Zita Fazakas[2], Victor Balogh-Sămărghiţan[2], Jolán Bánóczy[3]

[1] Ph.D., D.M.D. Professor, Department of Morphology of Teeth and Dental Arches; Technology of Dental Prosthesis and Dental Materials, Faculty of Dental Medicine, University of Medicine and Pharmacy, Târgu-Mureş, Romania. [2] Ph.D. Associate Professor, Department of Biochemistry, University of Medicine and Pharmacy, Târgu-Mureş, Romania. [3] Ph.D., M.D. D.M.D., D.Sc. Professor Emeritus, Department of Oral Biology, Semmelweis University, Budapest, Hungary.

Abstract

Aims: The aim of this study was to assess urinary fluoride (F) excretion under customary diet and controlled F intake conditions in young adults, and also to compare the bioavailability of F administered systematically in milk, together with, or without, tea consumption. ***Methods:*** The study comprised 36 subjects aged 19-23 years living in the city of Targu-Mures, Romania, where tea drinking is part of the daily diet and the F content of the tap water is low. The fluoride was administered in milk, together with, or without, tea consumption. The study was divided into three phases, each of two weeks during which the subjects drank daily: first phase, 200 ml tea (2.11 mg F/l); second phase, 200 ml tea and 200 ml fluoridated milk (5 mg F/l); third phase, 200 ml fluoridated milk (5 mg F/l). Urine sampling was conducted every 24 hours and analysed for fluoride using a fluoride electrode. The apparent bioavailability of F from the fluoridated milk, administered together with or without tea consumption, was calculated from the 24-hour urinary fluoride excretion data. A statistical analysis was performed using ANOVA and Student's *t*-tests. ***Results:*** The mean values (±SD) of the daily urinary fluoride excretion were: at baseline, 0.297±0.101 mg F; after the first phase, 0.451±0.111 mg F; after the second phase, 0.757±0.162 mg F; and after the third phase, 0.603±0.168 mg F, respectively ($P<0.0001$). Compared with the F bioavailability from tea, the bioavailability from fluoridated milk consumed with tea was 90.38% and from fluoridated milk was 86.84%, respectively. ***Conclusions:*** Fluoride administered in milk, together with tea consumption, resulted in higher urinary fluoride excretion but only a slight decrease in F bioavailability. These findings appear to indicate that milk is an appropriate vehicle for F supplementation on a community basis in regular tea-drinking young adults.

Key Words: Fluoride; Diet, Tea, Fluoridated Milk, Urinary Fluoride Excretion

Introduction

Dental caries remains a major public health problem in most high-income countries, affecting 60-90% of school children and the vast majority of adults [1,2]. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have emphasised the importance of effective prevention of dental caries through diet and administration of fluoride as part of public health programmes [3 4]. Public health approaches to the use of fluorides in caries prevention have used vehicles such as drinking water, salt [5,6], and milk [7-9].

Before introducing any systemic fluoridation, it is necessary to estimate the total daily fluoride exposure of subjects under their customary conditions of fluoride intake. Urinary excretion studies have compared fluoride excretion after fluoridated salt [5] and milk consumption [10,7] by children living in different areas and exposed to different fluoride regimens [11-13]. Few studies have investigated the bioavailability of systematically administered fluorides in adults. There is a general lack of data from adults [6,14] because most studies have been performed in children [9].

The tea plant is known as a fluorine accumulator, a natural source of F in diet [15]. Reports suggested that tea consumption leads to a reduction in dental caries, with the effect being attributed to its F content [16,17]. Tea in the diet can provide an

Corresponding author: Melinda Székely, DMD, PhD, Professor Faculty of Dental Medicine University of Medicine and Pharmacy Târgu-Mureş, Targu-Mures, Str. Gh. Marinescu nr. 38, Romania 540139; e-mail: mszekely_2000@yahoo.com

effective vehicle for F delivery to the oral cavity where it may then become associated with the oral tissues and their surface integuments [17].

However, no studies were found in the available literature comparing the biological effects of systematically administered fluorides using milk as vehicle on adults drinking tea on a daily basis. It was therefore decided to assess the urinary fluoride excretion and also to compare fluoride bioavailability in young adults following tea consumption and administration of fluoridated milk in order to assess the effects of their simultaneous intake.

Aims

- To determine the average 24-hour urinary fluoride excretion of the subjects to answer the question: Is the fluoride intake high under normal day-to-day conditions because of the regular tea consumption by young adults in Targu-Mures, Romania?
- To compare the bioavailability of fluoride administered in milk, together with, or without, tea consumption, by monitoring urinary fluoride excretion in subjects living in the same area.

Methods

Subjects

Thirty-six young adults, aged 19 to 23-years (19 males and 17 females), were included in the urinary fluoride excretion study. The participants were healthy volunteers selected from the dental students of the University of Medicine and Pharmacy, Targu-Mures, Romania, excluding those who received any treatment by local fluoridation. After they had been given verbal and written explanations of the study protocol, formal positive consent was obtained from every participant. The study was approved by the university's ethics committee.

Methodology

The study was based on time-controlled urine collection over 24 hours [18]. Fluoride concentration of the urine samples was determined using a fluoride ion-selective electrode after addition of TISAB (total ionic strength adjustment buffer) by a direct method [19]. The F analyses were performed with a Model 9609 BN fluoride electrode and a Model 720A fluoride meter (ORION Research Inc., Beverly, MA, USA). Details of each individual subject were recorded as described in a previously reported study performed in pre-school children [20].

The 24-hour urinary fluoride excretion (mg) was calculated as the total volume of the 24-hour sample (l) multiplied by its fluoride concentration (mg/l). Twenty-four hour urinary fluoride excretion per kg body weight was also calculated. Individual fractional urinary fluoride excretion (FUFE) was calculated by dividing the amount of F excreted during the 24-hour period by the amount of fluoride ingested in that period [11]. All calculations of the F bioavailability were performed using net F concentration, that is, urinary F levels determined at the baseline were subtracted from the F data obtained after ingestion of F [21,22]. The apparent bioavailability of F from the fluoridated milk, administered together with (1), or without tea (2), consumption, was calculated from the F recovered in the urine. The calculation of bioavailability (B) from values of urinary F was [21,22]:

$$B_{\text{F milk+tea}}\ \% = \frac{U_{\text{F milk+tea}} \times D_{\text{tea}} \times 100}{U_{\text{tea}} \times D_{\text{F milk+tea}}} \qquad (1)$$

$$B_{\text{F milk}}\ \% = \frac{U_{\text{F milk}} \times D_{\text{tea}} \times 100}{U_{\text{tea}} \times D_{\text{F milk}}} \qquad (2)$$

where U is the net amount of F excreted in the urine during 24 hours, D is the quantity of F in the tested items or in the tea reference, respectively. A 100% bioavailability of F administered in tea as an aqueous solution was assumed.

Plan of investigation

Baseline: The subjects were provided with plastic two-litre flasks, with added thymol crystals as a preservative, to use for the collection of urine samples. The baseline data were collected for one 24-hour period, one week prior to the test period to measure urinary fluoride excretion. Subjects continued with their usual diet, and to brush their teeth with fluoride toothpaste.

Phase I: The subjects were provided with 2.109 ppm F-containing Yellow-Label Tea (Lipton, London), commercially available in Targu-Mures, and Spearmint (Tri Leaf, China) non-fluoride toothpaste to be used during the study period. They were instructed to avoid the consumption of any fluoride-containing food and drinks, and also to avoid any treatment with local fluoridation during the study period. The students drank 200 ml tea daily on regular basis, and after two weeks the first urine sampling of the test period was conducted.

Phase II: For two weeks, the participants of this study drank daily 200 ml tea and also 200 ml of fluoridated milk containing 1 mg fluoride. After two weeks, the second urine sampling of the test period during 24-hours was conducted.

Phase III: The third urine sampling of the test period was conducted after two weeks, during which the students drank 200 ml fluoridated milk per day on regular basis, but abstained from drinking tea.

Data management

From the data obtained, in each period of sample collection, the urinary flow rate (ml/h) and the fluoride excretion rate (μg/h) were calculated. From data for all periods, the following parameters were assessed:

1. Integral daily urinary fluoride excretion (mg).
2. Daily fluoride intake (mg).
3. Daily fluoride dose (mg/kg body weight/day).
4. Fractional urinary fluoride excretion (FUFE).
5. Estimated fluoride bioavailability (%).

The data collected in the different phases of the study were compared in order to assess the bioavailability of fluoride administered systematically through natural sources such as tea drinking and fluoride supplementation by fluoridated milk consumption. Statistical analyses were performed with the GraphPad InStat computer program. ANOVA analysis of variance and Student's *t*-tests were used as statistical evaluation methods to compare the results obtained in the different sessions of the study. Results were presented as mean ± standard deviation and a value of $P<0.05$ was regarded as statistically significant.

Results

The subjects' mean age was 20.6±2.8 years and their mean body weight (bw) was 64.6±9.7 kg.

Table 1 presents the mean values and SD obtained for the daily urinary F excretion (mg F/day) and daily urinary fluoride excretion dose (mg F/kg bw/24h) obtained at baseline, after tea, tea with milk, and milk consumption ($P<0.0001$ and $P<0.0001$, respectively).

Table 2 presents the summary of FUFE (fractional urinary fluoride excretion) values obtained in this study ($P<0.0001$).

Fluoride bioavailability from the fluoridated milk was 86.84% and when administered with tea it was 90.38%, assuming that the F bioavailability of tea was 100%.

Table 2. *Summary of FUFE Values Obtained From 36 Young Adults After Tea, Tea With Milk and Milk Consumption*

Parameters	Tea	Tea and milk	Milk
No. of samples	36	36	36
Mean	1.072	0.547	0.626
SD	0.264	0.117	0.174
Median	1.092	0.553	0.613
Minimum	0.507	0.293	0.316
Maximum	1.482	0.849	1.004

Discussion

A regular supply of fluoride is the most important measure for caries prevention in children and adults to strengthen the resistance of dental hard tissues [3,23]. It is generally accepted that F absorption is relatively high, accounting for about 90% of total F intake. From studies on fluoride metabolism, it is now well established that approximately 10-20% of daily fluoride intake is not absorbed. Of the fluoride that is ingested, about 50-70% is excreted via the urine during the following 24 hours in young and middle-aged adults, and almost all of the remainder will become associated with calcified tissues [24].

Over 20 years ago, it was suggested that the aim of regimens for systemic fluoride supplementation (salt fluoridation) should be to adjust the dose

Table 1. *Summary of Daily F Excretion (mgF/24h) and Daily F Excretion (mgF/kg bw/24h) Values Obtained From 36 Young Adults at Baseline, After Tea, Tea With Milk and Milk Consumption*

Study periods	Daily fluoride excretion (mgF/24h)		Daily fluoride excretion (mgF/kg bw/24h)	
	Mean	SD	Mean	SD
Baseline	0.297	0.101	0.0046	0.0016
Tea	0.451	0.111	0.0077	0.0022
Tea and milk	0.757	0.162	0.0119	0.0027
Milk	0.603	0.168	0.0095	0.0029

so that urinary fluoride excretion would match that observed in successful water fluoridation schemes [25]. This rule of thumb could be applicable to milk fluoridation, assuming that the mode of action of F in water and milk is similar. Subsequently, urinary fluoride excretion has been used to estimate F intake for the purpose of setting the correct dose of F in milk. There is a need for quantitative data concerning the bioavailability of F from foods and drinks. Such information is of considerable importance when discussing dosage regimens for F supplementation given in areas with low-fluoride drinking water [9,21].

This study was undertaken in order to assess the daily fluoride excretion from which the amount of ingested fluoride may be evaluated and also to estimate the fluoride bioavailability regarding the fluoridated milk and tea consumption in young adults living in the city of Targu-Mures, Romania, where the F content of the tap water is low, 0.12 ppm F [26]. The study, based on the time-controlled urine sampling during 24 hours [18], was carried out in the University of Medicine and Pharmacy, Targu-Mures. The subjects were asked to consume 1 mg fluoride per day, according to the WHO recommendation, in 200 ml milk (5 mg F/l) as an integrated part of their daily diet. The young adults continued to receive their usual diet because the present study aimed to determine the urinary fluoride excretion under their usual conditions of fluoride intake, i.e. usual diet, which included tea and mineral water drinking. The participants were drinking daily 200 ml black tea (2.11 mg F/l), according to the research protocol.

Prior to the commencement of this study, the fluoride content of tea infusions prepared from the most frequently consumed 30 tea products, purchased in Targu-Mures, was measured. There was a significant difference in the F concentration of the filtered teas ($P<0.0001$). The mean value (±SD) of the traditional teas was 2.19±0.98 ppm F and it was concluded that the consumption of some conventional filtered teas on a regular basis could have dental caries preventive effect [27].

Considering that the urine is the main excretion route for ingested fluoride, urinary fluoride excretion was used to assess the fluoride bioavailability [28]. The term bioavailability has been defined as the rate and extent to which fluoride is absorbed from a product and reaches the systemic circulation [29]. F bioavailability has been determined by measuring the area under the plasma F-concentration-versus-time curve and by measuring urinary F excretion [22]. Because the bioavailability of sodium fluoride (NaF) in humans is 100% when ingested in water or tablets [30], it is generally used as a standard for assessing relative F bioavailability from foods and other products. The results of analyses of urinary F excretion relating to bioavailability largely agree with conclusions drawn from plasma fluoride profiles [31].

In this study the bioavailability of F from tea and fluoridated milk was estimated using data obtained through urinary F analyses. The mean values of the daily urinary F excretion (mg F/day) obtained at baseline, after tea, tea with milk, and milk consumption showed significant differences. Daily urinary F excretion data revealed that the F intake of the investigated young adults was under the recommended optimal value. The mean baseline value of daily urinary F excretion was 0.297±0.101 mg F/24h, which is a consistent value with the lower limit for a low F-intake of standards published in the WHO guidelines [18]. However, urinary F excretion after regular F-rich (2.109 ppm) tea consumption was only at the upper limit for a low F-intake community. The same occurred for the evaluation of the daily F dose. The data for the daily urinary fluoride excretion dose (mg F/kg bw/24h) obtained at baseline, after tea, tea with milk, and milk consumption showed statistically significant differences for their mean values and it could be seen that fluoridated milk consumption provided the optimal recommended F dose 0.0095 mg F/kg bw/24h.

The bioavailability of fluoride from cow's milk has been investigated, both in animal and human experiments, which also sought to clarify the systemic or topical mode of action of fluoridated milk [32]. Although one study concluded that milk was not a suitable carrier for fluoride because of the strong interaction between the fluoride ion and milk components [33], a more recent study showed that availability of ionised fluoride is high (>80%) at low F concentration (5 ppm) [34]. Sodium fluoride can be used as a fluoride source in milk and the availability of ionised fluoride is high. A number of other studies support the previous observations [34] on the ionic fluoride availability in fluoridated milk containing 5 mg/l fluoride [26,35,36]. Several reports concluded that fluoride added to milk, despite low ionisation, becomes biologically available following its ingestion [31]. The present study showed that the ionisation levels in milk are not relevant to urinary fluoride excretion. Therefore, milk can be considered an appropriate and effective carrier means for fluoride intake of population, where water and other sources of F are inadequate [24].

The influence of gastric contents on the bioavailability of F from milk was first investigated in 1974 [37]. The results indicated that none, or very little, of the F ion binds to food constituents. Several other studies, including investigations of plasma fluoride levels and urine analyses, have reported that the absorption of F given with milk is reduced by 13-30% depending largely on the gastric content, its acidity and sequence of consumption of milk, fluorides and other foods [38,39].

In a study of the bioavailability of F in water, with milk and with milk plus breakfast, measuring plasma F profiles and urinary F excretion over nine hours, the mean values for plasma AUC were: F water 100.8%; F milk 73.8%; F milk and breakfast 53.7%. For urinary output, the mean values were: 102.2%, 62.2%, and 66.1%, respectively [30].

Comparing the bioavailability of fluoride added to water, milk and infant formula *in vivo* in fasting young adults [21], the following bioavailability results were reported: plasma data were 72% for milk, but only 65% for water-diluted baby formula, and 76% and 63% respectively for urinary F excretion data. Recoveries of F added to milk and formula *in vitro* were 98-100% and 87-91%, respectively, and the authors attribute the reduced bioavailability of F from milk to formation in the stomach of a physical barrier to absorption, as also suggested by Ericsson (1958) [40], in view of the lack of binding of F to milk in vitro. One study [39] showed that in fasting subjects, bioavailability of fluoride was reduced by simultaneous ingestion of solid food. Plasma F peaks after ingestion of NaF or disodium monofluorophosphate (NaMFP), in solution or as tablets, where reduced by milk (relative bioavailability in milk 70%); however, with milk and food, the relative bioavailability was 96-97% compared with F from water. A study of bioavailability in pre-school children revealed that the urinary F output from NaMFP in milk was more than twice that from NaF in water, but when the F was administered on a fasting stomach, no differences were found [32].

In this study, fluoride was added in vitro to milk as an NaF solution. In order to provide 1 ppm F from the daily consumption of 200 ml fluoridated milk, 5 ppm F was added to 1 litre of milk. Recovery of almost 100 % in milk was found because the milk used in this study contained 0.96 ppm F. This is in agreement with the results presented above [21] and indicates that none, or very little, of the F binds to the milk constituents. One hundred per cent bioavailability of F administered in tea (an aqueous solution) was assumed. The results of the current *in vivo* study suggested a slight decrease in F bioavailability for both fluoridated milk (87%) and fluoridated milk consumed with tea (90%). It might be argued that the comparison of the two types of F ingestion is not strictly valid because the amount of F intake is different. However, on a community basis, the current results appear to indicate that milk is an appropriate vehicle for F supplementation and it is not adversely affected by tea consumption.

Most human bioavailability studies have been carried out on fasting individuals. However, as concomitant intake of food may influence the absorption of F, the applicability of such data is questionable where substances are ingested more or less randomly in relation to food intake [29]. The bioavailability of F is best determined under conditions that simulate the normal mode of intake of the test products [22]. The present study was conducted to assess the bioavailability of F on community bases on subjects under customary diet and controlled fluoride intake conditions. Furthermore, our findings allow better evaluation of the contribution of fluoridated milk consumed with or without tea to the dietary F provision of humans. The possible high availability of F from Ca-rich products such as milk should be considered when recommending F supplements for caries prevention, at least to individuals who regularly ingest tea rich in F.

Data of pharmacokinetic studies showed that both the FUFE and fractional fluoride retention values were about 50% of the absorbed dose in young adults [41]. An approximate FUFE value (relative to absorbed F) of 0.5 has been generally accepted as a typical value for young adults. However, in a recent publication, it was shown that the average daily FUFE value for 19-30 years was 0.70 under customary fluoride intake conditions, in a community with 0.6 mg F/l in its drinking water [14]. In our study, the mean FUFE value for young adults under daily 0.42 mg F-intake conditions by tea consumption was 1.072±0.264, under daily 0.96 mg F-intake conditions by F-milk consumption was 0.626±0.174, and under daily 1.39 mg F-intake conditions by F-milk with tea consumption was

0.547±0.117. The differences for mean FUFE values were statistically significant. Examination of both the FUFE values and daily F intake, expressed as the F dose (mg F/kg body weight), have revealed that there is an inverse correlation of the two variables, i.e. FUFE values decrease as F doses increase. The findings of the current study were in agreement with the results of a previous study [14]. However, the data are not directly comparable with the above-mentioned results because of the different research methodologies that were employed.

Conclusions

In the study that has been described:

- The fluoride intake of young adults was below the optimal recommended level in Targu-Mures, Romania, where regular tea consumption and mineral water drinking are part of the usual diet, and fluoride toothpaste is used for tooth-brushing.
- A reliable assessment of 24-hour fluoride excretion from which the fluoride bioavailability may be evaluated was achieved.
- The comparison of bioavailability of fluoride administered systematically in milk, together with, or without, tea consumption, by monitoring urinary fluoride excretion in adult subjects living in the same area, indicated that fluoride bioavailability is high in both situations.
- Fluoride administered in milk, together with tea consumption, resulted in higher urinary fluoride excretion but only a slight decrease in F bioavailability.
- The fluoride bioavailability was not reduced by tea drinking; however, the slight increase might appear due to the increased F intake.

Future epidemiological surveillance studies based on fluoride excretion are recommended to confirm the results of the study regarding the bioavailability of fluoride administered in milk.

Acknowledgements

The study was funded through a grant by The Borrow Foundation and the authors are grateful for the generous support.

Conflict of interest

Professor J. Bánóczy is Chairman of The Borrow Foundation.

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