Fluoride Excretion in Whole Saliva and Urine After Intake of Fluoridated Milk With or Without Breakfast in Young Adults

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Abstract

Aims: The aim of this study was to compare salivary and urinary fluoride (F) excretion in young adults drinking fluoridated milk on its own, subsequent to breakfast, or simultaneously with food consumption. ***Methods:*** After informed consent had been obtained, 27 healthy adults of both sexes (aged 18-22 years) were investigated in a four-phase experiment, of which the first was the baseline phase. Each morning on a single occasion, the participants consumed either: (1) a standard breakfast; or (2) 200 ml fluoridated milk (5 mg F/l); or (3) standard breakfast and after two hours 200 ml fluoridated milk; or (4) 200 ml fluoridated milk during standard breakfast. The test period lasted four weeks and the sampling took place on the same day each week. Whole saliva was collected immediately after F intake (0) and after 15, 60, and 120 minutes. Urine was also collected over 24 hours. The fluoride content of both the saliva and urine was analysed using F selective electrode by the direct method. The statistical analysis was performed by analysis of variance (ANOVA) and Mann-Whitney U tests. ***Results:*** Significant differences could be observed between the salivary F concentrations measured at 0, 15, 60, and 120 minutes after F intake in all phases ($P<0.0001$). Fluoride ingestion by milk caused a significant increase in the amount of fluoride in saliva immediately after intake and also after 15 minutes compared to the baseline ($P<0.001$). The highest values (mean±SD) of the salivary F content (µgF/ml) were measured when fluoridated milk was consumed two hours after breakfast: 0.263±0.197 and 0.090±0.027, respectively. Daily urinary fluoride excretion (mean±SD, µgF/24h) showed significant differences between phases: (1) 345±101; (2) 454±148; (3) 757±263; and (4) 663±173 ($P<0.0001$). ***Conclusions:*** The results indicated that in the young adults who participated in the study, the fluoride content in the saliva and also the amount of fluoride excreted in the urine were the highest when drinking fluoridated milk two hours after breakfast. The findings suggest that fluoride availability is influenced by the timing of solid food intake.

Key Words: Fluoride, Breakfast, Fluoridated Milk, Salivary Fluoride Content, Urinary Fluoride Excretion

Introduction

The general concepts of the mechanism of the action of fluoride, both systemically and topically, are still discussed [1]; however, there is convincing evidence of their caries-protective effect [2]. Various types of vehicle for fluoride have been proposed, among these the caries-preventive effect of fluoridated milk has brought good clinical results in more than ten countries, and the basic mechanisms were cleared [3].

Human studies of fluoridated milk have confirmed fluoride's dual mode of action: topical (local) and systemic. The local effect seems to be due to direct and re-secreted fluoride amount in saliva that could maintain ionised fluoride at sufficient levels to inhibit demineralisation and promote remineralisation of enamel [4]. Sodium fluoride (NaF) and disodium monofluorophosphate (DMFP) can both be used as fluoride sources in milk, and the availability of ionised fluoride is high in this medium [3,5].

The influence of milk on fluoride absorption in

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humans has been studied by several authors. They found that significantly less fluoride was absorbed when the fluoride was administered simultaneously with milk or dairy products. Ekstrand and Ehrnebo (1979) observed a decrease in fluoride absorption when consumed with solid foods that were dairy products [6]. Trautner and Einwag (1987) studied the administration of sodium fluoride in the form of tablets or solutions given immediately following a standard breakfast [7]. They found a decrease in absorption, with peak blood fluoride levels similar to those reported by Trautner and Siebert (1986) [8]. However, Trautner and Einwag (1989) showed that although milk significantly decreased the absorption of fluoride, the addition of a standard breakfast nearly reversed this effect [9].

The literature on fluoride absorption, metabolism and excretion in relation to fluoridated milk shows that, in fasting subjects, the absorption of fluoride could be reduced by about 25-30% after a single, simultaneous intake of milk [5,10]. However, other studies have suggested that fluoride is completely available when fluoridated milk is ingested after a longer period of fasting [11,12].

Current results showed that when fluoride is administered in milk together with breakfast food, which is a typical situation under real-life conditions, its rate of absorption is not significantly different than that of F-water [13]. However, it is still uncertain how the bioavailability of fluoridated milk would be affected by solid food consumption under different timing conditions. This study therefore sought to compare urinary fluoride excretion and fluoride content in saliva in young adults, following administration of fluoridated milk, in order to assess whether there is an influence from simultaneous intake of food compared with consuming it at some interval after food consumption.

Aims

Against this background, the current study aimed:

1. To measure salivary and urinary fluoride excretion in order to investigate whether there is any difference when consuming fluoridated milk at the same time as food, compared with consuming it two hours after food intake.
2. To compare fluoride bioavailability in young adults when fluoridated milk is administered: (a) alone, on a fasting stomach; (b) two hours after breakfast food; (c) simultaneously with the intake of solid food.

Methods

Subjects

Twenty-seven subjects aged 18-22 years (10 male and 17 female) were included in the study. The participants were healthy volunteers selected from the first-year undergraduate students of the Faculty of Dental Medicine, University of Medicine and Pharmacy Targu-Mures, Romania, excluding those who had received any recent application of topical fluoride. All first-year students were invited to take part and those who volunteered and met the inclusion criteria were selected. 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.

Plan of investigation

The study, based on the investigation of salivary fluoride content [1,14,15] and urinary fluoride excretion [16,17] after the ingestion of fluoridated milk, was carried out in Targu-Mures, Romania, where the fluoride concentration of drinking water is low (<0.2 ppmF).

Salivary and urinary fluoride excretion were investigated at four different testing phases. The start of each phase was at the same time, 08.00 in the morning, one week apart (wash-out period).

Phase I. The baseline study was carried out after a two-week wash-out period during which the subjects avoided foods and drinks which are known to contain more than minimal concentrations of fluoride and used fluoride-free toothpaste to brush their teeth. The subjects fasted until 08.00 for 10 hours (overnight) before the day of the sample collection. Then they consumed a low-fluoride-containing standard breakfast consisting of croissant with chocolate cream and coffee (without milk) or peppermint tea. After the breakfast, whole saliva was collected immediately (0) and after 15, 60, and 120 minutes. Unstimulated saliva samples (minimum 1 ml) were collected by passive drooling into graduated plastic tubes for 10 minutes [1]. The salivary volumes and flow rates were registered (ml/min) and pH was measured. Saliva samples were refrigerated until the time when fluoride concentration could be determined. Urine was collected during the 24 hours following the standard breakfast, in order to assess the daily urinary fluoride excretion. The data recorded were considered to represent the baseline levels of fluoride. Every participant was his/her own control.

Phase II. At 08.00 on the first day of the test period, after a one-week wash-out and overnight fasting, the participants drank 200 ml fluoridated milk (1mg F) from a cup. The fluoride was added *in vitro* to milk as a NaF solution (5 mg F/l). Whole saliva was collected immediately after the consumption of fluoridated milk (0) and after 15, 60, and 120 minutes. Urine was collected over 24 hours, as in phase I.

Phase III. After a one-week wash-out period, on a fasting stomach, a standard solid breakfast was consumed at 08.00 and two hours later 200 ml fluoridated milk was drunk. Whole saliva was collected immediately after the consumption of fluoridated milk (0) and after 15, 60, and 120 minutes. No other food or drink was allowed until 14.00. Urine was collected over 24 hours following the fluoridated milk intake.

Phase IV. After a one-week wash-out period, the subjects were asked to consume a standard breakfast simultaneously with 200 ml fluoridated milk at 08.00, on a fasting stomach. Every bite was followed by a sip from a cup, as in usual breakfast consumption habits. Whole saliva was collected immediately after fluoride intake (0) and after 15, 60, and 120 minutes. Urine was collected over 24 hours.

Laboratory analysis

The volume (ml) of saliva and urine samples was measured immediately. The whole saliva samples were centrifuged for 15 min at 6000 rpm using a Model EBA 20 centrifuge. Then the supernatant of every saliva sample was transferred to a small plastic tube and a TISAB II (total ionic strength adjustment buffer) was added for fluoride analysis. The concentration of fluoride in the saliva samples was determined using a Model 720A fluoride meter with a Model 9609 BN fluoride electrode (ORION Research Inc., Beverly, MA, USA) by the direct method. The fluoride analysis of urinary samples was also conducted with an ion-specific electrode using the direct method. All determinations were performed in duplicate. The detailed methodology of fluoride analysis has been described in a previously reported study [17].

The salivary fluoride content and urinary fluoride excretion data collected in the different time periods of the study were compared in order to assess the changes in dependence of time, and further the data of the different phases with each other, to assess the differences between groups.

Statistical analyses were performed with the GraphPad Prism program for Windows (GraphPad Software, La Jolla CA, USA). Analysis of variance (ANOVA) and Mann-Whitney U 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 mean age of the subjects was 19.2 (SD 0.6) years.

Table 1 presents the summary of salivary fluoride concentration mean values (μg/ml) obtained from the 27 young adults at different time points of the testing phases. *Figure 1* displays the salivary fluoride data (mean±SD, μg/ml).

Statistical analysis by the one-way ANOVA test revealed significant differences between the salivary F excretion measured at 0, 15, 60, and 120 minutes in all the different testing phases ($P<0.0001$). The data—except for the baseline (Phase I), where F concentration was low—showed peak values at 0 minutes and a slight decrease at 15 minutes.

Table 1. *Summary of Salivary Fluoride Concentration Mean Values (μg/ml) Data Obtained From 27 Young Adults at Different Testing Phases*

Saliva collected after F intake	Phase I (baseline)	Phase II	Phase III	Phase IV
0 min	0.034^{a}	0.167^{b}	0.263^{c}	0.171^{b}
15 min	0.029^{d}	0.054^{e}	0.090^{f}	0.071^{g}
60 min	0.012^{h}	0.030^{i}	0.030^{i}	0.037^{j}
120 min	0.014^{k}	0.027^{l}	0.026^{l}	0.024^{m}

Mean values with different superscript were significantly different from each other by one-way ANOVA test ($P<0.0001$) and Mann-Whitney U test ($P<0.05$): ^{b} $P=0.085$; ^{i} $P=0.615$; ^{l} $P=163$

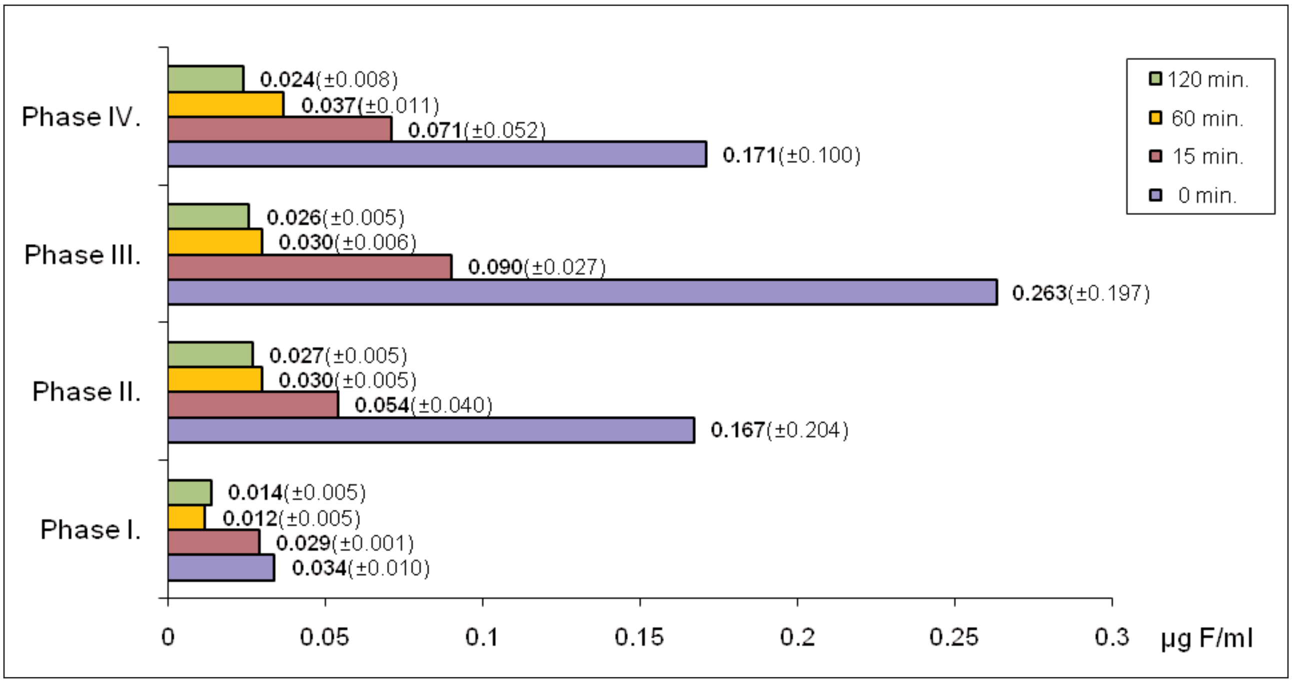

Figure 1. *Summary of salivary fluoride concentration (mean±SD, µg/ml) data obtained at different times during the testing phases (n=27).*

Fluoride ingestion by milk caused a significant increase in the amount of F in saliva immediately after intake and also after 15 minutes, compared to the baseline (*P*<0.0001). No further increase in F was observed during the two-hour saliva-collection period. The F levels were lower at 60 and 120 minutes after the F intake, but still remained significantly higher (*P*<0.0001) compared to the baseline values (Phase I) at the same time point of the salivary sample collection.

In Phase II, when fluoridated milk was administered alone, the salivary concentration values were also significantly (*P*<0.0001) higher at all time points than in Phase I. The highest values at 0 and 15 minutes were found in Phase III, when the fluoridated milk intake was two hours after breakfast (*P*<0.0001) and decreased at 60 and 120 minutes. In Phase IV, the values at 0 and 15 minutes were significantly lower compared with the values in Phase III. However, the mean value at 60 minutes was the highest among all groups, and at 120 minutes decreased similarly to the other session's values. All the values at 120 minutes were significantly higher (*P*<0.0001) compared to the baseline data of Phase I.

Table 2 presents a summary of daily urinary fluoride excretion (mg F/24h) data obtained from 27 young adults at different testing phases of the study.

The statistical analysis of the mean values showed significant differences using one-way ANOVA (*P*<0.0001). Urinary fluoride excretion increased after consumption of fluoridated milk and the highest mean value was obtained when

Table 2. *Summary of Daily Urinary Fluoride Excretion (µgf/24h) Data Obtained From 27 Young Adults at Different Testing Phases*

Parameters	**Phase I (baseline)**	**Phase II**	**Phase III**	**Phase IV**
Subjects (n)	27	27	27	27
Mean	343[a]	454[a]	757[b]	663[b]
SD	101	234	263	173
Median	335	356	756	647
Minimum	182	182	395	335
Maximum	663	1096	1708	989

Mean values with different superscript were significantly different from each other by one-way ANOVA test (*P*<0.0001) and Mann-Whitney U test (*P*<0.05): [a] *P*=0.088; [b] *P*=0.185

fluoridated milk was administered two hours after breakfast. Daily urinary F excretion (μgF/24h) showed significant differences (by Mann-Whitney U test) between the sessions, except for Phases I 343±101 and II 454±234 (P=0.088); Phases III 757±263 and IV 663±173 (P=0.185), respectively.

Discussion

It might be argued that the results are not strictly valid on a community basis due to the relatively small size of the sample; however, 27 subjects were included in this study, outnumbering the previous experiments where the number of subjects ranged between 6 and 18 [1,9,14]. A major cause of the inter-subject variation in fluoride values may well have been variation in fluoride metabolism [5]. For this reason, results could be different if more subjects were included in a study. However, rapid measurement of salivary fluoride concentration could be a valuable *in vivo* screening test for assessing, also on a community basis, the potential anti-caries efficacy of fluoridated milk intake.

The fluoride levels in whole saliva and in the separate gland secretions after intake of fluoridated milk have previously been investigated [11,14,15, 18]. It was found that fluoride ingested with milk is excreted through the salivary glands in increased concentration levels when compared to baseline values, and points to the fact that the bioavailability of fluoride from milk equals that from other vehicles (i.e., water). In addition to a significant increase in fluoride in saliva, fluoride accumulation in dental plaque has also been observed, providing evidence that consumption of fluoridated milk contributes to the "fluoride-storing process" in the oral cavity [5,19].

Urinary fluoride excretion has been used by several authors to assess fluoride bioavailability [3,5,17,20], because urine is the main excretion route for ingested fluoride and urine-sampling methods are recommended by the World Health Organization [16]. The results of analyses of urinary F excretion relating to bioavailability largely agree with conclusions drawn from plasma fluoride profiles [21].

Fluoride intake in all phases (II, III, and IV) of the present study increased salivary and urinary F excretion. A single intake of fluoridated milk on a fasting stomach (Phase II) resulted in a significant increase (P<0.0001) in salivary F content. However, the urinary fluoride excretion was not quite significantly different (P=0.088) compared to the baseline values (Phase I). This result differs from the findings of Ekstrand and Ehrnebo (1979) [6], and Spak, Ekstrand, and Zylberstein (1982) [22] in fasting subjects, who found a 25-35 % reduction of bioavailability measured in saliva and urine, using fluoridated water, milk, and dried baby formula. However, the results of the current study tally with those of Gintner and Bánóczy (2002) [11,12], who found that fasting did not significantly reduce the bioavailability of F from fluoridated milk.

A key indirect function of saliva is as a medium for the transfer of potentially active therapeutic agents, such as F, to the site of action [23]. Considering that even a small increase above basal salivary F concentrations is beneficial [24], the F concentrations observed in whole saliva suggest that fluoridated milk consumption may be effective in preventing dental caries. Even 60 minutes after simultaneous F-milk and breakfast food consumption the salivary F concentration was 0.03 ppm (μg/ml), which is still above the baseline levels for these individuals (0.01 ppm).

Other studies have shown that the bioavailability of fluoride was reduced by simultaneous ingestion of solid food by about 30%; however, with milk and food the plasma fluoride profile was flattened but prolonged, and bioavailability less reduced [7,9]. Although in one study both NaF and NaMFP in milk reduced the bioavailability, the authors suggested that stomach emptying is delayed by simultaneous food consumption, and as the milk proteins are digested by the gastric secretions, fluoride is liberated and absorbed. These suggestions might be valid in the present study, where food consumed two hours prior drinking fluoridated milk resulted in a prompt elevation of the fluoride profile of saliva and urine (Phase III). However, breakfast and F-milk taken simultaneously showed a less high immediate elevation in F levels, but a longer effect (Phase IV) prolonged over the time points.

The finding that food consumption does not significantly reduce the bioavailability of fluoridated milk has also been supported by Engström, Petersson, and Twetman (2002) [19], who found, on the basis of dental plaque accumulation studies, that milk is a suitable vehicle for local fluoride administration into the oral cavity, also when consumed together with a meal. Villa (2001) described plasma fluoride profiles and urinary data of young adults, which showed no significant differences for bioavailability when F in water, F in water plus breakfast, and F-milk plus breakfast was administered [25].

The research to date has studied predominantly the simultaneous administration of fluoride with milk or solid food. The present study was conducted in order to find out what happens if fluoridated milk is consumed simultaneously with food early in the morning or two hours after food has been consumed for breakfast, which could simulate real-life situations.

The availability of F in milk is not inhibited by other components of the milk, in agreement with Bánóczy, Petersen, and Rugg-Gunn (2009) [3]. Although the high calcium content of milk (typically 1,200 mg/l) has in the past led to a prediction that there would be interaction between calcium and fluoride in fluoridated milk resulting in the precipitation of calcium fluoride, this clearly does not happen with fluoridated milk containing 2-5 mg/l fluoride, as used in practice. Only a relatively small fraction of calcium in milk (80 mg/l) is in the form of free ions, which is insufficient to cause calcium fluoride precipitation at these relatively low levels of fluoride [26]. Finally, it should be noted that different studies support the observations made by Phillips (1991) [27] on the ionic-fluoride availability in fluoridated milk containing 5 mg/l fluoride [1,17,18]. This was the concentration used in the current study [28].

In summary, the findings suggest that the contribution to oral (salivary) fluoride levels from fluoridated milk consumption appears to be more important than the timing of solid food intake.

Conclusions

In the study that has been described:

- The results have shown that in young adults the fluoride content in the saliva and also the amount of fluoride excreted in the urine were the highest when drinking fluoridated milk two hours after breakfast on a single occasion.
- The salivary fluoride content was lower when fluoridated milk was administered simultaneously with breakfast; however, it had a longer effect.
- The findings suggest that administration of F-milk contributes to the F-storing process in the oral cavity with elevated F concentrations in saliva and is influenced by the timing of solid food intake.
- The current results appear to indicate that milk is an appropriate vehicle for F supplementation in young adults, even if it is consumed simultaneously with breakfast.

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Conflict of interest

Professor J. Bánóczy is Chairman of The Borrow Foundation.

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