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Fluoride Intake by the Duplicate-Diet Technique and Urinary Excretion in Korean Children Aged 3-6 Years

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Abstract : This study aimed to determine the fluoride intakes in 120 preschool children aged 3 to 6 residing in Jumunjin (community water fluoridation area) and Gangneung (non-fluoridation area). The parents were asked to collect 24-hour urine samples and to duplicate the samples of all the diets that their children ingested in the day of urine collection. The acid-diffusible fluoride in the food and non-carbonate beverages were isolated by the acid-diffusion technique and then measured with a fluoride electrode. The fluoride in carbonate beverages, drinking waters and urine samples were measured directly with a fluoride electrode. The geometric mean (geometric standard deviation) of daily fluoride intakes from all kinds of diet was 5.99 (2.27) μg/kg /day in the children in Gangneung and that of the children in Jumunjin was 18.36 (2.69). The amount of fluoride intake by food and drinking water in fluoridation area were significantly larger than that in non-fluoridation area but the statistical difference of fluoride intake by beverages between two areas was not observed. The GMs (GSDs) of daily fluoride excretion by urine of children in non-fluoridation area and in fluoridation area were 8.39 (1.73) and 18.62 (1.77) μg/kg/day, respectively. The correlation between fluoride intake from diet excluding beverage and urinary excretion was statistically significant. It is concluded that the amount of fluoride intake of children living in fluoridation area did not exceed the upper intake level to avoid the risk of dental fluorosis (2.2 mg/day in 4- to 8-year-olds) and urinary excretion of fluoride was good indicator of fluoride intake from diets.

Keywords : fluoride intake, duplicate-diet, acid-diffusion technique, urinary excretion, water fluoridation

Introduction

The prevalence of dental caries in developed countries has declined over past several decades, and it is considered mainly due to the widespread use of fluoride in various methods.[1] However, more people concern for increased fluoride ingestion and concern of enamel fluorosis associated with community water fluoridation[2] and that water fluoridation have resulted in a significant increase in the quantity of fluoride in the environment.[3] The level of fluoride necessary for adequate prevention of dental caries is only marginally less than that which can cause fluorosis and it is necessary to investigate accurate amount of total intakes of fluoride from all diets and beverages which children ingest daily to use fluoride properly.

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An analysis of difference in fluoride intake for children residing in fluoridated and non-fluoridated areas is important because there are some possibilities of fluoride exposure more than optimum level for the children in fluoridated area. Burt[4] suggested that the limit for safe daily fluoride intake by children is 0.05-0.07 mg/kg/day and the adequate intake (AI) for fluoride from all sources is 0.05 mg/kg/day in U.S.

Many accurate detection method for fluoride were researched and as many papers were published. Acid diffusion method in the presence of hexamethyldisiloxane (HMDS) was introduced by Taves.[5] This technique enables complete recovery of ionic fluoride form in various samples separating fluoride from interfering substances[6] and it is capable of accurate determination of background levels of F in food and biological materials. It is also known as flexible with respect to sample preparation and sample size.[7] Murakami *et al.*[1] conducted the duplicate-diet method which is believed as an accurate approach of assessing fluoride and nutrient intake and measured the daily amount of fluoride in the diets for children aged 3 to 5.

Gastrointestinal track is the main route of fluoride exposure like many environmental contaminants[8-10] and renal excretion is the predominant route for removal of inorganic fluoride from the body. Approximately 50% of the daily intake of fluoride is cleared by the kidneys.[11] As a result, the fluoride level will be higher in urine than in any other body fluid. Fluoride content of urine measured directly with the fluoride electrode is generally in good agreement with those measured by conventional other methods.[12] Therefore, urinary fluoride excretion is generally accepted as a better indicator[13] for estimation of daily or short-term fluoride exposure or utilization.[14]

The objective of this study was to describe the total fluoride intake from all diets for children at fluoridated and non-fluoridated communities. In order to prove the feasibility of urine sample for indicator of fluoride exposure, we estimated the correlation between the amount of urinary fluoride excretion and the fluoride intake by each diet.

Materials and Method

Study Population

In this study two communities in Gangwon Do were selected to collect food and beverages, Jumunjin as a fluoridated community and Gangneung as a non-fluoridated community. The reasons of selection were: their documented fluoride histories; their proximity to one another; and the similarity of their demographic and socioeconomic characteristics.[15] 120 preschool children at 4 kindergarten aged 3 to 6 were participated and their urine, drinking water, food and beverages were collected.

Collecting Samples and Preparation

All the diets (drinking water and food) that children ingested within a day were duplicated by their parents when they were at home and by teachers or researchers at kindergarten. The volume and kinds of commercial beverages taken by the children were recorded for laboratory analysis. During the weekdays the urine samples were collected over 24 hour period because researchers and teacher could supervise the children attending kindergarten at daytime. The parents collected nocturnal urine samples of their children.

All samples were stored in icebox after collecting and transported to the laboratory within a day. The samples which did not need pretreatment for analysis were kept in refrigerator until analyzing. Food samples were weighed and processed by homogenization prior to analysis. A sufficient amount of de-ionized water was added to each food sample with pre-weighed amount, and the total weight of the water and the food was recorded to calculate the grams of food per gram of homogenate. Then each food sample was then thoroughly homogenized by cookery mixer, and duplicate aliquots (30 m*l*) were frozen (−20°C) until the time of analysis.

Laboratory Analysis

The fluoride of all food homogenates and beverages that could not be analyzed directly, such as juices and milk, were analyzed by hexamethyldisiloxane (HMDS) diffusion method of Taves[5] as modified by Rojas-Sanchez *et al.*.[16] The acid-diffusible fluoride in the liquid homogenate was isolated by the acid-diffusion technique, which is called micro-diffusion principle,[17] using H_2SO_4-HMDS and trapped by NaOH solution and measured with a fluoride electrode (Orion Research EA940).

The fluoride in urine samples, drinking water and carbonated beverages were analyzed directly using fluoride-ion specific electrode. Konieczka *et al.*[18] studied about comparison of fluoride ion-selective electrode based potentiometric methods of fluoride determination in human urine and they found that there were high correlation between fluoride determination method by direct reading and other methods such as standard addition method, double standard addition method and bracketing solution method.

A 10-m*l* aliquot of each sample was mixed with equal volume of Total Ionic Strength Buffer (TISAB II) and the mixture was placed directly under the electrode. The results of millivolt readings were recorded and the fluoride concentration of each sample was calculated from a standard curve constructed on the basis of the millivolt readings for a series of fluoride standards analyzed at the same time under the same conditions. The standard curve was re-constructed daily because fluoride electrode is very sensitive with the change of temperature and other environment.

Statistical Analysis

The data were transformed as a logarithm in order to control the skewed distribution and then whey were normally distributed which facilitated other statistical analysis. The arithmetic and geometric mean and standard deviation (and/or GSD) of fluoride content were calculated for each urine, drinking water, food and beverage classification group. The differences in the fluoride intake among different sources (food, drinking waters and beverages) and between different water fluoridation patterns in communities were compared with independent samples t-test. A significance level of α, 0.05, was used to test all hypotheses. Pearson's coefficient of correlation was used for estimating the relationship between the amount of urinary fluoride excretion and fluoride intake by each diet. Statistical analyses were performed by SAS V8.01.

Results and Discussion

Differences of Fluoride Intake by Area

Totally 120 children participated in the study, 60 (34 boys, 26 girls) residing in Jumunjin city and 60 (25 boys,. 35 girls) in Gangneung city. The mean ages of participants in each area were aged 65.10 (SD 11.81) months in Jumunjin and 60.93 (SD 10.15) months in Gangneung. The duplicate-diet method is considered as the most accurate way of sampling diet and appears to indicate a substantially lower level of fluoride intake than other previous methods.[1] In this study, diet patterns were surveyed to ensure that the collected food samples were a true representation of a child each day.

The total fluoride intake of boys (16.68 μg/kg) was slightly higher than that of girls (14.20 μg/kg). This resulted from the number of boys in fluoridated area was larger than that of girls and after adjusting regional difference, the difference was not statistically significant.

The arithmetic and geometric means (SD and

Table 1. Fluoride intake from diet in Korean children aged from 3 to 6 years

Diet classification		Total (μg/kg/day)	Gangneung (μg/kg/day)	Jumunjin (μg/kg/day)	p-value[b]
Drinking water	N	114	56	58	
	AM[a] (SD)	8.43 (12.43)	2.78 (8.99)	13.88 (12.91)	
	GM (GSD)	3.39 (4.26)	1.21 (2.89)	9.12 (2.77)	<0.001
	Range	0.08-77.37	0.08-68.06	0.83-77.37	
Beverage[c]	N	105	52	53	
	AM (SD)	2.14 (2.76)	2.32 (3.41)	1.96 (1.94)	
	GM (GSD)	1.16 (3.19)	1.16 (3.25)	1.16 (3.16)	0.9925
	Range	0.05-19.83	0.14-19.84	0.05-9.74	
Food	N	117	57	60	
	AM (SD)	7.99 (13.84)	3.81 (4.88)	11.97 (17.92)	
	GM (GSD)	3.60 (3.74)	2.12 (3.42)	5.93 (3.39)	<0.001
	Range	0.06-104.71	0.06-33.28	0.32-104.71	
Total Intake	N	118	58	60	
	AM (SD)	17.96 (20.13)	8.50 (10.03)	27.11 (23.10)	
	GM (GSD)	10.59 (2.92)	5.99 (2.27)	18.36 (2.69)	<0.001
	Range	0.61-116.60	0.75-70.23	0.61-116.60	

[a]Arithmetic mean.
[b]T-test for differences of geometric mean between the two communities.
[c]Includes all commercial beverages such as juices, carbonated beverages and milk.

GSD) and ranges of fluoride intakes between regions are shown in Table 1. The mean daily fluoride intake from all diets of the children in Jumunjin was 0.445 mg/day (SD 0.348) and 0.123 mg/day (SD 0.092) in Gangneung. It is generally accepted that the optimal fluoride intake ranges from 50 to 70 μg F/kg/day and it is an adequate limit for safe fluoride exposure for children.[19] The daily fluoride intake from all diets of fluoridated area in this study was similar to lower range of optimal F intake.

The distribution of fluoride concentration of duplicated drinking water samples collected from fluoridated community was separated into two subgroups. The mean fluoride concentration of the one subgroup (n=48) was 0.88 mg/*l* and that of the other subgroup (n=10) was 0.11 mg/*l*. The former group has been drinking optimally fluoridated water, but the later group has been drinking negligibly fluoridated water comparable to the non-fluoridated community, where, except one outlier, the mean fluoride concentration of drinking water was 0.11 mg/*l* as same as the later group of fluoridated community. If this results are compared to that of Jackson *et al.*,[15] in this paper the mean fluoride concentrations (standard deviation) of water at community fluoridated area and non-fluoridated area were 0.90 (0.05), 0.16 (0.01) mg/*l*, respectively, the fluoride which was ingested by children living in community fluoridation area was comparable to that of negligibly fluoridated area. This finding was similar to a Korean study in another region.[21]

The fluoride intake of children living in Jumunjin where fluorides are supplied in drinking water was lower than optimal fluoride intake guideline by World Health Organisation (WHO). These results are related with several factors such as using tap-water treatment equipment and commercial purified water (mineral water). Dabeka *et al.*[21] calculated fluoride intake by infants from food and reported the average fluoride intake were 0.28 mg/day at the non-fluoridated area and 0.56 mg/day at the fluoridated area. In our results, the mean (SD) total fluoride intake from diets (food, beverages, drinking water) at the fluoridated area was were 0.51 (0.43) mg/day and that of non-fluoridated area was 0.16 (0.18) mg/day.

Chowdhury *et al.*[22] reported that daily fluoride intake of infants in New Zealand from food and drinks did not exceed 0.06 mg/kg body weight. We found five cases that exceed daily optimal fluoride intake, 0.07 mg/kg body weight for dental health benefits and single case that exceeded the guideline, i.e., 0.10 mg/kg body weight, which is generally accepted to avoid an undesirable degree of dental fluorosis. However, the fluoride excretion through fingernail (data are not shown here) was 3.34 μg/g which is similar to the reports from other researches.[23,24] Urinary excretion of the case was 21.91 μg/kg/day which was slightly higher than that of arithmetic mean for fluoridated area (Table 2). The main source of fluoride intake for this child was food and it seems that the child ingested food which contained much fluoride by chance at the sampling day.

As would be expected, the fluoride intakes from drinking water, food and all diets were significantly different between two geographical groups determined by t-test (Table 1). However, the fluoride intakes from beverage were not significantly different between the two groups because all the beverages ingested by the children were circulated commercially. When comparing the differences of fluoride intake from each diet, logarithmic transformation was used to correct the skewed distribution (Fig. 1).

Urinary Fluoride Excretion

The amounts of fluoride excretion by urine were summarized in Table 2. The daily (24-h) fluoride excretion by urine was 0.038-0.82 mg/day and the mean (SD) fluoride excretion of non-fluoridated and fluoridated area were 0.183 (0.096), 0.407 (0.186) mg/day, respectively. Ketley *et al.*[25] studied urinary fluoride excretion by preschool children in six European countries and reported mean 24-h fluoride excretion for the children in the non-fluoridated areas was 0.23 mg (±0.19) and that of fluoridated area was 0.37 mg (±0.11). Then, they attempted to estimate daily fluoride intake from the data of urinary fluoride excretion. Their estimation was based on a fractional excretion of 30% and obtained 0.052 (±0.044) mg/kg body weight and 0.075 (±0.026) mg/kg body weight for non-fluoridated and fluoridated area. If the same estimation was used in our data (Table 2), 32.2 μg/kg/day for non-fluoridated area and 70.6 μg/kg/day for fluoridated

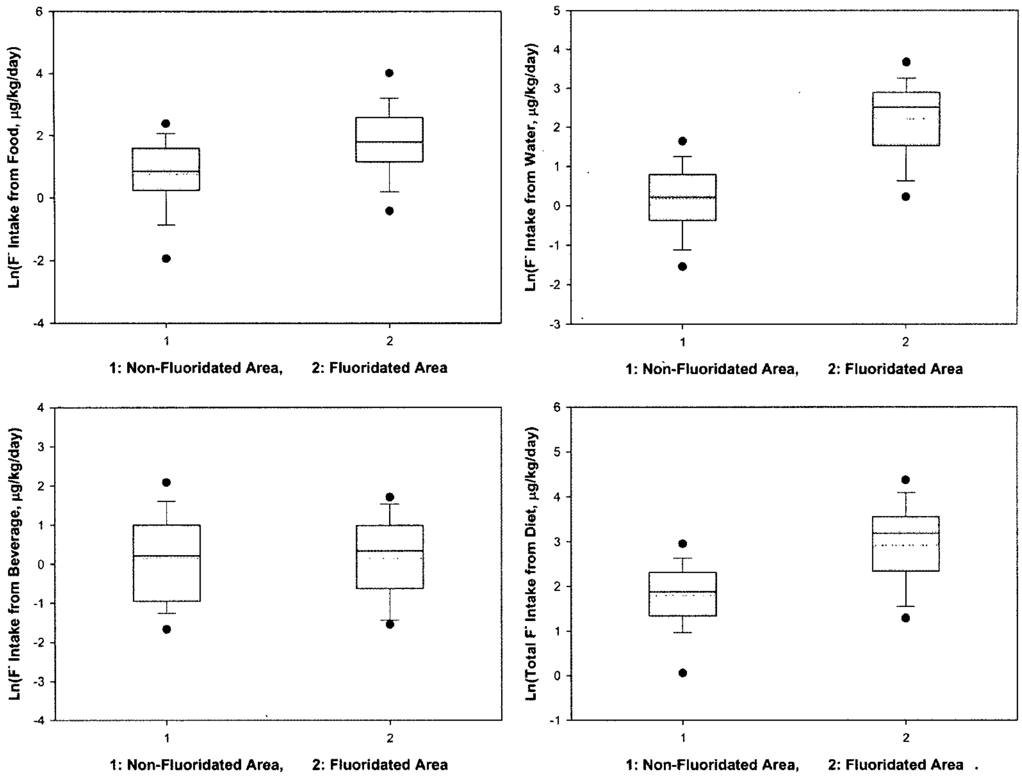

Fig. 1. Differences of fluoride intake from food in logarithmic scale between non-fluoridated area and fluoridated area; The amount of fluoride intake of each child was calculated in the unit of µg/kg/day. The box represents 25th, 50th (median) and 75th percentiles, dotted lines are means of logarithmic values and black dots are 5th/95th percentiles.

area can be obtained. Diets contributes very small portion in daily total fluoride intake (Table 1). Pessan *et al.*[22] estimated fluoride intake from diet as 0.018 (±0.009) mg/kg body weight and 0.020 (±0.026) from dentifrice, respectively. Although the fluoride intake from dentifrice is added, fluoride intake from diets is very small. Haftenburger *et al.*[26] reported urinary fractional excretion rates in the region of 50%. According to this fractional excretion rates, 19.3 µg/kg/day for non-fluoridated area and 42.4 µg/kg/day for fluoridated area can be obtained. This is similar results to Pessan *et al*'s ratio of fluoride intake from diets to that from total intake.

As the fluoride intake from diets, the amounts of fluoride excretion through urine were significantly different between the two geographical groups (Table 2, Fig. 2).

Table 2. Fluoride excretion by urine as to children's geographical region

Urinary excretion	Total (µg/kg/day)	Gangneung (µg/kg/day)	Jumunjin (µg/kg/day)	p-value[a]
N	118	59	59	
AM (SD)	15.42 (9.67)	9.66 (5.22)	21.19 (9.69)	
GM (GSD)	12.55 (1.99)	8.42 (1.73)	18.54 (1.77)	<0.001
Range	1.71-54.62	2.19-28.80	1.71-54.62	

[a]T-test for differences of geometric mean between the two communities

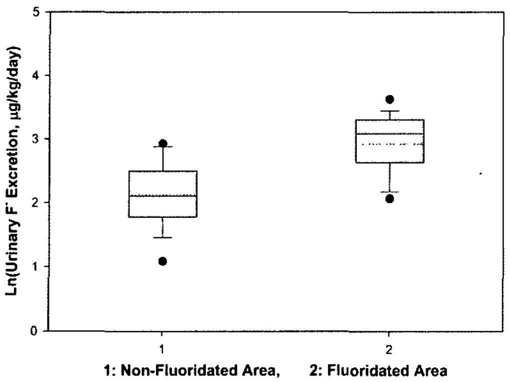

Fig. 2. Differences of fluoride excretion by urine in logarithmic scale between non-fluoridated area and fluoridated area.

Correlation between Intake and Urinary Fluoride Excretion

The amount of daily fluoride excretion by urine was significantly correlated with the fluoride intake from diets (food, drinking water and total intake from diets), but the relationship between daily fluoride excretion by urine and fluoride intake from beverages was not significant (Fig. 3).

Pearson's coefficient of correlation shows significant high level of relationship between urinary fluoride excretion and fluoride intake from all diets except beverages (Table 3). This suggests that 24-h urinary fluoride excretion would be a good biomarker[23] for exposure to fluoride from diet and dentifrice as well as to inhalation.[13] Similarly Hwang[20] reported in her master's thesis that the coefficient of correlation between fluoride of children's urine and drinking water was 0.3345 for fluoridated community in Korea. Even urinary fluoride excretion represents relatively short-time exposure of fluoride,[14] it is feasible substitution in children whose life style and diet are stable for the measurement of fluoride

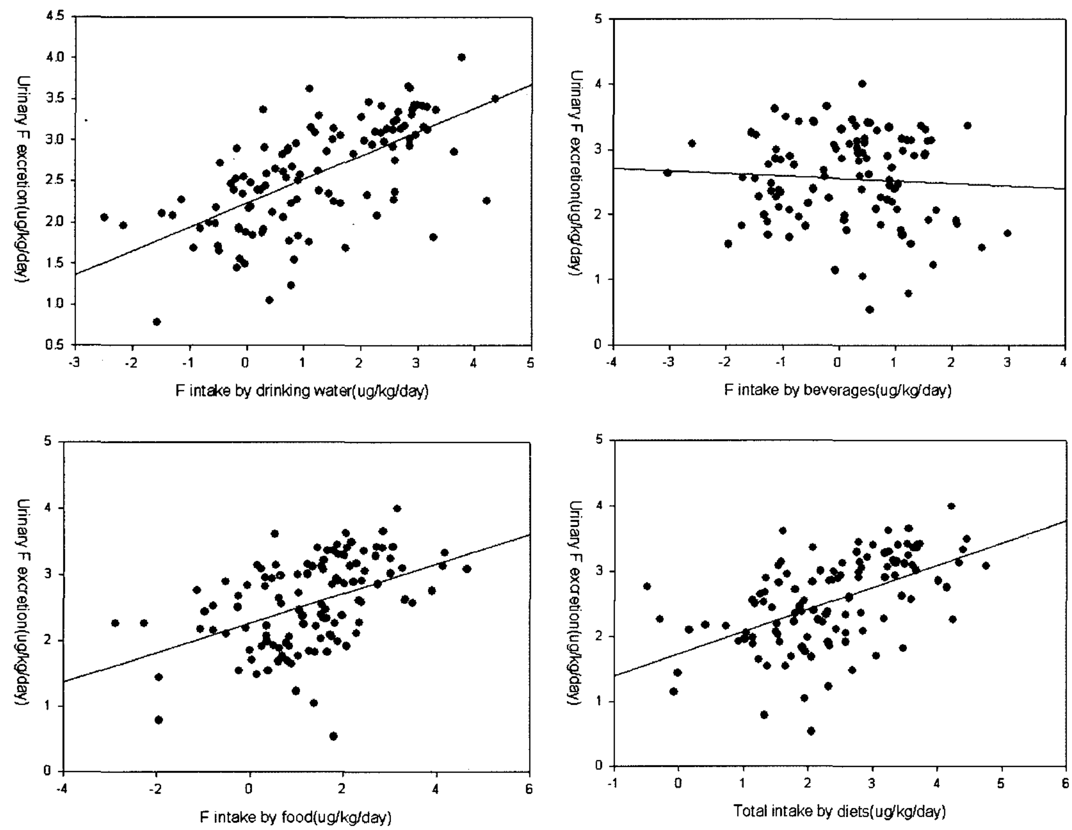

Fig. 3. correlation between daily urinary excretion and the fluoride intake from diets (drinking water, beverages, food and total intake from diets); all measurement analyzed in logarithmic scale.

Table 3. Pearson's coefficient of correlation between variables

	Intake from food	Intake from beverages	Intake from drinking water	Total intake from diets[a]
Urinary excretion	0.4494 p<0.001	−0.0643 p=0.5169	0.6474 p<0.001	0.5348 p<0.001
Intake from food		−0.0682 p=0.4916	0.4677 p<0.001	0.7269 p<0.001
Intake from beverages			0.0202 p=0.8407	0.2371 p=0.0149
Intake from drinking water				0.8159 p<0.001

[a]Intake form food + veverages + drinking water.

intake from all sources (food, beverages, dentifrice and etc.) which is difficult, time-consuming and expensive.

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Conclusion

This study designed to determine the fluoride intakes in Korean children aged 3~6 years. The duplicate diet method was adapted to collect children's diet sample in a day and the amount of fluoride in diets was analyzed by HMDS diffusion method for food and non-carbonate beverages and direct reading method for urine and carbonate beverages. The means of daily fluoride intakes form all kinds of diets by children residing in Jumunjin and Gangneung were 0.445 (±0.354) mg/day and 0.131 (±0.097) mg/day, respectively and they were similar to previous investigations in Korea and other country. It is concluded from this investigation that the amount of fluoride intake of children living in Jumunjin (fluoridated area) and Gangneung (non-fluoridated area) did not exceed the upper intake level designated by the Institute of medicine of the US National Academy of Science to avoid the risk of dental fluorosis (2.2 mg/day in 4- to 8-year-olds). The correlation between urinary fluoride excretion and total fluoride intake from diets was very high and statistically significant and the measurement of urinary fluoride excretion would be good biomarker of fluoride exposure.

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