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Original Article

Estimation of daily fluoride intake of infants using the microdiffusion method

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KEYWORDS
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Abstract *Background/Purpose:* The standard of daily fluoride intake (DFI) has been discussed mainly for adults since 1950s in Japan. Although dietary habits have changed significantly in recent years, there have been no further studies on DFI in the past 10 years, and the need for further review has been discussed. Additionally, fluoride bioavailability in infants is higher than that in adults; hence, an excess fluoride intake often manifests symptoms. However, the number of studies on the DFI of infants is less than that of adults. The purpose of this study is to investigate the DFI for Japanese infants to provide adequate fluoride application.
Materials and methods: 20 products of infant foods for 4 age groups, 5 products of infant formulas, and 5 products of bottle water available in retail stores in Japan were prepared for this study. Fluoride concentration of each product was measured by microdiffusion method and fluoride ion-selective electrode, and then DFI in infants aged 5, 7, 9, and 12 months were calculated.
Results: According to our study, the DFI in infants aged 5, 7, 9, and 12 months is 185.34 μg/day, 181.16 μg/day, 174.59 μg/day, and 179.19 μg/day, respectively.
Conclusion: From this result, it is estimated that the DFI from infant food and beverages in Japan is lower than the standard in other countries. Lifestyles and dietary habits are different in each country, and a new standard of DFI for Japanese children is required to meet the adequate fluoride recommendation.

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Introduction

The preventive effect of fluoride on caries, especially its significance for the growth and development of children, has been proven by many epidemiological studies. World Health Organization (WHO), recommended fluoride in 1969 to prevent caries and advocated its regular intake.[1] It is necessary to define daily fluoride intake (DFI),[2] to ensure safe and adequate fluoride intake; hence, regular evaluations and verification of DFI are practiced in Europe and United States,[3–10] where the acceptable amount of fluoride as a nutrition is suggested. However, only a few studies[11–13] on DFI of infants using infant food and formulas purchased in Japan has been conducted. Some elementary studies are necessary to recommend safe systemic fluoride intake. Therefore, we believe that the fluoride present in foods and beverages should be quantified for DFI. It is reported that the fluoride intake during the early childhood period contributes to the development of resistance to caries through pre-eruptive maturation and improvement of the crystalline structure of the enamel.[2,7,14,15] Moreover, fluoride bioavailability in infants is higher than that in adults and excess fluoride intake is often known to have adverse effects;[15] hence, an exact standard is necessary. The existing data of measured values of food samples in Japan are calculated using different methods of analysis, and it is difficult to compare these results directly. This has been the major hurdle for the collation of data and for further research. Therefore, it is imperative to establish reference standards for fluoride concentration analysis.

The method for analyzing fluoride concentration is different for each form of foodstuff. Fluoride ion-selective electrode is usually used to measure the fluoride concentration in liquids, and steam distillation is used for organic samples. However, steam distillation has several disadvantages including the need for long hours of ashing, the significant loss of sample materials, the long duration of the process, and the high cost of reagents. In this study, we used the microdiffusion method. This method was easy to conduct because it required no ashing process and needed less time and reagents. We performed a standard test of the microdiffusion method based on a previous study by Hinoide et al., in 1992,[16] before conducting a measurement of fluoride concentration on food and beverage samples. In our study, we focused on the diets of infants; measured the fluoride content of infant foods, infant formulas, and bottled water available in retail stores in Japan; analyzed their fluoride concentration through the microdiffusion method; and then calculated the DFI of infants referring to the recommended amount of each product.

Materials and methods

Preparation of the sample (infant food, formula, and water)

In this study, commercial infant foods, infant formulas, and water were selected as samples for infants. Infant foods for 5, 7, 9, and 12-month-olds are available in the market. Randomly selected 20 infant foods from 3 manufacturers, 5 for each age group, were analyzed for this study. Each infant food was homogenized using an electrical blender before the microdiffusion process. The fluoride concentration of 5 infant formulas from 3 manufacturers were analyzed using microdiffusion without homogenizing the procedure. For the liquid sample, 5 products of bottled water from 4 manufacturers were selected. These liquid samples were analyzed using fluoride ion-selective electrode.

Steam distillation method

First, we attempted the standard test to establish the accuracy and measuring conditions. A 100-ppm fluoride standard (Thermo Fisher Scientific, Waltham, MA, USA) was used as a sample to compare the steam distillation and microdiffusion methods. Steam distillation was performed using the method reported by Iizuka et al., in 1964.[17] A steam-generating flask with 1 L of purified water was continually maintained in the alkaline state using 10% of aqueous sodium hydroxide (NaOH) and phenolphthalein reagent. A distilling flask with 30 ml of the water sample was condensed with NaOH, 50 ml of aqueous perchloric acid ($HClO_4$), 2 ml of aqueous silver (I) perchlorate, and 10 glass balls was heated to 135° for the steam distillation process. 84.9% of fluoride was collected from the first 200 ml of distilled water when the distilling speed was set at 5—20 ml per minute.

Microdiffusion method

For the microdiffusion method, an airtight and heat-resistant polytetrafluoroethylene apparatus consisting of outer and inner compartments, as previously described by Hinoide et al., in 1992,[16] were prepared (Fig. 1). Fifty milligrams of samples, infant formulas, and homogenized infant foods, and 4 ml of hexamethyldisilazane (HMDS)-saturated 5M $HClO_4$ as the diffusion solution was poured into the outer compartment of the apparatus. One milliliter of 0.1M NaOH was poured into the inner compartment as the trapping solution for fluoride. The apparatus was placed at 60 °C for 1 h. This condition was set according to the results of condition analysis as later discussed. After

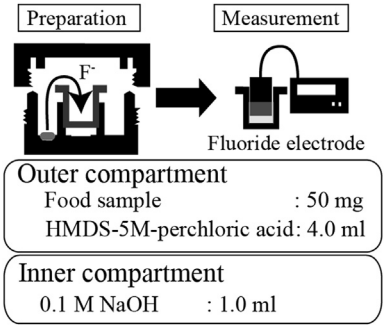

Figure 1 The polytetrafluoroethylene apparatus was prepared for the microdiffusion method. The apparatus was used for the diffusion process in a water bath after an organic sample and reagents were poured into the compartments. 0.1 ml of TISAB III was poured into the inner compartment and the fluoride concentration was measured using fluoride ion-selective electrode.

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the reaction, 0.1 ml of the total ionic strength adjustment buffer III (TISAB III) was poured into the inner compartment and the fluoride concentration was measured using a fluoride ion-selective electrode. For the control, an apparatus with 50 ml of purified water was prepared and subjected to the same process.

For the liquid samples, 2 ml of the sample and 0.2 ml of TISAB III were agitated and analyzed with the fluoride ion-selective electrode. 2 ml of purified water was used as a control.

Condition analysis for the microdiffusion method

It was necessary to ensure the accuracy of the microdiffusion method before analyzing the fluoride concentration of the samples. The accuracy depends on the diffusion time according to the previous studies.[16,18] We conducted some standard tests to find the best condition for this process. In this test, the diffusion time was set for 30, 60, 90, and 120 min, and the recovery rate was 96.9%, 102.9%, 104.2%, 101.5%, respectively (n = 5) (Table 1).

Another test was carried out for further confirmation of the measurement range in the fluoride concentration and accuracy by the microdiffusion method. A 100-ppm fluoride standard solution, diluted with purified water, including 0.1 μg, 1 μg, and 10 μg was prepared for this test. The recovery rate was 96.3%, 99.7%, and 102.9%, respectively (n = 5) (Table 2).

From the results of these standard tests, it was proven that the microdiffusion method is effective for measuring the fluoride concentration of organic samples including 0.1–10 μg of fluoride with acceptable accuracy under 60 °C for 60 min.

Table 1 We set the diffusion time for 30, 60, 90, and 120 min (n = 5). The results of samples which underwent reaction for 60 min and longer showed 100% recovery rate. This shows that 60 min is the most appropriate reaction time for the microdiffusion method.

Diffusion time (min.)	Recovery rate Mean ± S.D. (%)
30	96.9 ± 1.00
60	102.9 ± 1.31
90	104.2 ± 1.02
120	107.4 ± 2.97

Table 2 0.1, 1, and 10 μg of fluoride in fluoride standard solution was poured in the outer compartment of the apparatus (n = 5). After 1 h of reaction, the maximum fluoride in the inner compartment was measured. In this trial, the recovery rates were within an uncertainty range of 5%. This shows that our method is effective for samples containing between 0.1 and 10 μg of fluoride.

Added Fluoride concentration (jig)	Recovery rate Mean ± S.D. (%)
0.1	96.3 ± 7.50
1.0	99.7 ± 0.98
10	102.9 ± 2.00

Calculation of DFI

The DFI of infants for each age group of the infant foods, formulas, and water were calculated by the following equations.

DFI from infant foods (μg) = Σ(fluoride concentration of infant food × content for each meal) × 3/(number of samples)

DFI from drinking water (μg) = Σ(fluoride concentration of bottled water × amount of water consumed each day)/(number of samples)

DFI from infant formulas (μg) = Σ(fluoride concentration of infant formula × content for each day + fluoride concentration of bottled water × amount of water needed to dissolve formula)/(number of samples)

The total value of these 3 equations was considered as the DFI for each age group.

Statistical analysis

The Origin 2018b for Windows software package (OriginLab Corp., USA) was used for statistical analysis. All results were represented as mean ± S.D., and difference were considered to be significant at $p < 0.01$. The method of Turkey, after One-Way ANOVA, was used to compare the variation of products ($p < 0.05$).

Results

Infant food

Fluoride concentrations of the 20 types of commercial infant foods, as described under the Materials and Methods section, were in the range of 0.0292–0.1244 μg/g (Table 3). There are significant differences between products among 7 and 12-month-olds (one-way ANOVA, $p < 0.01$), and no significant differences between products among 5 or 9-month-olds. When the suggested contents are consumed three times a day, the DFI of 5, 7, 9, and 12-month-olds from the infant foods are 8.7696 μg, 14.0376 μg, 15.3264 μg, and 19.9296 μg, respectively, with the amount increasing with age.

Infant formula

Fluoride concentrations of the 5 types of commercial infant formulas, as described under Materials and Methods, were in the range of 0.2528–1.5696 μg/g (Table 4). There are significant differences between products (one-way ANOVA, $p < 0.01$). The amount for each day 98–135 g: depending on products, is dissolved in 700 to 1,000 ml of water to be consumed, so the DFI from the infant formula is 77.868–264.336 μg (mean 135.6622 μg) in total, when the fluoride concentration of the water used for dissolution is considered to be 0.0524 μg/ml.

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Table 3 Fluoride concentration in 5 products of infant foods each for 5, 7, 9, and 12-month-old infants (mean ± S.D., n = 5). The infant foods were homogenized and subjected to microdiffusion; the concentration was then measured using fluoride ion-selective electrode. The *p*-values were calculated by one-way ANOVA and significant differences observed at $p < 0.01$.

Age (month)	Infant foods	Fluoride concentration Mean ± S.D. (μg/g)
5	Creamed fish and potato	0.0456 ± 0.0294
	Porridge	0.0320 ± 0.0137
	Corn	0.0412 ± 0.0282
	Pumpkin and sweet potato	0.0608 ± 0.0180
	Apple	0.0292 ± 0.0148
7	Vegetable chicken rice	0.0660 ± 0.0164
	Chicken and vegetable	0.1192 ± 0.0073
	Noodle with fish and seaweed	0.0508 ± 0.0053
	Tuna rice	0.0428 ± 0.0128
	Salmon porridge	0.0368 ± 0.0020
9	Chicken rice with burdock	0.0436 ± 0.0161
	Stewed chicken rice with burdock	0.0600 ± 0.0025
	Flatfish risotto	0.0648 ± 0.0156
	Pork with radish	0.0668 ± 0.0235
	Noodle with vegetable and egg	0.0732 ± 0.0259
12	Noodle with vegetable and pork	0.0788 ± 0.0165
	Stewed hamburger	0.1244 ± 0.0203
	Minced fish stew	0.0444 ± 0.0027
	Bean curd with liver	0.0980 ± 0.0255
	Chop suey with squid	0.0696 ± 0.0378

(ANOVA, $p < 0.01$).

Table 4 Fluoride concentration in 5 products of infant formulas (mean ± S.D., n = 5). After the infant formulas underwent microdiffusion, the concentration was measured using a fluoride ion-selective electrode. The *p*-values were calculated by one-way ANOVA and significant differences observed at $p < 0.01$.

Infant formula	Maker	Fluoride concentration Mean ± S.D. (μg/g)
Pure	MEGMILK SNOW BRAND Co.,Ltd.	0.6356 ± 0.1334
Hagukumi	Morinaga & Co., Ltd.	0.2528 ± 0.0349
Chilmiru	Morinaga & Co., Ltd.	0.8064 ± 0.2475
Meiji step	Meiji Co., Ltd.	0.4200 ± 0.0274
Meiji hohoemi	Meiji Co., Ltd.	1.5696 ± 0.0666

(ANOVA, $p < 0.01$).

Bottled water

Fluoride concentrations of the 5 types of commercially bottled water, as described under Materials and Methods, were in the range of 0.006—0.1357 μg/ml, and the mean value was 0.0524 μg/ml (Table 5). There are significant differences between products (one-way ANOVA, $p < 0.01$). Since the infants aged 5, 7, 9, and 12-months-old are expected to consume 780 ml, 600 ml, 450 ml, and 450 ml of water respectively each day, the mean DFI from drinking water are 40.9032 μg, 31.464 μg, 23.598 μg, and 23.598 μg, respectively.

Estimation of DFI

The mean DFI of infants is calculated from the fluoride concentrations of infant foods, formulas, and bottled waters selected in this study. Infant formula accounts for 73.20%, 74.88%, 77.70%, and 75.71% of the DFI in 5, 7, 9, and 12-month-old infants, respectively. The infant formula takes up a larger share for each age group than the infant food. The DFI values measured in our study are approximately one-sixth to one-third of the tolerable upper intake level (0—5 months: 660 μg/day for male infants and 610 μg/day for female infants, 6—11 months: 880 μg/day for male infants and 820 μg/day for female infants, 12 months: 1200 μg/day for male infants, 1100 μg/day for female infants) according to "The Proposal of Fluoride Consumption Standard for Japanese" (Study Group for Fluoride Application, Japanese Society for Oral Health, 2007), a significantly lower value in each age group. Furthermore, these estimated values are around one-third to two-thirds of the recommended dietary allowance (0—5 months: 330 μg/day for male infants and 310 μg/day for female infants, 6—11 months:440 μg/day for male infants and 410 μg/day for female infants, 12 months: 600 μg/day for male infants, 550 μg/day for female infants) (Fig. 2).

Table 5 Fluoride concentration in 5 products of bottled water (mean ± S.D., n = 5). Bottled water was directly measured using a fluoride ion-selective electrode after TISAB III was poured. The *p*-values were calculated by one-way ANOVA and significant differences observed at $p < 0.01$.

Bottled water name	Maker	Fluoride concentration Mean ± S.D. (μig/g)
Natural water of the southern alps	Suntory Holdings Limited	0.0622 ± 0.0012
ILOHAS	Coca-Cola (Japan) Co, Ltd.	0.0060 ± 0.0001
Evian	Danone Japan Co.,Ltd.	0.0455 ± 0.0096
Natural water of kirishima	FamilyMart Co.,Ltd.	0.1375 ± 0.0019
Natural water of tsunan	FamilyMart Co.,Ltd.	0.0110 ± 0.0012

(ANOVA, $p < 0.01$).

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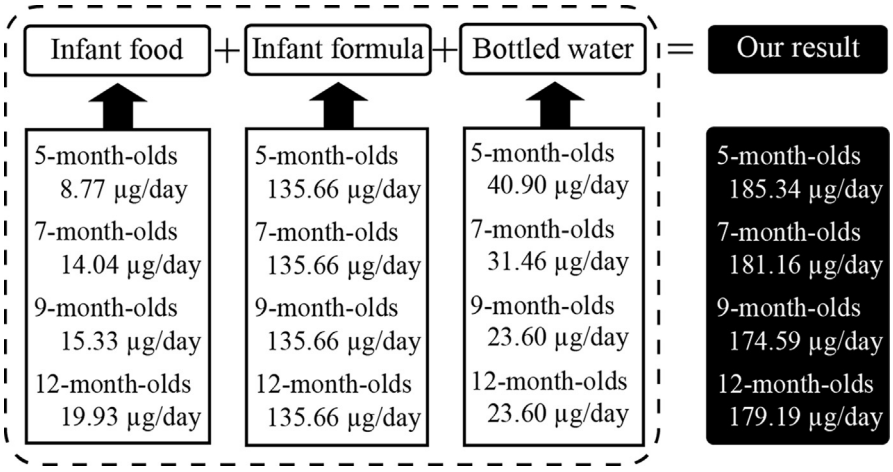

Figure 2 Comparison between the result, recommended dietary allowance, and tolerable upper limited intake level in each age group.

Discussion

Fluoride intake for the prevention of caries has been reviewed its efficacy and safety for the past 50 years.[2] WHO released the advisory for fluoride application in 1969, 1979, and 1994, and people around the world currently receive its benefits.[1] However, the necessity for estimation of recommended allowance per day and standard intake level in life represents an important issue with the well-established fluoride standard.[5] "Food and Nutrition Board Commission on Life Sciences" from the National Research Council in the United States considers calcium, phosphorus, magnesium, iron, zinc, iodine, and selenium as nutrition with Recommended Dietary Allowances (RDAs) along with Vitamin A, D, E, K, and B families.[19] Fluorine is listed as the second major ion after iron in the human adult by the body on "Estimated Safe and Adequate Daily Dietary Intakes of Selected Vitamins and Minerals." Besides, although the Resources Council of the Ministry of Education, Culture, Sports, Science, and Technology in Japan developed and announced the "Standard Tables of Food Composition in Japan" with relevant ministries and agencies, the DFI of fluoride was not listed on the 6th Nutritional Requirements list.[19]

Lifelong fluoride intake for prevention of caries is suggested today, and the estimation of DFI is imperative for the evaluation of its efficacy and safety. Fluoride as an essential trace element plays an important role in the growth of the apatite crystal and improves its structure during the period of odontogenesis. A significant number of researches have been published about this role of fluoride. Describing the bioavailability of fluoride, approximately 90% of the fluoride ingested each day is absorbed from the alimentary tract. The proportion of ingested fluoride retained in the body is approximately 55% in children and 36% in adults, and the remainder of the absorbed fluoride is excreted through the kidneys. Approximately 99% of the fluoride in the body is associated with calcified tissues and is available to the enamel during the period of odontogenesis or pre-eruptive maturation. Absorption across the oral mucosa is limited and probably accounts for less than 1% of the daily intake, but fluoride affects the outer surface of the enamel when stagnated in the oral cavity.[15] Fluoride largely contributes not only to the maturation of tooth apatite structure but also to the stability of the bone apatite crystal. Fluoride is clearly beneficial throughout life, so adequate intake[20] of fluoride is necessary for the appropriate application to receive its benefit. Several reports on the analysis of fluoride in food and the daily intake for Japanese have been reported since the 1950s, and the adequate intake of fluoride for adults is 480 to 2640 μg for one day. However, re-evaluation of these recommendations is necessary since the dietary habits are changing in current times.

It is necessary to measure the fluoride concentrations of foods and beverages to calculate the DFI and to discuss the necessity and safety of systemic fluoride intake. Until now, the fluoride concentration of organic samples was usually analyzed by steam distillation. However, steam distillation has several disadvantages including the ashing process for long hours, the large loss of sample materials, the long process time, and the high cost of reagents. On the other hand, the microdiffusion method overcomes these disadvantages, and allows a more accurate measurement of the concentration. Since the different diffusion conditions were preset in all the previous studies, we conducted a condition analysis before analyzing the food and formula samples. According to the results of our trials, 60 °C for 60 min is the most effective for diffusion.

Since fluoride contributes to the pre-eruptive enamel maturation during the odontogenic period, the DFI of infants from food and beverages will be an important index to prevent dental fluorosis, which is estimated to occur due to the high concentration of fluoride in drinking water and an overdose of fluoride from other routes.

While the standard for DFI for the population from infants to adults is established in the United States and in the European countries, Japan should set up its own standard for consumption of fluoride since the lifestyle and dietary habits of the Japanese are different from these countries. This study presents several basic values of estimated DFI based on the analysis of commercially available infant foods and formulas in Japan. Hence, it is suggested that this

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data could contribute to further fluoride intake studies especially systemic fluoride intake.

Conflicts of interest statement

The authors have no conflict of interest relevant to this article.

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