INFLUENCE OF DIETARY FLUORIDE INTAKE ON URINARY FLUORIDE CONCENTRATION AND EVALUATION OF CORRECTED LEVELS IN SPOT URINE

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SUMMARY: The urinary fluoride (F) concentration is recognized as an indication of F intake of preceding days. Since F is found naturally in water supplies, plants and animals, urinary F is readily affected by various food intakes. In this study, the influence of dietary F intake on urinary F concentrations was investigated, as well as the validity of spot urine samples as indices of F exposure.

Subjects were eight healthy female adults (age: 21 ± 1 year) who stayed for 16 days, for the analysis of F metabolism, in a human experimental facility of the National Institute of Nutrition. F concentrations in the diets and urine were analyzed by the F ion specific electrode method, or its combination with the steam distillation method after ashing procedure.

F intake during 24 hrs from foods usually consumed in Japan, exclusive of green tea which contains a high amount of F (1.5 ppm), ranged from 0.79 to 2.74 mg/day. The levels were the same as have been reported in fluoridated communities. The excretion ratio of ingested F in the urine during 24 hrs was 18 to 35%. With 2.73 mgF/day intake, urinary F concentration reached 1.17 ppm at 3.5 hrs after dinner intake, and maintained that level to the next morning. The results showed that F excretion continued for several hrs after the intake from the diets. The amount of F in the diets and F excretion in 24-hr urine were well correlated ($r = +0.95$). Correlation coefficients with spot urine, with or without corrections for specific gravity or creatinine, were over +0.82.

The results show that, when estimating the F body burden, particularly due to low F exposure, it is necessary to monitor accurately F intake from diets. It is also suggested that the use of spot urine F concentration, with or without correction, is a valid procedure.

Key words: Dietary fluoride; Fluoride body burden; Urinary fluoride.

Introduction

It is important, for the health care of inhabitants and workers exposed to fluoride (F) compounds, to evaluate the effects of the F body burden. Exposure to F compounds has been estimated by measuring the F concentration in urine, serum, hair, and environment. Among these, since ingested F is mainly excreted via the kidney, urinary F concentration has been widely recognized and used as a good indicator of F exposure.[1-3]

Because F is widely distributed throughout the earth's crust, it is found naturally in water supplies, plants and animals, and is a constituent of all diets.[4-6] The F ingested from these foods is excreted into urine in the same way as F inhaled during exposure.[7] Therefore, urinary F concentrations are readily affected by various food intakes. To evaluate accurately the body burden of F in exposed inhabitants and

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workers, it is necessary to measure the influence of F in the diets on the urinary F concentration. In this study, the influence of dietary F intake on urinary F concentrations in healthy Japanese adults, after ingestion of typical daily Japanese diets, was investigated, as well as whether or not F concentrations in spot urine samples can be used as indices of F exposure.

Materials and Methods

Subjects

The subjects were eight healthy female adult volunteers (age: 21 ± 1 yr; height: 157.6 ± 3.1 cm; weight: 53.2 ± 5.8 kg). They stayed in a human experimental facility of the National Institute of Nutrition from July 26 to August 10, 1990. They were closely supervised under their usual life-style conditions during the experiment. The experimental room was maintained at a constant temperature with air conditioning to minimize F insensible perspiration. The procedure of the study was explained to all subjects, and the study was conducted with their informed consent.

Experimental schedule and sampling time of urine

The experiment was conducted over 16 days: 4 preparatory days, for adaptation to the provided dietary and living conditions; and 12 days for the urine-excretion study. Three experimental cycles were designed for the urine-excretion study, each taking 4 days.

The daily schedule followed during the experiment was:

1) The subjects arose at 7 am, when the first urinary samples were collected.
2) Second urinary samples were collected at 8:30 am. Then, for breakfast, each subject ingested the entire amount allocated within 30 to 40 min.
3) Third urinary samples were collected at 12:30, before lunch, which was ingested the same way as breakfast.
4) Fourth urinary samples were collected at 16:30 pm.
5) Fifth urinary samples were collected at 18:30 pm. Then dinner was taken.
6) The last urinary samples were collected at 22:00, when the subjects retired.

Twenty four-hour samples were collected from 8:30 (second urinary samples) until 7:00 (the first urinary samples) the next morning.

The diets in the experiment

In order to provide the same kind of diet for each cycle, the foods used in the study were purchased from the same store, from the same lot and from the same producing area. Four different daily diets were chosen to be used on days 1, 2, 3, and 4 of each of the experimental cycles.

Meals were given three times each day: at 8:30 for breakfast, 12:30 for lunch and 18:30 for dinner.

The subjects were fed diets considered adequate for each individual with respect to caloric and protein allowances (energy, 1800 Kcal; protein, over 70 g; the ratio of fat energy, 25%; sodium salt, 10 g). They were forbidden to drink Japanese tea and/or black tea throughout the experiment. However, they were allowed to have ion-exchanged water freely during the day.

The composition of the experimental diet on each day of a cycle is shown in Table 1, with the approximate amounts of each item ingested. The subjects' diets during the experiment were typical Japanese menus.

TABLE 1. Menu of experimental diet on each day of one cycle

	Diet day 1 Food item/Amount(g)		Diet day 2 Food item/Amount(g)		Diet day 3 Food item/Amount(g)		Diet day 4 Food item/Amount(g)	
Breakfast	Rice	150	Bread (with butter and strawberry jam)	90	Rice	150	Bread (with butter)	68
	Miso soup (with haricot and soybean)	166	Milk	200	Miso soup (with taro and soybean)	177	Milk	200
	Satsuma age (fried fish paste)	51	Vegetable salad (fillet of crab, tuna and cucumber)	142	Fermented soybean	70	Frankfurter (pickled cucumber cabbage and onion)	184
	Japanese radish	23	Tomato juice	150	Japanese radish	6	Orange juice	150
	Tomato	30			Dessert (kiwifruit and yogurt	153		
	Furikake (containing powdered small fish)	15						
	Grapefruit juice	200						
Lunch	Bread (with butter and strawberry jam)	118	Rice served in a bowl with ground beef and haricot	259	Spaghetti (with cod roe)	119	Tirashizushi rice (wary crab and scallop (canned), hijack (sea-weed), small fish(dried whole),pods pear and lotus root)	274
	Milk	200	Cooked carrot	67	Coffee (instant, milk)	215		
	Scramble egg (with cheese, corn and smoked pork)	145	Grapefruit juice	200	Vegetable salad (onion, cucumber, allspice and tomato)	147	Japanese soup with soy-sauce	156
	Vegetable (head lettuce and cucumber)	47					Cooked pumpkin	108
	Dessert (peach, canned)	50					Pickled Japanese radish	10
							Tomato juice	200
Dinner	Rice	150	Rice	150	Rice	150	Rice	150
	Miso soup (with eggplant and soybean)	216	Miso soup (with haricot and soybean)	166	Miso soup (with enokitake-fungi and soy bean)	166	Omelet (ground chicken and green asparagus)	220
	Tsukune yaki (baked ground chicken with Welsh onion and Chinese chive)	186	Omelet (ham)	218	Hamburg steak (ground beef, onion, Welsh onion and Chinese chive)	145	Miso soup (with nameko-fungi and soy bean)	166
	Japanese vegetable salad with vinegar (scallop and cucumber)	81	Vegetable salad (okura, onion, scallop and cucumber)	131	Vegetable (corn and head lettuce)	120	Japanese vegetable salad with soy-sauce (pods pear and small shrimp (dried whole))	62
	Dessert (muskmelon)	100	Cooked vegetable (bamboo)	68	Cooked vegetable (edible burdock)	55	Oyster salad (smoked oyster and head lettuce)	36
			Dessert (vanilla ice cream)	82	Dessert (pineapple, canned)	50	Dessert (orange sherbet)	120

Urinalysis

1) F concentration [2]

One mL of urine samples was placed in a Teflon tube and 100 μL TISAB III (Orion Research, USA) was added. Then, the samples were analyzed by a F ion electrode method (Expandable Ion Analyser EA 940, Combination Fluoride 96-09-00 Orion Research, USA).

2) Specific gravity

The urinary specific gravity was measured with a Clinical Refractometer (Erma, Japan). For the statistical analysis, the F concentration in the urine at a standard specific gravity of 1.024 was calculated by the formula:

$$\text{F concentration (ppm at specific gravity 1.024)} = \frac{\text{measured level of F (ppm)} \times (1.024 - 1.000)}{\text{measured specific gravity} - 1.000.}$$

3) Creatinine

Creatinine concentrations were measured by the Folin-Wu method. The fluoride concentration in the urine at a standard creatinine concentration of 1 g per L of urine was calculated by the formula:

$$\text{F concentration (ppm/g creatinine per L of urine)} = \frac{\text{measured level of F (ppm)}}{\text{creatinine concentration (g/L)}}$$

4) F excretion rate

The fluoride excretion rate as a percentage of the dietary intake was calculated by dividing the weight of F excreted in the urine over 24 hours by the fluoride intake in the diet over 24 hours and multiplying by 100.

$$\text{F excretion rate (\%)} = \frac{\text{F excreted in urine over 24 hr in μg}}{\text{F in the diet over 24 hr in μg}} \times 100$$

The fluoride excretion rate as an absolute rate in micrograms per minute was calculated by dividing the weight in micrograms in the spot urine by the time interval between the collection of spot urine and the previous spot urine.

$$\text{F excretion rate (μg per min)} = \frac{\text{weight of F in spot urine in μg}}{\text{time in minutes between collection of spot urine and the previous spot urine}}$$

Measurements of F content in the diets

After weighing, the sample was placed in a platinum crucible and 2 mL of suspended calcium oxide was added as a fixative and accelerant agent for ashing. The sample was then homogenized and heated at 600°C for 3 hr in a muffle furnace. To the ashed sample, 10 mL of 60% perchloric acid and 0.5 mL of 25% silver-perchlorate were added. The mixture was subjected to distillation at 135°C for 25 min, and F concentration in the distillate was measured by the same method used for urine samples described above.

The percentage recovery of F in 5 to 50μg F added samples by the method was approximately 95%.[6]

Statistical differences between groups were examined by Student's t-test if the variances were equal, and Welch's t-test if the variances were unequal (F-test).

Results

Dietary F content and rate of urinary F excretion

Table 2 shows the amount of total F intake from the diets, and F content in urine, during 24 hrs. The amount of F in the diets in each cycle ranged from 0.79 to 2.74 mg/day. The data were the averages of triplicate analyses. Although menus for each

cycle were prepared with the same foodstuffs, there were small differences in F amounts, ranging from 0.79 to 1.00 mg/day on the first day, 1.01 to 1.15 mg/day on the second day, 0.86 to 1.04 mg/day on the third day, and 2.61 to 2.74 mg/day on the fourth day. The F amounts on the first, second and third days were nearly 1 mg/day. However, the diet on the fourth day showed higher F levels than those of other dietary days.

The rates of urinary F excretion were 31.9~35.3% on the first day, 18.1~20.6% on the second day, 18.1~22.0% on the third day and 21.2~22.2% on the fourth day. The F excretion rate on the first day was higher than those of other days, probably a spill-over from the previous fourth day.

Kinetics of urinary F excretion after the dietary intakes

Figure 1 shows change of urinary F level after intake of the diet containing 1.01 mgF. No marked difference in the urinary F concentrations was observed during 24 hrs. On the other hand, as Figure 2 shows, in the case of 2.73 mgF intake, although the urinary F concentration was about 0.2 ppm before dinner intake, it reached 1.17 ppm at 3.5 hrs after dinner intake, this high level was continued to the next morning, and a relatively high level of 0.66 ppm was found at 08:30.

TABLE 2. Urinary fluoride excretion after intake of diets in healthy adults (N=8)

	Experimental day	Dietary F intake (mg/day)	24-hr urine samples (Mean ± S.D.) Volume (ml)	F concentration (ppm)	F excretion (μg)	Rate of F excretion / intake (%)
Pre	2	1.22	691.8 ± 73.9	0.33 ± 0.07	224 ± 48	18.4 ± 4.0
	3	0.93	880.4 ± 270.1	0.21 ± 0.03	177 ± 40	19.0 ± 4.3
	4	2.79	907.8 ± 203.9	0.65 ± 0.16	563 ± 93	20.2 ± 3.3
Cycle 1	1	0.79	1201.4 ± 314.1	0.25 ± 0.12	280 ±113	35.3 ±14.3
	2	1.01	918.6 ± 209.2	0.24 ± 0.05	208 ± 30	20.6 ± 2.9*
	3	0.86	1006.9 ± 244.6	0.19 ± 0.04	188 ± 34	22.0 ± 3.9*
	4	2.73	943.3 ± 149.4	0.66 ± 0.10	607 ± 63	22.2 ± 2.3*
Cycle 2	1	0.97	964.3 ± 212.4	0.35 ± 0.07	327 ± 37	33.7 ± 3.8
	2	1.15	933.3 ± 188.2	0.23 ± 0.04	207 ± 13	18.1 ± 1.2**
	3	1.04	939.0 ± 225.6	0.21 ± 0.04	188 ± 33	18.1 ± 3.1**
	4	2.61	940.1 ± 253.9	0.63 ± 0.16	555 ± 66	21.2 ± 2.5**
Cycle 3	1	1.00	1119.6 ± 260.5	0.30 ± 0.06	320 ± 31	31.9 ± 3.1
	2	1.08	916.4 ± 219.5	0.22 ± 0.05	197 ± 29	18.2 ± 2.6**
	3	1.02	880.6 ± 170.2	0.21 ± 0.02	184 ± 36	18.1 ± 3.5**
	4	2.74	773.8 ± 89.4	0.76 ± 0.09	585 ± 52	21.4 ± 1.9**

*p<0.05 compared with experimental day 1

** p<0.01 compared with experimental day 1

FIGURE 1. Changes in urinary fluoride concentrations after ingestion of the diet containing fluoride of 1.01mg

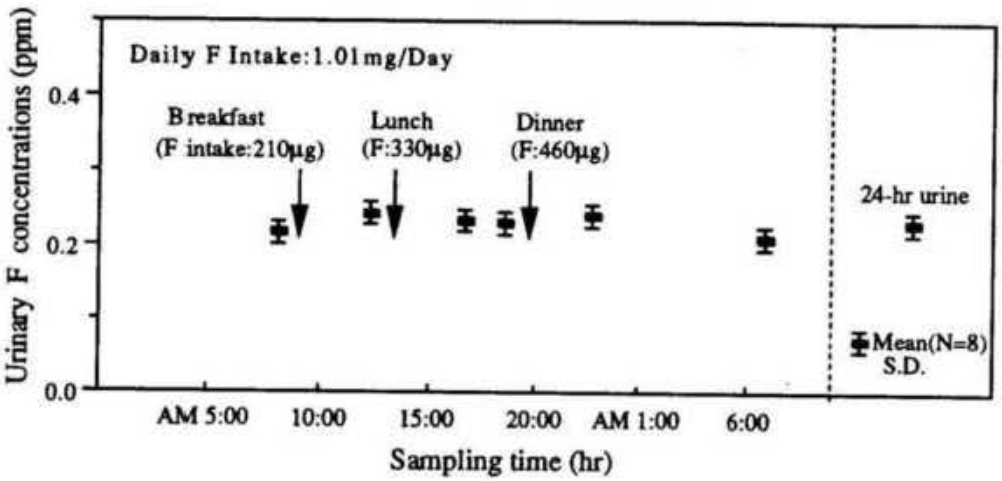
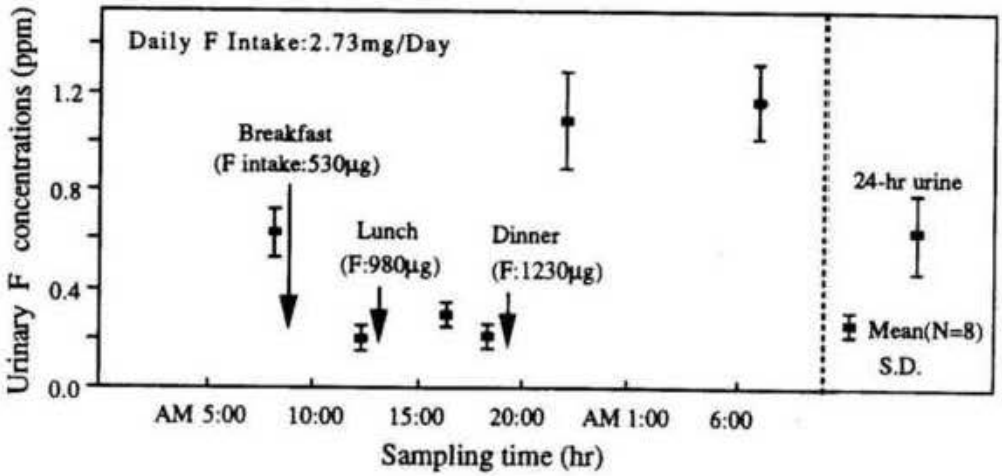


FIGURE 2. Changes in urinary fluoride concentrations after ingestion of the diet containing fluoride of 2.73mg



Relationships between daily dietary F amount, F concentration in the spot urine, and F excretion in 24-hr urine

Table 3 shows that the F excretion in 24-hr urine and the amount of F in the diets were well correlated (r = 0.95). Significant relationships between the F excretion in 24-hr urine and F concentrations in the spot urines collected at 12:30, 16:30 and 18:30 were not observed. The relationships between the daily dietary F amounts and F concentrations in those spot urines were also not significant. These samples were collected before the intake of F for the day had been completed. As there was some variation in the day to day F intake, urine samples taken before the intake had occurred could not be expected to reflect the intake. However, correlation coefficients between the spot urines collected after the meal intakes (at 22:00, 07:00, 08:30) were significant (r = 0.75 or over). These were all times occurring after the dietary intake for the day had been completed. The correlation matrices of F concentration in the spot urines after the meal intakes are shown in Table 4. Good correlation coefficients (r = 0.82 or over) were obtained between measured concentrations and concentrations corrected for specific gravity or creatinine.

Discussion

It is evident that F is present in almost all foods, and that its concentration varies markedly among foods. As shown in Table 2, the diet on the fourth day showed higher F levels than those of other days. The foodstuffs on the fourth day contained seaweed (F concentration: 15 ppm), dried small fish (37 ppm) and shrimp (19 ppm).[6,7] So the different levels may have resulted from foodstuffs containing high amounts of F. The dietary F amounts in Table 2 are consistent with those of previous observations in Japan, and of fluoridated communities.[8,10]

The rates of urinary F excretion were 20~35% of ingested F (Table 2). In particular, the F excretion rate on the first day was significantly higher than those of other days. It has been reported that intakes of insoluble F, such as from food containing bone and skin, are absorbed slowly from the gastrointestinal tract, and are excreted gradually into the urine, when compared with intakes of water soluble NaF.[11,12] Moreover, our previous report showed that high urinary F concentrations were maintained for many hrs when excessive amounts of F were ingested.[7] The results may suggest that the rates of F excretion on the first day were influenced by the F amount and/or the kind of foodstuffs in the diets of the fourth day (the last day of each cycle including the pre-experiment). When the variations in dietary intake are reduced by calculating the intake and excretion over 48 rather than 24 hour periods the variation in the F excretion rate is less with a range of 18~27%. If 72 hour periods are used the range is even smaller at 19~25%.

The measurement of urinary F concentration in industries is essential when providing health care for F exposed workers. When making such measurements, a 24-hr urine analysis is ideal for accurate monitoring of environmental F exposure. However, it may be difficult to sample urine and make accurate measurements for a large number of workers. Therefore, it is important to know whether spot urines samples can be used for estimation of F body burden. Zober *et al* confirmed a significant relationship between total daily elimination of F and the F concentration in postshift urine samples.[1] Kono *et al* also reported that measurement of the F concentration in postshift urine would be useful for routine evaluation of F exposure.[3]

TABLE 3. The correlation coefficients of the observation items (N = 96)

	Spot urine											
Sampling time	12:30				16:30				18:30			
	Con	SG	Cr	Te	Con	SG	Cr	Te	Con	SG	Cr	Te
24-hr F excretion	-.16	-.43	-.39	-.42	-.11	-.36	-.28	-.30	-.22	-.30	.06	-.11
Daily dietary F	-.16	-.50	-.49	-.49	-.19	-.48	-.37	-.45	-.26	-.38	-.03	-.20

Sampling time	22:00				7:00				8:30				Daily dietary F
	Con	SG	Cr	Te	Con	SG	Cr	Te	Con	SG	Cr	Te	
24-hr F excretion	.87	.93	.91	.90	.91	.97	.92	.90	.79	.84	.86	.77	.95
Daily dietary F	.86	.92	.91	.80	.91	.96	.97	.87	.83	.86	.88	.75	

Con: F concentration in ppm as measured in the spot urine
SG: F concentration in ppm at a urinary specific gravity of 1.024
Cr: F concentration in ppm per g creatinine per L of urine
Te: F excretion rate in micrograms per minute
Correlation coefficients greater than 0.5 show $p<0.001$

TABLE 4. The correlation matrices of the observation items in the spot urine at each time (N = 96)

A) Sampling time: 22:00

	SG	Cr	Te
Con	.96	.92	.92
SG		.97	.97
Cr			.98

B) Sampling time: 7:00

	SG	Cr	Te
Con	.92	.91	.88
SG		.99	.97
Cr			.98

C) Sampling time: 8:30

	SG	Cr	Te
Con	.96	.91	.82
SG		.97	.92
Cr			.91

Con: F concentration in ppm as measured in the spot urine
SG: F concentration in ppm at a urinary specific gravity of 1.024
Cr: F concentration in ppm per g creatinine per L of urine
Te: F excretion rate in micrograms per minute

Recently, with improvements in the workplace, the degree of F exposure has been decreased among workers. Under the new working conditions, when judging environmental hazards of low F concentration, it is necessary to eliminate the influence of diets and foodstuffs containing F on urinary F concentration. Kono *et al* have also reported that workers should be requested to limit the intake of foods such as tea, or marine products containing large amounts of F, on the day before sampling the urine, in order to exclude the influence of F in the diets.[13] As shown in Figure 2, the high urinary F concentration caused by the dietary intake continued for at least 12 hrs. Therefore, attention must be paid to the amounts and solubility of F compounds in lunch menus when postshift urine is used as an index of occupational F exposure.

It has been reported that, when spot-urine samples are used instead of 24-hr urine, the urinary excretion of various metal and organic substances is significantly affected by urinary volume, both in the workers and in healthy controls.[14] A commonly used method to eliminate varying amounts of diuresis is to correct for specific gravity or creatinine. However, our F analysis (Tables 3 and 4) shows high correlation coefficients were found between the concentrations of spot urines and 24-hr urine when collected after meal intakes, and between measured concentrations and specific gravity- or creatinine-corrected concentrations in the spot urines.

From the results obtained, it is suggested that the F concentration in the spot urines collected after meal intake, with or without correction for specific gravity or creatinine, could be used as a valid index of daily F body burden, for persons with healthy kidney function.

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