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Timing of fluoride intake in relation to development of fluorosis on maxillary central incisors

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Abstract – *Objectives:* Several studies have focused on the timing of fluoride intake relative to the development of dental fluorosis. This study reports the relationships of fluoride intake during the first 48 months of life with fluorosis on early-erupting permanent teeth. *Methods:* Subjects were followed from birth to 48 months with questionnaires every 3–4 months. Questionnaires gathered data on intakes from water, diet, supplements, and dentifrice to estimate total fluoride intake. Early-erupting permanent teeth of 579 subjects were assessed for fluorosis using the Fluorosis Risk Index (FRI) at approximately age 9. Fluorosis cases were defined as having FRI definitive or severe fluorosis on both maxillary central incisors. Individuals with FRI questionable fluorosis were excluded. The importance of fluoride intake during different time periods was assessed using *t*-tests and logistic regression. *Results:* One hundred and thirty-nine (24%) subjects had fluorosis on both maxillary central incisors. Mean fluoride intake per unit body weight (bw) ranged from 0.040 to 0.057 mg/kg bw, with higher intake during earlier time periods and relative stability after 16 months. In bivariate analyses, fluoride intakes during each of the first 4 years were individually significantly related to fluorosis on maxillary central incisors, with the first year most important ($P < 0.01$), followed by the second ($P < 0.01$), third ($P < 0.01$), and fourth year ($P = 0.03$). Multivariable logistic regression analyses showed that, after controlling only for the first year, the later years individually were still statistically significant. When all four time periods were in the model, the first ($P < 0.01$) and second years ($P = 0.04$) were still significant, but the third ($P = 0.32$) and fourth ($P = 0.82$) were not. *Conclusions:* The first two years of life were most important to fluorosis development in permanent maxillary central incisors; however, this study also suggests the importance of other individual years.

Key words: dental fluorosis; fluoride; fluoride intake; maxillary central incisors

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In the United States and other developed countries, the marked decline in dental caries has been accompanied by an apparent increase in dental fluorosis because of the widespread use of fluoride (1, 2). Of fundamental importance in the use of fluoride is to maximize the caries-preventive benefits and minimize fluorosis risk. Therefore, an accurate identification of the periods during which fluoride intake most strongly results in enamel fluorosis is crucial to more judicious and effective use of fluoride in caries prevention. Enamel fluorosis is a condition which results from exposure to excessive fluoride during enamel formation. For the permanent dentition (except the third molars), the age for possible fluorosis development has been generally considered to be the first 6–8 years of life

(3, 4). Although specific fluoride exposures can be highly variable during this broad time period and host susceptibility to fluorosis could differ by tooth types, a general window of maximum susceptibility (WMS) relative to stages of enamel formation could potentially be identified for specific types of teeth (5).

There are relatively few studies of the timing of fluorosis development and most of these studies have focused on the esthetically important maxillary central incisors. These studies generally suggest that the early maturation stage of enamel development is more critical for fluorosis than is the earlier, secretory stage (6–10). However, the specific results vary and the evidence is not conclusive regarding the age at which maxillary central incisors are most susceptible to fluorosis (6, 10–13). For example, Ismail and Messer (12) reported that the first year of life was the most critical period for developing fluorosis on maxillary central incisors. However, Evans and co-workers (10, 11) concluded that the maxillary central incisors appear most at risk for fluorosis from 15 to 24 months in males and 21–30 months in females. Ishii and Suckling (14) suggested this time period was 35–42 months of age. A more recent study showed that the first 3 years were the critical period for fluorosis on maxillary central incisors (13). Therefore, controversy is evident concerning the identification of the WMS for maxillary central incisors. Moreover, previous studies were mostly cross-sectional and retrospective, and used aggregate data to report fluoride exposure history, such as fluoride level in drinking water, instead of individual fluoride intake data. Additionally, they usually focused on single sources of fluoride intake rather than total fluoride intake from all possible sources. These limitations in study design restricted the validity of the conclusions from most previous studies. Thus, the need for additional studies to more precisely define the WMS is evident.

A better understanding of the timing of fluorosis development on maxillary central incisors, which usually are index teeth for early-erupting permanent teeth, could advance our knowledge of biological mechanisms responsible for fluorosis and provide a sounder scientific basis for decisions on the use of fluoride. Using longitudinal data on individuals' fluoride intake collected in the Iowa Fluoride Study, we report the relationships of fluoride intake for specific time periods during the first 48 months of age with fluorosis on maxillary central incisors.

Methods

Data were collected from subjects in the Iowa Fluoride Study, a prospective study of fluoride intake among a cohort recruited at birth from March 1992 to February 1995, using Institutional Review Board-approved informed consent procedures. Demographic characteristics at baseline were previously described (15). Briefly, this cohort is predominantly Caucasian, from families of relatively high socioeconomic status, and generally healthy.

Study methodologies have been described in detail previously (16, 17). Questionnaires were sent to parents mostly at 3- and 4-month intervals from birth to age 4 and included a series of items concerning children's fluoride exposures and ingestion from various sources during the preceding time period or weeks. Fluoride intake in mg per kg body weight (bw) per day was estimated from water, beverages and selected foods, dietary fluoride supplements, and fluoride dentifrice based on parents' responses to the series of questions. Parents' responses were not validated, but reliability was assessed for selected questions (16, 17).

Children ($n = 579$, 297 males and 282 females) were examined for dental fluorosis on early-erupting permanent teeth at about 8–10 years of age (mean age 9.2 years) by two trained and calibrated examiners using the Fluorosis Risk Index (FRI) (3). Twelve early-erupting teeth were examined for each subject and these were eight permanent incisors and four first molars. A mouth mirror and exam light were used, but teeth were dried lightly with gauze. Fluorosis was differentiated from nonfluorosis opacities based on Russell's criteria (18). Fluorosis was also distinguished from enamel demineralization ('white spot' lesions) based on color, texture, demarcation, and relationship with the gingival margin (19). The FRI was modified to assess all visible enamel surfaces, with four zones scored separately on each buccal surface (the incisal edge/occlusal table, the incisal/occlusal third, the middle third, and the cervical third). The scoring criteria, as applied to all zones, differentiated no fluorosis, questionable fluorosis (<50% of zone with white striations), definitive fluorosis (>50% of zone with white striations), and severe fluorosis (zone displays deformity) (9). As many cervical zones were incompletely erupted and not able to be scored, three zones (incisal/occlusal edge, incisal/occlusal third, and middle

third) were used for the main analyses in this report.

A fluorosis case for regression analyses was defined as having FRI definitive or severe fluorosis on at least one zone of both maxillary central incisors; controls had fluorosis on neither of these incisors. Subjects with only one maxillary central incisor having fluorosis were excluded. All other subjects with only FRI questionable fluorosis were grouped as questionable fluorosis, unless they were excluded because the required three zones could not be scored due to reasons such as incomplete eruption. The subjects with only questionable fluorosis were not used for the analyses.

Fluoride intakes in mg/kg bw were estimated using means (standard deviation), range, and percentiles for individual time periods and for cumulative time periods by the area under the curve (AUC) trapezoidal method. The differences in mean fluoride intake in mg/kg bw between cases and controls were assessed using two-sample *t*-tests first at the surface zone level and then for combined zones (incisal/occlusal edge, incisal/occlusal third, and middle third). The correlations among fluoride intakes for the first 4 years were assessed using Spearman rank correlation analyses. The estimated daily average fluoride intake was categorized into tertiles (low, middle, and high fluoride intakes) based on the frequency distribution of average fluoride intake for each of the first 4 years separately.

With fluorosis cases defined as having FRI definitive or severe fluorosis on at least one zone (incisal/occlusal edge, incisal/occlusal third, and middle third) of both maxillary central incisors, the relationships between fluoride intake of individual years and fluorosis were assessed using logistic regression analyses. The odds ratios (OR) and corresponding *P*-values were calculated. Akaike Information Criterion (AIC), a measure of lack-of-fit, was used to assess the fit of the model. Based on the −2 log likelihood estimate, AIC adds a 'penalty' for each parameter in the model which offsets the decreased lack-of-fit associated with models using more parameters. Thus, the AIC can be used to compare single-parameter models (lower AIC is preferable) as well as to compare models with differing numbers of parameters. Generalized R^2 values were used to examine the predictive power of logistic regression models. Thus, we tested whether fluoride intakes, based on the tertiles, during each of the first 4 years of life, were significant individual predictors of fluorosis on maxillary central incisors and whether these variables remained significant after controlling for other years. Therefore, the relative importance of individual years was assessed and the most important time periods for fluorosis development on maxillary central incisors were determined. The two-way interactions among the individual years were assessed. The significance level was set at $\alpha = 0.05$. All data were analyzed with SAS statistical software for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA).

Receiver operating characteristic (ROC) curves (20) also were used to assess the relationships between fluoride intake (mg/kg bw) and fluorosis during the different years. An ROC curve is a plot of sensitivity versus (1 − specificity) for each possible threshold for the predictor variable. The ROC curve for a more accurate predictor variable is closer to the top left corner and has a larger AUC value. The sensitivity and specificity values were computed for each subject's yearly fluoride intake and saved as output to construct the ROC curves.

Results

Considering three zones on the maxillary central incisors, 139 of 579 subjects (24%) had fluorosis on at least one zone of both maxillary central incisors, 45 (8%) had fluorosis on only one maxillary central incisor, 214 (37%) were questionable fluorosis cases, and 181 (31%) had no fluorosis on either maxillary central incisor. Almost all fluorosis was mild, with 4 of the 139 fluorosis cases having staining and/or pitting. Subsequent analyses then focused primarily on the 320 subjects having fluorosis on both maxillary central incisors or no fluorosis on either of these incisors.

Table 1 shows means, standard deviations, ranges, and selected percentiles of total fluoride intakes combined from water, beverages, selected foods, supplements, and dentifrice for both individual and cumulative time periods. Mean intake of fluoride per unit bw ranged from 0.040 to 0.057 mg/kg, with higher intake up to 9 months, and then lower intake from 12 to 16 months. The mean intake per kg bw was relatively stable from 20 to 40 months. However, there was a decline after 40 months as gains in bw surpassed increases in fluoride intake. Substantial variation among individuals is evident for each time period. Spearman correlations showed statistically significant associations among fluoride intake of different time

Table 1. Fluoride intake distribution (mg/kg bw)

Age	N^a	Mean (SD)	Range	25%	33.3%	50%	66.7%	75%	95%
Individual periods (months)									
Birth to 3	559	0.055 (0.056)	0–0.327	0.007	0.014	0.036	0.067	0.095	0.120
>3 to 6	565	0.057 (0.047)	0–0.238	0.018	0.025	0.044	0.074	0.091	0.143
>6 to 9	564	0.054 (0.041)	0–0.225	0.021	0.026	0.043	0.071	0.082	0.129
>9 to 12	559	0.040 (0.030)	0.002–0.180	0.019	0.023	0.031	0.045	0.052	0.098
>12 to 16	533	0.041 (0.027)	0.003–0.151	0.021	0.025	0.036	0.047	0.055	0.091
>16 to 20	528	0.051 (0.029)	0.002–0.190	0.030	0.036	0.045	0.057	0.066	0.098
>20 to 24	551	0.052 (0.031)	0.004–0.218	0.030	0.034	0.045	0.057	0.065	0.106
>24 to 28	542	0.050 (0.029)	0.004–0.198	0.030	0.034	0.045	0.056	0.063	0.113
>28 to 32	541	0.052 (0.028)	0.002–0.204	0.031	0.036	0.046	0.056	0.067	0.105
>32 to 36	420	0.052 (0.027)	0.007–0.171	0.031	0.037	0.048	0.058	0.064	0.105
>36 to 40	336	0.052 (0.032)	0.003–0.028	0.031	0.035	0.046	0.055	0.063	0.115
>40 to 44	313	0.047 (0.027)	0.001–0.200	0.029	0.032	0.041	0.052	0.061	0.095
>44 to 48	396	0.044 (0.029)	0.003–0.254	0.026	0.030	0.039	0.048	0.058	0.097
Cumulative periods (months)									
0–12	514	0.052 (0.036)	0.001–0.190	0.022	0.028	0.043	0.065	0.076	0.120
12–24	440	0.046 (0.023)	0.004–0.145	0.030	0.034	0.044	0.052	0.058	0.088
24–36	444	0.052 (0.025)	0.008–0.183	0.035	0.040	0.048	0.058	0.064	0.095
36–48	430	0.049 (0.025)	0.008–0.167	0.031	0.036	0.045	0.054	0.061	0.095
0–20	441	0.051 (0.028)	0.004–0.151	0.028	0.035	0.048	0.060	0.069	0.107
0–36	297	0.052 (0.021)	0.013–0.115	0.035	0.042	0.051	0.060	0.063	0.090
0–48	117	0.050 (0.019)	0.017–0.122	0.036	0.040	0.047	0.055	0.060	0.084

[a]The numbers of subjects who returned questionnaires varied for different reporting time periods.

periods during the first 4 years of life. For example, fluoride intake during the first year significantly correlated with fluoride intake during the second year ($\rho = 0.379$, $P < 0.01$), the third year ($\rho = 0.176$, $P < 0.01$), and the fourth year ($\rho = 0.109$, $P = 0.036$), respectively.

Table 2 summarizes the associations between fluorosis on different surface zones of maxillary central incisors and fluoride intake during different time periods using *t*-tests. The most important individual time periods of fluoride intake are somewhat different for fluorosis on different surface zones. The significant time periods for fluorosis on the incisal edges were mostly in the first year, and all of the first 2 years were important to incisal third zones. However, the majority of the periods during the first 4 years were not important to fluorosis on middle zones, and most time periods during the first 4 years were not important to fluorosis on cervical zones. The patterns of important cumulative time periods was similar for fluorosis on the incisal edges and incisal thirds, with very few cumulative periods determined important to fluorosis on middle and cervical thirds. Considering fluorosis on any zone of both maxillary central incisors, all of the individual periods during the first 28 months and all cumulative periods during the first 4 years were important.

Table 3 presents mean daily fluoride intakes during different time periods for fluorosis cases (at least one zone on both maxillary central incisors) and noncases (without any fluorosis). Clearly, subjects with fluorosis had significantly higher fluoride intake for almost all individual time periods and for all cumulative time periods. Fluorosis subjects usually had a mean daily fluoride intake of >0.05 mg/kg bw, and nonfluorosis subjects always had a mean fluoride intake of <0.05 mg/kg bw during all 4 years. For most time periods, the lower limits of the 95% CI of mean daily fluoride intake among fluorosis subjects were higher than the upper limits of 95% CI of mean daily fluoride intake among nonfluorosis subjects.

Using logistic regression analyses, the relative importance of fluoride intake during the four individual years was assessed. Table 4 presents logistic regression analyses with fluorosis on at least one zone of both maxillary central incisors and all possible combinations of the four predictor variables of yearly fluoride intake. The estimated daily average fluoride intake was categorized into tertiles (low, middle, and high fluoride intakes) based on the frequency distribution of average fluoride intake for each individual year. Considering the one-variable models shown in the table, all 4 years were individually significantly related to fluorosis on maxillary central incisors. The first (high level versus low level OR = 5.90, middle level versus low level OR = 2.43, overall $P < 0.01$)

Table 2. *P*-values from *t*-tests comparing the differences in fluoride intake between fluorosis cases and noncases in relation to different surface zones of maxillary central incisors by individual and cumulative time periods[a]

Age	Incisal edges (FRI zone I)	Incisal thirds	Middle thirds	Cervical thirds (FRI zone II)	Any zone of both central incisors[c]
Cases[b]:	115	213	104	49	139
Controls:	292	302	425	436	181
Individual periods (months)					
Birth to 3	0.04	<0.01	0.40	0.27	<0.01
>3 to 6	<0.01	<0.01	0.01	0.25	<0.01
>6 to 9	<0.01	0.01	0.02	0.06	<0.01
>9 to 12	0.18	<0.01	<0.01	0.10	0.03
>12 to 16	0.09	0.03	0.69	0.22	0.01
>16 to 20	0.05	0.02	0.06	0.18	<0.01
>20 to 24	<0.01	<0.01	0.10	0.05	<0.01
>24 to 28	0.12	0.20	0.19	0.59	<0.01
>28 to 32	0.24	0.28	0.56	0.56	0.07
>32 to 36	<0.01	<0.01	0.01	0.14	<0.01
>36 to 40	0.27	0.45	0.55	0.30	0.05
>40 to 44	0.94	0.71	0.51	0.46	0.61
>44 to 48	0.04	<0.01	0.02	0.16	<0.01
Cumulative periods (months)					
0 to 6	<0.01	<0.01	0.09	0.12	<0.01
0–12	<0.01	<0.01	0.05	0.27	<0.01
>12 to 24	0.03	<0.01	0.02	0.05	<0.01
>24 to 36	<0.01	0.02	0.19	0.40	<0.01
>36 to 48	0.09	0.01	0.14	0.17	<0.01
0 to 20	<0.01	<0.01	0.01	0.03	<0.01
0 to 36	<0.01	<0.01	0.20	0.30	<0.01
0 to 48	0.46	0.08	0.17	0.09	0.02

[a]The numbers of subjects who returned questionnaires varied for different reporting time periods in each column.
[b]The numbers of cases and noncases varied for each column, depending on the case and control definition for the column.
[c]Fluorosis on both maxillary central incisors, considering three zones and excluding cervical zones.

Table 3. Mean daily fluoride intake and two-sample *t*-test results

Age	*N*	No fluorosis[a] (95% CI), mg/kg bw	With fluorosis[a] (95% CI), mg/kg bw	*P*-value (*t*-test)
Individual periods (months)				
Birth to 3	308	0.047 (0.039–0.055)	0.065 (0.055–0.074)	<0.01
>3 to 6	309	0.047 (0.041–0.053)	0.070 (0.061–0.079)	<0.01
>6 to 9	309	0.048 (0.042–0.054)	0.063 (0.056–0.071)	<0.01
>9 to 12	304	0.036 (0.032–0.041)	0.044 (0.039–0.050)	0.03
>12 to 16	294	0.037 (0.033–0.041)	0.044 (0.040–0.048)	0.01
>16 to 20	289	0.046 (0.042–0.063)	0.056 (0.052–0.063)	<0.01
>20 to 24	305	0.045 (0.041–0.050)	0.059 (0.053–0.065)	<0.01
>24 to 28	300	0.045 (0.041–0.049)	0.054 (0.049–0.059)	<0.01
>28 to 32	298	0.047 (0.043–0.051)	0.053 (0.048–0.058)	0.07
>32 to 36	233	0.045 (0.041–0.049)	0.060 (0.054–0.066)	<0.01
>36 to 40	181	0.047 (0.042–0.053)	0.056 (0.049–0.063)	0.05
>40 to 44	169	0.045 (0.040–0.051)	0.047 (0.042–0.053)	0.62
>44 to 48	224	0.038 (0.034–0.042)	0.050 (0.043–0.057)	<0.01
Cumulative periods (months)				
0–12	279	0.044 (0.039–0.049)	0.061 (0.055–0.068)	<0.01
>12 to 24	248	0.041 (0.037–0.044)	0.051 (0.047–0.055)	<0.01
>24 to 36	246	0.048 (0.044–0.051)	0.057 (0.052–0.062)	<0.01
>36 to 48	238	0.044 (0.041–0.048)	0.053 (0.049–0.058)	<0.01
0 to 20	238	0.043 (0.039–0.047)	0.058 (0.053–0.064)	<0.01
0 to 36	164	0.045 (0.041–0.049)	0.059 (0.054–0.063)	<0.01
0 to 48	59	0.043 (0.038–0.049)	0.053 (0.045–0.062)	0.02

[a]Fluorosis on both maxillary central incisors considering three zones, excluding cervical zones.

Table 4. Logistic models predicting fluorosis on both maxillary central incisors[a] ($N = 191$)

Number of variables	Fluoride intake included in model: Time period (months)	Fluoride intake included in model: Fluoride intake level[b]	Odds ratio (OR)	*P*-value	Combined *P*-value[c]	Generalized R^2	AIC
1	0–12	High	5.90	<0.01	<0.01	0.1050	243.14
		Middle	2.43	0.01			
1	12–24	High	5.53	<0.01	<0.01	0.1184	240.23
		Middle	1.13	0.74			
1	24–36	High	4.24	<0.01	<0.01	0.0749	249.47
		Middle	1.71	0.13			
1	36–48	High	2.64	0.01	0.03	0.0374	257.06
		Middle	1.34	0.42			
2	0–12	High	3.87	<0.01	<0.01	0.1676	233.28
		Middle	2.42	0.02			
	12–24	High	3.72	<0.01	<0.01		
		Middle	0.89	0.76			
2	0–12	High	5.30	<0.01	<0.01	0.1559	235.95
		Middle	2.46	0.02			
	24–36	High	3.79	<0.01	0.01		
		Middle	1.82	0.11			
2	0–12	High	5.94	<0.01	<0.01	0.1300	240.32
		Middle	2.58	0.01			
	36–48	High	2.70	0.01	0.04		
		Middle	1.34	0.44			
2	12–24	High	3.64	0.01	<0.01	0.1311	241.48
		Middle	0.91	0.81			
	24–36	High	2.18	0.10	0.25		
		Middle	1.47	0.33			
2	12–24	High	4.70	<0.01	<0.01	0.1238	243.10
		Middle	1.05	0.90			
	36–48	High	1.54	0.30	0.56		
		Middle	1.11	0.78			
2	24–36	High	3.86	0.01	0.02	0.0759	253.26
		Middle	1.70	0.17			
	36–48	High	1.16	0.76	0.90		
		Middle	0.95	0.89			
3	0–12	High	4.34	<0.01	<0.01	0.1863	232.95
		Middle	2.69	0.01			
	12–24	High	2.13	0.14	0.03		
		Middle	0.64	0.29			
	24–36	High	2.67	0.04	0.12		
		Middle	1.85	0.14			
3	0–12	High	4.28	<0.01	<0.01	0.1728	234.85
		Middle	2.60	0.01			
	12–24	High	2.82	0.03	0.01		
		Middle	0.77	0.52			
	36–48	High	1.95	0.12	0.30		
		Middle	1.25	0.57			
3	0–12	High	5.45	<0.01	<0.01	0.1585	239.37
		Middle	2.50	0.01			
	24–36	High	3.11	0.03	0.09		
		Middle	1.75	0.18			
	36–48	High	1.36	0.54	0.75		
		Middle	0.97	0.95			
3	12–24	High	3.63	0.07	<0.01	0.1315	245.40
		Middle	0.91	0.81			
	24–36	High	2.07	0.20	0.43		
		Middle	1.48	0.35			
	36–48	High	1.08	0.88	0.96		
		Middle	0.95	0.89			

Table 4. (Continued.,)

Number of variables	Fluoride intake included in model: Time period (months)	Fluoride intake level[b]	Odds ratio (OR)	*P*-value	Combined *P*-value[c]	Generalized R^2	AIC
4	0–12	High	4.49	<0.01	<0.01	0.1880	236.54
		Middle	2.74	0.01			
	12–24	High	2.08	0.16	0.04		
		Middle	0.63	0.28			
	24–36	High	2.23	0.17	0.32		
		Middle	1.79	0.19			
	36–48	High	1.34	0.58	0.82		
		Middle	1.01	0.98			

[a]Fluorosis cases were defined as having fluorosis on both maxillary central incisors.
[b]Fluoride intakes were categorized into tertiles based on frequency distributions of yearly average intake as mg/kg bw. The reference tertile for each model is low fluoride intake.
[c]Overall *P*-values for models with multiple fluoride intake periods were all highly significant (all $P < 0.008$).

and second years (high level versus low level OR = 5.53, middle level versus low level OR = 1.13, overall $P < 0.01$) were most strongly related to fluorosis on maxillary central incisors, followed by the third (high level versus low level OR = 4.24, middle level versus low level OR = 1.71, overall $P < 0.01$) and fourth years (high level versus low level OR = 2.64, middle level versus low level OR = 1.34, overall $P = 0.03$). Results from two-variable models showed that the first and second years (0–12 and 12–24 months) were significantly related to fluorosis after controlling for any other individual year. The third and fourth years (24–36 and 36–48 months) were still significant after controlling for the first year, but were not significant after controlling for the second year. The best two-variable model included fluoride intake during the first ($P = 0.05$) and second years ($P < 0.01$). The best three-variable model included the first 3 years, but adding another year to any two-variable model did not substantially decrease the AIC or increase the generalized R^2 scores. When all 4 years were in the model, the first ($P < 0.01$) and second years ($P = 0.04$) were still significant, but the third ($P = 0.32$) and fourth ($P = 0.82$) were not. Overall *P*-values for models with multiple fluoride intake periods were all highly significant (all $P < 0.01$). Based on AIC criteria for model fit, it is apparent that the model containing both 0–12 month and 12–24 month fluoride (AIC = 233) is better than any single-variable model. None of the three-parameter models or the four-parameter models show meaningful improvement over the best two-variable model (minimum AIC = 233).

When characterizing fluoride intake into lower tertiles (usually <0.04 mg/kg bw) versus upper tertiles (usually >0.06 mg/kg bw) separately for each of the first 3 years and excluding all those with any middle tertile of fluoride intake, the fluorosis prevalence rates were 76.2% (16/21) for those with 3 years of higher intake, 50.0% (5/10) with 2 years of higher intake, and 15.8% (6/38) with 0 or 1 years of higher intake. The difference in the prevalence rates was statistically significant (chi-square test: *P*-value <0.01). Thus, those with all 3 years of higher intake were nearly five times as likely to have fluorosis as those with 0–1 year of higher fluoride intake.

Additional analyses were conducted with the first 4 years divided into five approximately 8-month-long intervals based on the study's schedule of questionnaires: birth to 9, 9–16, 16–24, 24–32, and 32–40 months. Fluoride intake in all these time periods was individually significantly related to fluorosis on maxillary central incisors with corresponding *P*-values (OR: upper versus lower tertiles and middle versus lower tertiles) of <0.01 (3.24 and 2.28), 0.03 (2.92 and 2.92), <0.01 (2.07 and 1.41), 0.01 (3.16 and 1.12), and 0.02 (3.13 and 2.06), respectively. Using backward stepwise logistic regression, the final model contained only 0–9 months ($P < 0.01$) and 16–24 months ($P = 0.01$). No third time period was significant when considered for addition to this regression model already including 0–9 and 16–24 months.

As both these logistic regression analyses indicated that the first 2 years are most important to fluorosis on maxillary central incisors, other analyses were conducted to assess the most critical,

shorter time periods during the first 2 years of life. Specifically, the first 2 years were divided into seven individual assessments based on the study's schedule of questionnaires: 3, 6, 9, 12, 16, 20, and 24 months. Using backward stepwise logistic regression starting with the full, seven-variable model, the final model contained only 6 months ($P < 0.01$) and 24 months ($P < 0.01$). No third time point was significant when these two periods were already in the model.

Table 5 shows the sensitivity and specificity for various levels of average daily fluoride intake during the first year. For example, when a threshold of 0.05 mg/kg bw is chosen to classify a subject as having fluorosis, there was sensitivity of 63% (i.e. 63% of the true fluorosis subjects are correctly classified as having fluorosis) and a specificity of 65% (i.e. 65% of the nonfluorosis subjects are correctly classified as not having fluorosis). Figure 1 displays the ROC curves for fluoride intake during the four individual years. The ROC curve for the first year had a larger AUC than for the other individual years, indicating a higher predictive power ($c = 0.681$), but the second year's AUC was close ($c = 0.673$). Third year ($c = 0.651$) and fourth year ($c = 0.605$) fluoride intake had less predictive ability, but were still good (above 0.60). The ROC curves and the corresponding AUCs confirmed the results from the logistic regression analyses.

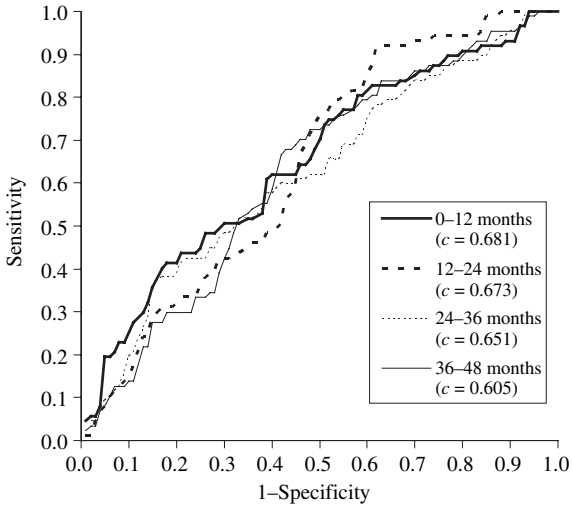

Fig. 1. Comparison of receiver operating characteristic (ROC) curves for fluoride intake during the first 4 years of life.

Discussion

With the goal of enhancing our understanding concerning the timing of fluorosis development on early-erupting, esthetically important permanent teeth, this study assessed the relative importance of fluoride intake during different time periods in the first 4 years of life. The results indicated that the maxillary central incisors as a whole, but excluding the cervical zones, appear most at risk to fluorosis from fluoride intake during the first 24 months, especially around 6 and 24 months. Although differing in the fluorosis indices used and the sources of fluoride assessed, most other studies on the timing of fluorosis suggested that maxillary central incisors appear to be most susceptible to fluorosis during the first 2–3 years. Thus, our results were generally consistent with those of most previous studies on the timing of fluorosis.

Ismail and Messer (12) reported the OR for fluorosis on maxillary central incisors were 5.69, 11.38, 19.50, and 15.17 for children exposed to higher than 2 ppm fluoride in drinking water beginning at birth or during the first year of life relative to those exposed only after the first year, after the second year, after the third year, and after the fourth year of life, respectively, suggesting that the first year was the most significant period. Burt et al. (13, 21) found, in a retrospective study of the effects of a break in water fluoridation on development of fluorosis and caries, that the maxillary central incisors had significantly greater sensitivity to fluorosis from birth to 48 months of age than from 48 to 72 months. A study in Hong Kong of different age cohorts relative to a reduction in the community water fluoride concentration

Table 5. Sensitivity, specificity, and accuracy for various levels of fluoride intake during the first year of life in predicting fluorosis of maxillary central incisors

Cut points of levels of average fluoride intake during first year of life (mg/kg bw)	Sensitivity	Specificity	Accuracy
0.01	0.95	0.12	0.46
0.02	0.86	0.27	0.52
0.03	0.78	0.45	0.59
0.04	0.69	0.55	0.61
0.05	0.63	0.65	0.64
0.06	0.51	0.69	0.62
0.07	0.46	0.78	0.65
0.08	0.37	0.82	0.63
0.09	0.29	0.86	0.62
0.10	0.23	0.92	0.63

concluded that maxillary central incisors were most susceptible to fluorosis during a period of 22–26 months of age (10), and later the authors refined their estimates to 15–24 months of age for males and 21–30 months of age for females (12). A more recent study of the impact of a 7-year break in water fluoridation in Brazil found that fluorosis prevalence of maxillary central incisors were 7.41, 3.70, and 7.87 for those who were 36, 27, and 18 months old when the break started, respectively, suggesting that the risk of fluorosis on the maxillary central incisors is similar among these three birth cohorts (22). Holm and Andersson (23) found that the prevalence of fluorosis among children who had started fluoride supplements at age 6 months was 81%, compared with prevalence rates of 59%, 38%, and 33% for children who started supplements at the ages of 12, 24, and 36 months, respectively. Another study also indicated that fluorosis prevalence was substantially lower (52.4%) when exposure to fluoride supplements started later than 42 months versus those who started at 30 months of age (88.9%) (7). Pendrys and co-workers (24, 25) reported that both toothbrushing starting during the first 2 years of life more than once a day and fluoride supplementation during the first year of life were significantly related to fluorosis on maxillary central incisors in fluoridated areas.

On the other hand, several studies suggest different, perhaps later, critical periods for fluorosis development. For example, Ishii and Suckling (14) reported that children aged 35–42 months old when the fluoride level in drinking water changed from 7.8 to 0.2 ppm had significantly more fluorosis than those who were up to 33 months of age prior to the change in fluoride level. McKay (26), at a time when there were no fluoride products available, such as dentifrice, supplements, etc. found that fluorosis prevalence among children exposed to a water fluoride level of 6 ppm from birth up to 18, 30, and 35 months had fluorosis prevalence of 9%, 36%, and 96%, respectively. Another early study reported similar results that fluorosis prevalence among children exposed to a water supply containing 13 ppm from birth up to 2 years of age was negligible (4%) compared with 80% and 100% among those born up to 36 and 48 months prior to adoption of a water source containing negligible fluoride concentration (27). Pendrys and co-workers (8, 28) reported that fluoride supplementation during the first year of life was less important than supplementation later during the second to sixth years in fluorosis development on early-erupting permanent teeth in nonfluoridated communities. Therefore, controversy persists regarding the timing of fluorosis development and additional studies are needed.

It should be noted that most of these studies were retrospective and cross-sectional, and many investigated only a single source of fluoride intake. In most, the estimates of fluoride exposure and intake were not actual, individual determinations, but categorical only. Therefore, the present study provided a unique opportunity as fluoride intake from multiple sources was assessed simultaneously at the individual level with longitudinal data from birth to 48 months. Thus, the results can help advance the understanding of the timing of fluorosis development as this type of longitudinal, period-specific data allowed for more detailed analyses of the association of timing of the fluoride intake with fluorosis.

Although the first 2 years of life generally were found to be more important compared with later years, fluoride intake during each individual year was associated with fluorosis on both maxillary central incisors. Moreover, the results indicated that subjects with ingestion of higher levels of fluoride during the whole 3 years had the highest risk for fluorosis. Therefore, fluorosis development appears to relate not only to the timing of fluoride intake relative to the stages of enamel formation, but also to the cumulative duration of such a fluoride level. This result is consistent with the possible mechanisms of fluorosis etiology suggested by other researchers (11, 29, 30), that fluorosis is more severe when high-level exposure occurs in both the secretory and maturation stages and fluorosis may develop in teeth exposed to excessive fluoride during periods exclusive of the critical period. It has been shown that an acute high dose of fluoride at the secretory stage alone could induce fluorosed enamel (31), and fluoride also could affect the maturation stage of enamel formation without prior exposure of secretory enamel to fluoride (32), although most available data suggest that the early maturation stage is most sensitive to the effects of fluoride exposure (9, 29, 33). Therefore, the critical period or WMS seems to be relative to other periods and, thus, should be viewed as the period when risk of fluorosis is maximal, but not as the only time when there is fluorosis susceptibility.

The findings from this study should be interpreted in the context of study limitations. Incomplete

questionnaire data, which is an unavoidable problem in longitudinal studies, made only 191 subjects to be available after 4 years of life for multivariable logistic regression analyses. The cohort is a convenience sample of families with relatively high socioeconomic status. Fluoride intake data were obtained through self-administered questionnaires by parents without direct verification. These estimates were based on assessment at 3–4 points during each year and do not fully account for period variations in intake. Some potentially important sources of fluoride, such as fluoride rinses and gels, were not included in these analyses.

A previous report concerning this study cohort (16) suggested that average total fluoride intakes appeared to be relatively stable very early in life and then at 20 months and later, with a transitional period between 6 and 20 months. From birth to 36 months, dietary fluoride supplement intake was low and stable, fluoride ingested from dentifrice increased substantially from 6 to 24 months and then generally leveled off, and fluoride from water (from water itself and added to foods/beverages) generally increased from 12 to 36 months (16, 17). However, due to the growth of the children, the total fluoride intake/kg bw decreased from 12 to 36 months (17). This transitional period of importance in the etiology of fluorosis development could provide opportunities for health practitioners to assess the amount of fluoride intake and educate patients to establish appropriate dietary habits and toothbrushing practices, so that the best balance between the benefits in caries prevention and risk of fluorosis from fluoride use could be achieved. Of course, additional study needs to be done in this area.

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