Original Research

Associations between Intakes of Fluoride from Beverages during Infancy and Dental Fluorosis of Primary Teeth

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Key words: beverages, fluoride, fluorosis, children

Objective: We describe associations between primary tooth fluorosis status and intakes of beverages and fluoride from these beverages during infancy.

Methods: Subjects (n = 677) are members of the Iowa Fluoride Study, a cohort of young children followed from birth. Food and nutrient intakes were obtained from 3-day diet records. Diets were analyzed at 6 weeks, 3, 6, 9, 12 and 16 months and cumulatively for 6 weeks through 16 months of age. Primary tooth fluorosis was assessed at 4.5–6.9 years of age and defined as present or absent. Multiple logistic regression analyses were used to develop models to predict fluorosis status.

Results: Water-based beverage intakes were higher in subjects with fluorosis than in those without. Specifically, higher intakes of water used to reconstitute formulas at 3, 6 and 9 months; any intake of water as a beverage at 16 months; and higher intakes of combined 100% juice and miscellaneous beverages at 16 months were positively associated with fluorosis ($p < 0.05$). Fluoride intakes from water sources were also higher in subjects with fluorosis than in those without. Specifically, higher intakes of fluoride from water used to reconstitute formulas at 3, 6, 9 and 12 months and for 6 weeks through 16 months, and higher intakes of fluoride from water as a beverage at 16 months and for 6 weeks through 16 months were positively associated with fluorosis ($p < 0.05$).

Conclusion: Infant beverages, particularly infant formulas prepared with fluoridated water, can increase the risk of fluorosis in primary teeth.

The role of fluoride in dental caries prevention is well known. Topical exposure to fluoride inhibits the caries process by limiting demineralization through its presence in enamel as fluorapatite; aiding remineralization through absorption to partially demineralized enamel lesions; and interfering with enzymatic activity of oral bacteria [1,2]. Caries rates have declined in both fluoridated and nonfluoridated communities [3]. This decline has been attributed to the fluoridation of public water systems and the widespread availability of fluoride from oral health care products (e.g., dentifrices) [3].

Ingestion of excessive fluoride during tooth development can cause structural changes in tooth enamel. The mechanisms by which excessive uptake of fluoride interferes with tooth development are not clearly understood, but altered protein metabolism appears to produce a disorganized crystal structure and hypomineralization [4,5]. The resulting condition, dental fluorosis, is observed in both primary and permanent teeth. Effects in permanent teeth are typically esthetic (Fig. 1) and, on rare occasion, structural. The prevalence of fluorosis in permanent teeth in areas with fluoridated water has increased from

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Abbreviations: IFS = Iowa Fluoride Study, ppm = parts per million.

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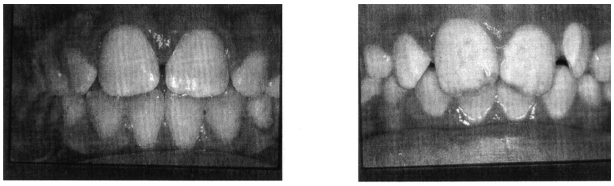

(a) (b)

Fig. 1. Examples of mild (a) and severe (b) esthetic effects of dental fluorosis in permanent teeth.

about 10%–15% in the 1940s to as high as 70% in recent studies, while during the same time period caries rates have declined by about 50–60% [6–9]. Most observed cases of fluorosis in permanent teeth are mild, but can have an esthetic effect [8–12]. However, the presence of fluorosis suggests that excessive fluoride was consumed during critical periods of tooth development; knowledge of the relative importance of fluoride sources is important in understanding fluorosis development in order to refine recommendations for fluoride use, while maintaining caries prevention.

Fluoride is typically ingested from dietary sources or from oral care products. The major source of dietary fluoride is fluoridated water. In addition to fluoride from water consumed as a beverage, foods and beverages prepared with fluoridated water will reflect the water's fluoride content. Oral health care products containing fluoride include dentifrices, mouth rinses and gels, as well as oral fluoride supplements for infants and young children.

Previous studies found that fluoridated water supplies, infant formulas, supplements and dentifrices contribute to fluorosis in the permanent teeth [13–20] and could contribute to fluorosis in the primary teeth. Pendrys *et al*. [14] reported that fluorosis in early developing permanent teeth was typically associated with infant formulas, supplements and dentifrices, and that fluorosis in later developing permanent teeth was associated with fluoride from supplements and dentifrices. Of significance, formulas consumed by participants in the study by Pendrys and coworkers [21] were manufactured prior to the industry's voluntary reduction of fluoride concentrations in the early 1980s. Ismail *et al*. [16] reported that exposure to greater than 2 ppm fluoride concentrations from well water during the first year of life increased risk of fluorosis in permanent mandibular and maxillary central incisors.

Fluorosis development is not fully understood, with even less known about primary tooth fluorosis. Primary tooth fluorosis is less common and usually less severe than permanent tooth fluorosis. The critical exposure period for primary teeth may occur prior to or at the same time as that for permanent teeth. The critical period for development of fluorosis in late developing primary teeth is 4 months gestational age through 11 months of age [22,23]. The critical period for development of fluorosis in permanent maxillary central incisors is 4 months through 4 years [23–25]. Not surprisingly, primary tooth fluorosis is predictive of permanent tooth fluorosis [22, 26]. Therefore, it is important to understand factors that contribute to primary tooth fluorosis as its presence foreshadows more problematic fluorosis in the early erupting permanent teeth. The risk of primary tooth fluorosis associated with beverages providing infant nutrition has not been thoroughly investigated. Infant beverages [27–30] and foods [31] are known to have varying fluoride concentrations that can be further influenced by water used in preparation. Levy *et al*. [13] reported that total fluoride intake, mostly from water (all sources), between the ages of 6 and 9 months was associated with fluorosis in the primary second molars. Fluoride intakes from beverages are dependent on both the quantity of beverages consumed and the concentration of fluoride in the beverages. We hypothesized that the type and quantity of beverage consumed during infancy could affect fluorosis risk in primary teeth, and that this risk is due mostly to the fluoride concentration of water used in reconstituting beverages. In this paper, we describe associations between primary tooth fluorosis and intakes of beverages and fluoride from these beverages in a cohort of young children.

METHODS

Subjects

Subjects were participants in the Iowa Fluoride Study (IFS), a longitudinal investigation of dietary and non-dietary fluoride exposures and the relationships between fluoride exposures and dental fluorosis and caries [13,22,27–37]. Mothers of newborn infants were recruited from 1992 to 1995 for their children's participation. Dental examinations were completed on the primary dentition of 690 children at 4.5–6.9 years of age [22,37]. Subjects who participated in dental examinations and whose parents completed 3-day food and beverage diaries are the focus of this report (n = 677). The Institutional Review Board at the University of Iowa approved all components of the IFS; written informed consent was obtained from mothers at recruitment and at the examination.

Data Collection

Parents were mailed IFS questionnaires and 3-day food and beverage diaries when their children were 6 weeks of age, 3, 6, 9 and 12 months of age, every 4 months through 3 years of age and every 6 months thereafter. IFS questionnaires were designed to obtain information regarding the child's beverage intake, general health and oral health behaviors.

Children underwent dental examinations in the General Clinical Research Center at The University of Iowa or in one of several community locations. Dental fluorosis examinations were visual and all were conducted using a portable chair and exam light by one of two trained and calibrated examiners [22,37].

Dental Fluorosis

The criteria for dental fluorosis in primary teeth were adapted from the Tooth Surface Index of Fluorosis for permanent teeth [22,38]. Fluorosis was distinguished from non-fluoride opacities based on differences in shape, demarcation and color [39]. In addition, fluorosis was distinguished from enamel demineralization or early cavity development (i.e., white-spot lesions not yet requiring treatment) by appearance, location and plaque accumulation patterns. Fluorosis was considered a dichotomous variable for this study; subjects were categorized as with or without fluorosis. Among subjects with fluorosis, all but one had fluorosis on second primary molars (e.g., late developing primary teeth).

Diet Analyses

Three-day food and beverage diaries completed when their children were 6 weeks of age and 3, 6, 9, 12 and 16 months of age were used in these analyses. Inclusion in area-under-the-curve analyses (i.e., a weighted average of the 6-week through 16-month intakes) required a minimum of 4 diaries, including the 6-week and 16-month diaries (n = 410). The area-under-the-curve was calculated using the trapezoidal rule.

On each diary, parents were asked to record the types and quantities of all foods and beverages consumed by their children for one weekend day and two weekdays. Detailed information was requested regarding beverages, including brand names, types of preparation, and sources of water used in preparation. All entries from the 3-day food and beverage diaries were coded and verified by registered dietitians to create a Food and Beverage Intake Table [36]. Human milk intakes were estimated using volume-based models of mean intakes established by Fomon *et al*. [40]. We assigned intakes ranging from 5.4 oz human milk/kg body weight/day at 6 weeks to 4.6 oz/kg body weight/day at 12 months. If infant foods and beverages were consumed in addition to human milk, the intake of human milk was adjusted by subtracting the volume of foods and beverages consumed [36].

The fluoride concentrations of foods, beverages and waters were placed in a Fluoride Concentration Table. As part of the IFS, non-municipal home and childcare water, filtered municipal water and beverages available for purchase by our subjects have been analyzed for fluoride [27,29–31]. Fluoride concentrations of water from public water systems were obtained from the Iowa State Health Department. Fluoride values of foods were assayed in this laboratory, obtained from data by Ophaug and co-workers [Levy—personal communication] and Featherstone and co-workers [41] or estimated from ingredient fluoride concentrations (i.e., the fluoride concentration of cheeseburgers was estimated from the fluoride concentrations of hamburger, cheese and bun).

A Nutrient Table was created from nutrient data obtained from the USDA (Nutrient Database for Standard Reference 12, Agriculture Research Service), the Minnesota Nutrient Database (Nutrition Coding Center NDS-R, Version 4.01, Minneapolis, MN) and manufacturers' data. Food and beverage weights and percentage added water variables were used from this table to calculate subject-specific fluoride intakes from added water.

A relational database (Microsoft Access, Version SR-1; Redmond, WA) was used to link the Food and Beverage Intake Table, Fluoride Concentration Table and Nutrient Table for calculation of total daily fluoride intake. For beverages and foods with water added during preparation (e.g., infant formulas, juice concentrates, infant cereals), the fluoride content of the water at the site of preparation (e.g. home, childcare) was used to determine the fluoride content of the prepared beverage or food.

Beverages were classified according to beverage type (i.e., soy-based formula, milk-based formula, human milk, cows' milk, 100% juice, water). Formulas were further categorized based on type of preparation (i.e., ready-to-feed, powder concentrate, liquid concentrate). Since few subjects consumed soy-based formulas, they are not reported as a separate category, but are included as part of infant formulas. Intakes of sugared and sugar-free juice drinks, sports drinks, beverages reconstituted from powder and soda-pop were minimal at the ages studied and, therefore, combined to form one category (e.g., miscellaneous beverages). Weighted averages based on weekend and weekday consumption were calculated to reflect average consumption over a week.

Non-Dietary Fluoride Intake

IFS questionnaires completed at the same time as the 3-day food and beverage diaries were used to estimate fluoride consumption from fluoride supplements and dentifrices [13,32–34].

Statistical Analyses

Analyses were conducted using SAS (SAS, Version 8; Cary, NC). Subject characteristics were categorized and are presented as percentages. Daily beverage intakes and fluoride intakes from beverages are presented as medians (25th, 75th percentiles). The Wilcoxon rank-sum test was used to compare distributions of beverage and fluoride intakes between subjects with and without fluorosis. A *p*-value of <0.05 was considered statistically significant.

Categorical variables were used to develop statistical models to predict fluorosis since not all relationships between beverage and fluoride intakes and fluorosis were linear. Intakes were categorized into three levels: none (nonconsumers), low and high intakes. Low and high intakes were defined as below or above the median intake of consumers. Each three-category variable was represented by two indicator variables in fluorosis prediction models using the LOGIST procedure in SAS. Multiple logistic regression models were developed to predict fluorosis status separately from beverage intakes and from fluoride intakes. Backward elimination was used to reduce the number of variables in the models. Final models included only variables that were significant at $p < 0.05$.

RESULTS

Demographic characteristics of the 677 subjects and their parents at enrollment into the IFS are presented in Table 1. Parents were well educated and 85% of household incomes were $20,000 or greater. Mothers were primarily Caucasian (97%), similar to the racial distribution of Iowa. Fluorosis prevalence was 11.1%; demographic characteristics at enrollment did not differ by fluorosis status.

The distribution of dietary and non-dietary sources of fluoride at each age is summarized in Fig. 2. The estimated mean percentages of total fluoride from beverages and supplements decreased, while the contributions from food and dentifrice increased with age. Estimated mean total fluoride intakes were 285, 396, 497, 539, 392 and 476 μg/day at 6 weeks and 3, 6, 9, 12 and 16 months, respectively. Estimated fluoride intakes from dentifrice did not differ between subjects with and without fluorosis at 6 weeks, 3, 6, 9, 12 months [0.00 (0.00, 0.00) *vs*. 0.00 (0.00, 0.00) μg/day; $p > 0.05$] and 16 months [20.23 (0.00, 72.50) *vs*. 18.05 (0.00, 109.40) μg/day; $p > 0.05$]. Estimated median fluoride intakes from supplements did not differ between subjects with and without fluorosis at any time point [0.00 (0.00, 0.00) *vs*. 0.00 (0.00, 0.00) μg/day; $p > 0.05$].

Table 1. Demographic Characteristics of Subjects and Their Families at Enrollment from 1992–1995 (%)*

Gender		
Male	48.3	
Female	51.7	
Birth Order		
First child	41.0	
Other	59.0	
Household Income		
<$19,999	12.6	
$20–39,999	36.0	
$40–59,999	29.5	
≥$60,000	18.5	
Unknown	3.4	
Age	Mother	Father
16–24	17.4	7.1
25–29	32.4	26.4
30–34	31.2	33.5
35+	19.1	27.9
Unknown		5.0
Education	Mother	Father
High school/GED or less	19.8	26.6
Some college	34.7	27.9
4 years college or more	45.5	40.6
Unknown		4.9

* n = 677 subjects.

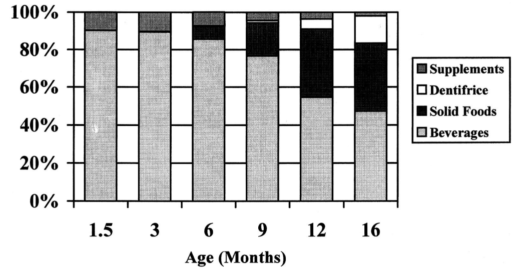

Fig. 2. Estimated mean percentages of fluoride ingested from beverages, solid foods, dentifrice and supplements at 6 weeks and 3, 6, 9, 12 and 16 months of age.

Daily intakes of beverages consumed by subjects at each age were compared by fluorosis status at 4.5–6.9 years of age. Intakes of human milk, total infant formula (i.e., milk- and soy-based ready-to-feed, powdered and liquid concentrates), total milk-based formula (i.e., ready-to-feed, powdered and liquid concentrates), 100% juice and miscellaneous beverages at 6 weeks and 3, 6, 9, 12 and 16 months did not differ between subjects with and without fluorosis. Intakes of milk-based formulas reconstituted from powder were higher in subjects with fluorosis than without fluorosis at 6 months [539 (0, 790) *vs*. 50 (0, 672) g/day; $p < 0.01$] and 9 months [526 (0, 681) *vs*. 0 (0, 610) g/day; $p < 0.001$] and for 6 weeks through 16 months [287 (31, 459) *vs*. 90 (0, 329) g/day; $p < 0.01$]. Intakes of milk-based formulas reconstituted from liquid concentrate were lower in subjects with fluorosis than without fluorosis at 9 months [0 (0, 0) *vs*. 0 (0, 0) g, 95%: 107 *vs*. 760 g/day; $p < 0.05$]. Intakes of cows' milk were lower in subjects with fluorosis than without fluorosis at 6 months [0 (0, 0) *vs*. 0 (0, 0) g, 95%: 0 *vs*. 57 g/day; $p < 0.05$], 9 months [0 (0, 0) *vs*. 0 (0, 44) g, 95%: 549 *vs*. 771 g/day; $p < 0.05$] and 16 months [362 (205, 540) *vs*. 466 (271, 635) g/day; $p < 0.05$]. Intakes of water as a beverage were higher in subjects with fluorosis than without fluorosis at 16 months [97 (36, 191) *vs*. 72 (0, 152) g/day; $p < 0.05$].

Daily fluoride intakes from beverages consumed by subjects at each age were compared by fluorosis status (Table 2). Fluoride intakes from infant formulas were higher in subjects with fluorosis than without fluorosis at 3, 6, 9 and 12 months and for 6 weeks through 16 months ($p < 0.05$). Fluoride intakes from milk-based formulas were also higher in subjects with fluorosis than without fluorosis at 6 and 9 months and for 6 weeks through 16 months ($p < 0.05$). Fluoride intakes from milk-based formulas reconstituted from powder were higher in subjects with fluorosis than without fluorosis at 6 weeks and 3, 6, 9 and 12 months and for 6 weeks through 16 months ($p < 0.05$). Fluoride intakes from milk-based formulas reconstituted from liquid concentrate were lower in subjects with fluorosis than without fluorosis at 9 months ($p < 0.05$). Fluoride intakes from cows' milk were lower in subjects with fluorosis than

Table 2. Median (25th, 75th percentiles) of Fluoride Intakes (μg/day) from Beverages at Each Age by Fluorosis Status at 4.5–6.9 Years of Age

Beverage	With Fluorosis	Without Fluorosis	Wilcoxon Z Score	*p*-Value
6 weeks:	n = 71	n = 566		
Human milk	1 (0,7)	3 (0,7)	−0.753	0.451
Infant formula (e.g., soy- and milk-based; ready-to-feed, powder and liquid concentrates)	166 (0,701)	77 (0,421)	1.954	0.051
Milk-based formula (e.g., ready-to-feed, powder and liquid concentrate)	49 (0,597)	18 (0,251)	1.286	0.199
Reconstituted from powder concentrate	11 (0,452)	0 (0,89)	2.411	0.016
Reconstituted from liquid concentrate	0 (0,0)	0 (0,0)	−1.124	0.261
Cows' milk	0 (0,0)	0 (0,0)	−0.792	0.428
100% juice (e.g., ready-to-feed, concentrates)	0 (0,0)	0 (0,0)	0.276	0.783
Water (e.g., as a beverage)	0 (0,0)	0 (0,0)	0.023	0.982
Miscellaneous beverages (e.g., juice drinks, sports drinks, beverages from powder, soda-pop)	0 (0,0)	0 (0,0)	−0.612	0.541
Total beverages	171 (10,701)	80 (9,430)	1.815	0.070
3 months:	N = 72	N = 573		
Human milk	0 (0,7)	0 (0,7)	−0.548	0.584
Infant formula (e.g., soy- and milk-based; ready-to-feed, powder and liquid concentrates)	295 (44,837)	157 (9,545)	2.148	0.032
Milk-based formula (e.g., ready-to-feed, powder and liquid concentrate)	146 (0,799)	60 (0,429)	1.780	0.075
Reconstituted from powder concentrate	55 (0,760)	0 (0,240)	2.271	0.023
Reconstituted from liquid concentrate	0 (0,0)	0 (0,0)	−1.387	0.166
Cows' milk	0 (0,0)	0 (0,0)	−0.870	0.385
100% juice (e.g., ready-to-feed, concentrates)	0 (0,0)	0 (0,0)	−0.042	0.967
Water (e.g., as a beverage)	0 (0,0)	0 (0,0)	0.885	0.376
Miscellaneous beverages (e.g., juice drinks, sports drinks, beverages from powder, soda-pop)	0 (0,0)	0 (0,0)	0.636	0.525
Total beverages	298 (51,837)	171 (23,563)	2.273	0.023
6 months:	n = 70	n = 548		
Human milk	0 (0,0)	0 (0,3)	−0.944	0.345
Infant formula (e.g., soy- and milk-based; ready-to-feed, powder and liquid concentrates)	710 (165,990)	214 (60,617)	4.896	<0.001
Milk-based formula (e.g., ready-to-feed, powder and liquid concentrate)	486 (51,919)	119 (0,462)	3.747	<0.001
Reconstituted from powder concentrate	369 (0,912)	20 (0,358)	4.071	<0.001
Reconstituted from liquid concentrate	0 (0,0)	0 (0,0)	−1.522	0.128
Cows' milk	0 (0,0)	0 (0,0)	−2.238	0.025
100% juice (e.g., ready-to-feed, concentrates)	0 (0,19)	0 (0,20)	−0.391	0.696
Water (e.g., as a beverage)	0 (0,39)	0 (0,11)	0.026	0.976
Miscellaneous beverages (e.g., juice drinks, sports drinks, beverages from powder, soda-pop)	0 (0,0)	0 (0,0)	0.367	0.714
Total beverages	809 (215,1041)	269 (89,675)	4.910	<0.001
9 months:	n = 68	n = 516		
Human milk	0 (0,0)	0 (0,0)	−1.423	0.155
Infant formula (e.g., soy- and milk-based; ready-to-feed, powder and liquid concentrates)	638 (190,820)	201 (13,624)	4.420	<0.001
Milk-based formula (e.g., ready-to-feed, powder and liquid concentrate)	601 (24,801)	97 (0,516)	4.132	<0.001
Reconstituted from powder concentrate	601 (0,793)	0 (0,377)	4.877	<0.001
Reconstituted from liquid concentrate	0 (0,0)	0 (0,0)	−2.279	0.023
Cows' milk	0 (0,0)	0 (0,1)	−2.367	0.018
100% juice (e.g., ready-to-feed, concentrates)	10 (0,66)	9 (0,50)	0.522	0.602
Water (e.g., as a beverage)	14 (0,64)	2 (0,41)	1.421	0.155
Miscellaneous beverages (e.g., juice drinks, sports drinks, beverages from powder, soda-pop)	0 (0,0)	0 (0,0)	−1.584	0.113
Total beverages	734 (304,940)	312 (114,697)	4.556	<0.001
12 months:	n = 62	n = 465		
Human milk	0 (0,0)	0 (0,0)	−0.600	0.549

(*Table continues*)

Table 2. (*Continued*)

Beverage	With Fluorosis	Without Fluorosis	Wilcoxon Z Score	*p*-Value
Infant formula (e.g., soy- and milk-based; ready-to-feed, powder and liquid concentrates)	0 (0,209)	0 (0,50)	1.986	0.047
Milk-based formula (e.g., ready-to-feed, powder and liquid concentrate)	0 (0,150)	0 (0,0)	1.526	0.127
Reconstituted from powder concentrate	0 (0,127)	0 (0,0)	2.050	0.040
Reconstituted from liquid concentrate	0 (0,0)	0 (0,0)	−1.0010	0.317
Cows' milk	8 (1,10)	9 (3,13)	−1.649	0.099
100% juice (e.g., ready-to-feed, concentrates)	30 (0,74)	33 (0,82)	0.005	0.996
Water (e.g., as a beverage)	29 (0,127)	14 (0,71)	1.630	0.103
Miscellaneous beverages (e.g., juice drinks, sports drinks, beverages from powder, soda-pop)	0 (0,6)	0 (0,7)	0.224	0.823
Total beverages	228 (128,435)	133 (68,315)	3.007	0.003
16 months:	n = 56	n = 398		
Human milk	0 (0,0)	0 (0,0)	−0.747	0.455
Infant formula (e.g., soy- and milk-based; ready-to-feed, powder and liquid concentrates)	0 (0,0)	0 (0,0)	−0.196	0.845
Milk-based formula (e.g., ready-to-feed, powder and liquid concentrate)	0 (0,0)	0 (0,0)	−0.495	0.621
Reconstituted from powder concentrate	0 (0,0)	0 (0,0)	0.024	0.981
Reconstituted from liquid concentrate	0 (0,0)	0 (0,0)	−0.647	0.518
Cows' milk	7 (5,10)	9 (6,12)	−2.772	0.006
100% juice (e.g., ready-to-feed, concentrates)	81 (9,132)	50 (4,113)	1.267	0.205
Water (e.g., as a beverage)	79 (14,159)	30 (0,103)	2.905	0.004
Miscellaneous beverages (e.g., juice drinks, sports drinks, beverages from powder, soda-pop)	0 (0,61)	3 (0,50)	−0.099	0.921
Total beverages	232 (145,389)	175 (94,284)	2.946	0.003
6 weeks-16 months	n = 51	n = 359		
Human milk	0 (0,2)	0 (0,3)	−0.638	0.523
Infant formula (e.g., soy- and milk-based; ready-to-feed, powder and liquid concentrates)	337 (163,505)	161 (54,351)	3.572	<0.001
Milk-based formula (e.g., ready-to-feed, powder and liquid concentrate)	287 (73,493)	96 (11,266)	3.719	<0.001
Reconstituted from powder concentrate	266 (34,493)	46 (0,221)	3.587	<0.001
Reconstituted from liquid concentrate	0 (0,0)	0 (0,3)	−1.376	0.169
Cows' milk	3 (2,4)	3 (2,5)	−2.098	0.036
100% juice (e.g., ready-to-feed, concentrates)	33 (15,61)	25 (10,51)	1.381	0.167
Water (e.g., as a beverage)	43 (14,62)	16 (4,42)	3.171	0.002
Miscellaneous beverages (e.g., juice drinks, sports drinks, beverages from powder, soda-pop)	1 (0,11)	2 (0,13)	−0.820	0.412
Total beverages	457 (245,635)	268 (117,451)	3.902	<0.001

without fluorosis at 6 months, 9 months, 16 months and for 6 weeks through 16 months ($p < 0.05$). Fluoride intakes from water as a beverage were higher in subjects with fluorosis than without fluorosis at 16 months and for 6 weeks through 16 months ($p < 0.01$). Fluoride intakes from all beverages were higher in subjects with fluorosis than without fluorosis at 3, 6, 9, 12 and 16 months and for 6 weeks through 16 months ($p < 0.05$).

Infant formulas were investigated further. Daily fluoride intakes from the purchased component (e.g., milk- and soy-based ready-to-feed, powder concentrate and liquid concentrate) and the water used to reconstitute formulas were compared separately by fluorosis status. Fluoride intakes from the purchased formula components were higher in subjects with fluorosis than without fluorosis at 6 months [96 (41, 117) *vs.* 57 (21, 105) μg/day; $p < 0.05$] and 9 months [65 (30, 96) *vs.* 42 (3, 87) μg/day; $p < 0.05$]. Fluoride intakes from water used to prepare formulas were higher in subjects with fluorosis than without fluorosis at 6 weeks [60 (0, 591) *vs.* 11 (0, 314) μg/day; $p < 0.05$], 3 months [194 (5, 716) *vs.* 36 (0, 437) μg/day; $p < 0.05$], 6 months [579 (109, 880) *vs.* 119 (7, 507) μg/day; $p < 0.001$], 9 months [564 (121, 713) *vs.* 112 (0, 530) μg/day; $p < 0.001$] and 12 months [0 (0, 189) *vs.* 0 (0, 10) μg/day; $p < 0.05$] and for 6 weeks through 16 months of age [283 (81, 440) *vs.* 108 (13, 291) μg/day; $p < 0.001$].

The fluoride concentrations in water used to reconstitute formulas were compared. Subjects with fluorosis had higher water fluoride concentrations than subjects without fluorosis at 6 weeks, 6, 9 and 12 months (data not shown, $p < 0.05$).

Multiple logistic regression models were developed for 6 weeks, 3, 6, 9, 12 and 16 months and for 6 weeks through 16 months to predict primary tooth fluorosis using categories of beverage intakes. Initial models included human milk, cows' milk, water as a beverage, 100% juice and miscellaneous beverages (combined), purchased milk-based formula components and water used to reconstitute formulas. High intakes of water used to reconstitute formulas (*vs*. none or low) at 3 months ($p < 0.05$), 6 months ($p < 0.001$) and 9 months ($p < 0.001$) were positively associated with fluorosis. Any consumption of water as a beverage (*vs*. none) at 16 months ($p < 0.05$) and high intakes of combined 100% juice and miscellaneous beverages (*vs*. none or low) at 16 months ($p < 0.05$) were positively associated with fluorosis. High intakes of fluid milk (*vs*. none or low) for 6 weeks through 16 months ($p < 0.05$) were negatively associated with fluorosis, while high intakes of water used to reconstitute formula (*vs*. none or low) for 6 weeks through 16 months ($p < 0.05$) were positively associated.

Multiple logistic regression models were also developed for 6 weeks, 3, 6, 9, 12 and 16 months and for 6 weeks through 16 months to predict primary tooth fluorosis from fluoride intakes from the various categories. Initial models included fluoride from water as a beverage, combined 100% juice and miscellaneous beverages, purchased milk-based formula components, water used to reconstitute formula, ingested dentifrice and supplements. High intakes of fluoride from water used to reconstitute formulas (*vs*. none or low) at 3 months ($p < 0.05$), 6 months ($p < 0.001$), 9 months ($p < 0.001$) and 12 months ($p < 0.05$) were positively associated with fluorosis. High intakes of fluoride from water as a beverage (*vs*. none or low) at 16 months ($p < 0.01$) were positively associated with fluorosis. High intakes of fluoride from water used to reconstitute formulas (*vs*. none or low; $p < 0.001$) and from water as a beverage (*vs*. none or low; $p < 0.05$), and any fluoride from supplements (*vs*. none; $p < 0.001$) for 6 weeks through 16 months were positively associated with fluorosis. Two-way interactions between variables were not significant.

DISCUSSION

We are among the first to investigate associations between beverages, fluoride from these beverages and risk of primary tooth fluorosis. Results described herein support our hypothesis that beverages consumed during infancy influence risk of primary tooth fluorosis. These results are important because primary tooth fluorosis is predictive of permanent tooth fluorosis; understanding primary fluorosis development and identifying those at risk could help to refine recommendations to reduce permanent tooth fluorosis risk [22–26].

Infant formulas were identified during the 1970s as a significant source of ingested fluoride and a risk factor for permanent tooth fluorosis [42–43]. In response to these findings, formula manufacturers in the United States voluntarily reduced fluoride concentrations of the purchased components [21]. The fluoride concentrations of the purchased component of formulas consumed by our subjects ranged from 0.04–0.25 ppm for milk-based formulas and 0.04–0.47 ppm for soy-based formulas [19]. Our results suggest that the fluoride contribution of water used to reconstitute formulas increases risk of fluorosis and could be an area for intervention.

Consumption of milk-based formulas reconstituted from powder by our subjects was associated with risk of fluorosis: quantities consumed by subjects with fluorosis were higher and the fluoride concentration of water used for reconstitution by subjects with fluorosis was higher. Although these observations were limited to milk-based formulas, we suspect that, given sufficient power, similar results would be observed for soy-based formulas. We did not distinguish between subjects who consumed normal quantities of formula prepared with high water fluoride concentrations and those who consumed excessive quantities of formula prepared with lower water fluoride concentrations; both would be expected to increase total fluoride intake and risk of fluorosis.

Fluoride concentrations of both human milk (0.005–0.010 ppm) and cows' milk (0.03–0.06 ppm) are low, and consumption of either human milk or cows' milk could decrease fluorosis risk. In other words, their fluoride concentrations are so low that excessive consumption would not provide adequate fluoride to disrupt enamel development. Partial or full replacement of higher fluoride containing beverages (e.g., formula reconstituted with fluoridated water) by human milk or cows' milk would decrease fluoride intake. Although human milk did not appear to protect against fluorosis in our subjects, intakes declined with time and may not have been of sufficient duration to affect fluorosis risk. Walton and Messer [19] reported that a minimum of three months' duration of breastfeeding was necessary to decrease fluorosis risk in permanent teeth. Supporting long-term lactation could be an important strategy to decrease fluorosis risk of primary teeth and early developing permanent teeth. Cows' milk consumption was associated with reduced fluorosis risk in our subjects. Cows' milk is not recommended before one year of age [44], but intake after one year of age could be important for reducing fluorosis risk in permanent teeth.

At the ages studied, the subjects in our study consumed small quantities of both water as a beverage and miscellaneous beverages. However, intakes of water as a beverage and combined 100% juice and miscellaneous beverages at 16 months were associated with increased risk of fluorosis. Water could have been from the same source as water used for formula reconstitution. If so, identification of the water source at the time fluorosis is first observed could allow for intervention and limiting the risk of fluorosis in developing permanent teeth.

Although supplement use entered the final model as a source of fluoride increasing risk of fluorosis, few of our subjects consumed supplements. Dentifrice use by our subjects did not appear to increase risk of primary tooth fluorosis at the ages studied.

Our study has several limitations. Dietary data were reported by the parent or caregiver (e.g., babysitter) and may not have reflected actual consumption. Fluoride concentrations of beverages available for purchase were assayed; however, distribution to east-central Iowa from multiple production sites resulted in a range of fluoride concentrations available for some products. The mean fluoride concentration of products analyzed was applied to the product, while actual consumption may have been limited to a concentration at either end of the range. Thus, fluoride intakes may have been over- or underestimated. Subjects and their families are generally from middle-income, educated families and are not representative of subjects throughout the United States.

CONCLUSIONS

The types of beverages and fluoride concentrations of beverages influences the risk of primary tooth fluorosis. Our data suggest that the fluoride contribution of water used to reconstitute infant feedings is a major determinant of primary tooth fluorosis. Further investigation is needed to identify the optimal fluoride concentration of water to be used for formula reconstitution and determine whether the water used for reconstitution of infant formulas is associated with permanent tooth fluorosis.

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