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Associations of Fluoride Intake with Children's Bone Measures at Age 11

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Abstract

Background—Relationships between fluoride intake and bone health continue to be of interest, as previous studies show conflicting results.

Objectives—The purpose is to report associations of fluoride intake with bone measures at age 11.

Methods—Subjects have been participating in the ongoing Iowa Fluoride Study/Iowa Bone Development Study. Mothers were recruited postpartum during 1992–95 from eight Iowa hospitals, and detailed fluoride questionnaires were sent every 1.5–6 months. From these, combined fluoride intakes from water sources (home, childcare, filtered, bottled), other beverages, selected foods, dietary fluoride supplements, and dentifrice were estimated at individual points and cumulatively (with area-under-the-curve). Subjects received dual-energy x-ray absorptiometry (DXA) scans of proximal femur (hip), lumbar spine and whole body (Hologic QDR 4500A). DXA results (bone mineral content – BMC; bone mineral density – BMD) were related to fluoride intake in bivariate and multivariable analyses.

Results—The mean fluoride intake estimated by AUC was 0.68 mg (SD=0.27) per day from birth to 11 years. Associations (Spearman) between daily fluoride intake (mg F/d) and DXA bone measures were weak ($r = -0.01$ to 0.24 for girls and 0.04 to 0.24 for boys). In gender-stratified, and body size- and Tanner Stage-adjusted linear regression analyses, associations between girls' bone outcomes and fluoride intake for girls were almost all negative, associations for boys were all positive, and none were statistically significant using an $\alpha = 0.01$ criterion.

Conclusions—Longitudinal fluoride intake at levels of intake typical in the United States is only weakly associated with BMC or BMD in boys and girls at age 11. Additional research is warranted to better understand possible gender- and age-specific effects of fluoride intake on bone development.

Introduction

A comprehensive 1991 review stated that “sodium fluoride has clearly been shown to have pronounced effects on the skeleton....”(1) Fluoride (F) is readily absorbed from the gastrointestinal tract under typical conditions and, once absorbed, distributes evenly throughout intra- and extra-cellular spaces (2). Fluoride also has a high affinity for calcium, and within the gastrointestinal tract, calcium can bind to fluoride and form an insoluble salt, which is not absorbed (3). However, fluoride's affinity for calcium can result in a concentration of fluoride in bone, where it stimulates osteoblastic activity and replaces hydroxyl ions in the hydroxyapatite structure (4). In doing so, bone crystal size is increased,

and elasticity is decreased. It has also been reported that fluoride accumulates in the developing skeleton of children at a much faster rate than in adults, so it is plausible that fluoride's effects on developing bone could be significant (5). However, neither the role of fluoride nor the interaction between fluoride and calcium in bone development has been widely studied in children or adolescents.

Published studies that have specifically assessed the effects of fluoride intake on bone measures in children or adolescents are extremely rare, or as stated in the National Research Council's 2006 report, *Fluoride in Drinking Water*, such information is "sparse and sometimes conflicting" (6.) One study from the late 1960s assessed the effects of sodium fluoride on bone density in a population of non-ambulatory children with intellectual disabilities in Texas (7). In this study, 32 children received 0.2 mg fluoride per kilogram body weight twice daily for 18 months, and were compared to a control group (n=32) receiving no fluoride intervention. Bone density of the left heel was assessed using a photometric x-ray technique. At the end of the 18-month study period, bone density for children in the fluoride group increased by 8.1% compared to 3.3% in the control group (7). However, at a re-evaluation 15 months after the cessation of fluoride supplementation, there were no statistically significant differences in bone density between the fluoride and control groups (8). A study in Tanzania (9) used wrist radiographs to compare skeletal age and bone thickness of 11- to 15-year-old girls living in four areas with water fluoride concentrations of < 0.2, 1.5, 2.5 and 3.5 ppm. The results of the study relating bone measures to water fluoride concentrations were inconclusive, but the authors did report an association between increased severity of dental fluorosis and decreased (retarded) bone accrual. Another study compared "unexplained" bone fracture rates among 6- to 12-year-old Mexican children living in areas with water fluoride concentrations ranging from 0 to >12 ppm, and found that the fracture rates were similar across locations (10). A more refined study of young (18- 25-year-old) Canadian women compared lumbar spine and femoral bone density of those who had lived in a low water-fluoride (0.1 ppm) community to those who had lived in an optimally fluoridated (1.0 ppm) community (11). This study controlled for height, weight, physical activity and dietary factors, and it assessed bone density using dual-energy x-ray absorptiometry (DXA). The women who had lived in the optimally fluoridated community had 12% greater bone density in the lumbar spine and nearly 8% greater bone density at L-3 than did those who had lived in the low-fluoride community. There were no differences between the two groups in bone density of the proximal femur or in whole body assessments. The authors concluded that the subjects' exposure to water fluoridation during the growing years could have had a positive effect on the bone density of the spine. Lastly, a two year longitudinal study of Swedish adolescents aged 15 years at baseline found that at age 17, whole body bone mineral content was significantly higher among those residing in an area with water fluoride concentration of 1.1 ppm when compared to those living in an area with water fluoride concentration of 0.1 ppm (12.) The study controlled for several other factors such as body size, physical activity, diet and social factors, and the authors speculated that the differences between regions' water fluoride concentrations may explain the differences; however, they also cautioned that well-controlled studies were needed to support their speculation.

As described above, there has been very little study of the relationship between fluoride intake and bone development, despite ample evidence that fluoride strongly interacts with bone tissues. Thus, there is a clear need for studies that assess the effects of ingested fluoride on bone development in children. Additionally, there is a need to determine if the effects of ingested fluoride are more pronounced for boys vs. girls, during certain stages of development, or at certain bone sites. The purpose of this paper is to report on the relationships between longitudinal fluoride intakes during different time periods and

cumulatively from birth to 11 years of age and DXA bone outcomes at selected sites when children were 11 years old.

Methods

Study children are participants in the Iowa Bone Development Study (IBDS) (13–16), which grew out of the longitudinal Iowa Fluoride Study (IFS) (17–26). With institutional review board approval and parental informed consent, families with newborns were recruited from 8 Iowa hospital postpartum wards from 1992–95 (n=1,882). A total of 1,382 were still participating when their children reached age 6 months. From 1998–2000, families of children then participating in the IFS (n ~800) were invited to also join the IBDS. Age 5 (n=470), age 8 (n=538), and age 11 (n=481) bone examinations were conducted, as described further below.

Parents completed detailed questionnaires at recruitment, as well as mailed questionnaires sent at 1.5, 3, 6, 9, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 months, and every 6 months through age 11 years. The questionnaires were designed to assess numerous important aspects of fluoride intake. This included breast-feeding status during infancy; water sources (home, child care, school, well vs. public, bottled vs. tap, filtered vs. unfiltered); intake of water, other ready-to-drink beverages, and selected foods and beverages made with water; use of dietary fluoride supplements; and use and ingestion of fluoride dentifrice (17,20,22). Validity of the estimates was not assessed specifically, but reliability assessments of selected questions concerning water sources, fluoride supplements, toothbrushing, and use of dentifrice were conducted on a sample of about 5% of respondents by telephone 7–10 days after the initial questionnaire (17). (See Results section.) Individual and filtered water sources were assayed for fluoride content and levels for public water were obtained from state health department records (17,20,23). Selected beverages and foods that were most commonly used were assayed for fluoride content by direct read or microdiffusion procedures (24–26).

Individual daily fluoride intake estimates at each time of response from each source category (water, beverages, selected foods, supplements, and dentifrice) were determined using intake data and fluoride levels and then summed to estimate total daily fluoride intake (mg F/d) (17,20). Using parent-reported child weights, fluoride intake at each time period was estimated per unit body weight (mg F/kg/d) (17,20). Because fluoride intakes over different time periods might have different effects on different bone outcomes, intakes for the periods of 0–36 months, 36–72 months, 72–102 months, 102–132 months, 0–102 months, and 0–132 months were estimated and used for analysis. These period-specific intakes were determined using trapezoidal area-under-the-curve (AUC) estimates. Each AUC required data at the upper and lower endpoints, with such endpoints allowed to be interpolated from estimates within 12 months of the stated endpoints. In addition to the endpoints, the required minimum other numbers of observations for inclusion were: four for 0–3 years, one for each of 3–6 years, 6–8.5 years, and 8.5–11 years, two for 0–8.5 years, and three for 0–11 years. The majority of participants included in these analyses had substantially greater numbers of questionnaires returned and fluoride intake estimates than the minimum number required to be included in each AUC estimate.

Bone measurements (BMD, BMC) were made in the General Clinical Research Center (now called the Clinical Research Unit of the CTSA) at The University of Iowa when the children were approximately 11 years of age. DXA scans of the whole body and lumbar spine (L1 – L4) were acquired using a Hologic QDR 4500A densitometer and analyzed using software version 12.3 (Hologic, Waltham, MA) by one of two International Society of Clinical Densitometry (ISCD)-certified technicians using standard positioning and analysis

procedures as described in the Hologic User's Manual (27). Proximal femur (hip) scans were acquired as described above and analyzed by one ISCD-certified technician using software version 12.6 and the IBDS protocol for pediatric hip analysis (28). Region of interest areal BMD and BMC were obtained from the analysis. The proximal femur included the femoral neck, trochanteric and inter-trochanteric regions. Outcomes of interest were whole body (minus the head) BMC, and spine and hip BMD and BMC. BMD is expressed as grams/cm² and BMC as grams. The densitometer used for this study was calibrated daily with a Hologic hydroxyapatite phantom. The coefficient of variation (CV) for quality control scans on the densitometer in this laboratory is less than 1%. Figures 1A to 1C show examples of DXA scans of the lumbar spine, proximal femur, and whole body, respectively (29).

Tanner Staging

The participants self-reported their pubertal status, aided by standardized drawings using 5 stages of Tanner breast development for girls and 5 stages of genitalia development for boys (30). Parents assisted in the assessments, when necessary. Because there were no statistically significant differences in bone measures between Tanner stage 1 and stage 2 children, and the number of children who had progressed beyond Tanner stage 2 was small, Tanner stage was dichotomized to differentiate between little or no development and sexual dimorphism (≤ 2 vs. ≥ 3) for inclusion as a covariate in multivariable linear regression prediction models for BMD and BMC.

Anthropometric measurements

Anthropometric measurements were obtained for each child at the time the bone measurements were made. Height was measured using a Harpenden stadiometer (Holtrain, United Kingdom), and recorded in tenths of centimeters. Weight was measured using a Healthometer physician scale (Continental, Bridgeview, IL), and recorded in tenths of kilograms. Children were measured while wearing indoor clothes, but without shoes. The stadiometer and scale were routinely monitored for accuracy and precision.

Data Analysis

All statistical analyses were conducted using procedures from the Statistical Analysis System (SAS, version 9.1.3) (OR 9.3.1 – ASK ELENA). Analyses were gender-specific. Means and standard deviations (SD) were calculated to describe the distributional properties of the measures.

Spearman rank-order correlation coefficients were calculated to estimate unadjusted associations between bone measures and fluoride intakes during different time periods. Linear regression models were used to assess relationships between DXA outcomes and estimated fluoride intake during various time periods, adjusting for age, body size (height, weight), and physical development (Tanner Stage). Fluoride intake was first included as a continuous predictor in the set of models for bone outcomes. Then fluoride intake during various time periods was categorized using tertiles of the distributions to investigate the possible non-linear associations between fluoride intake and bone outcomes (hypothesized relatively lower bone measures for either low or high fluoride intake or both). Least squares (LS) means were calculated from adjusted general linear models (SAS PROC GLM). P-values <0.01 were considered statistically significant rather than $p<0.05$, due to the many statistical tests of overlapping fluoride intake estimates that were conducted. For LS means, Bonferroni adjustment was used for multiple comparisons; thus, three pairwise comparisons for bone LS means by fluoride intake time interval tertiles in Tables 4 and 5 were, with p-values less than $0.01/3=0.0033$ considered statistically significant.

Results

Most of the children (97%) were white, with 1% African American and 1% other; 3% were Hispanic (data not shown). The children were mostly from high socio-economic status families. Specifically, 50% of mothers and 43% of fathers had at least a 4-year college degree and 68% had annual family income of at least \$60,000 or more. Nearly one-half of girls (48%) and boys (47%) were self-reported Tanner Stage 2, with about one-third of boys (35%) reporting Stage 1 and one-third of girls (31%) reporting Stage 3. Six percent of girls vs. 2% of boys reported Stage 4–5. Table 1 presents summary descriptive statistics for the outcome bone measures, fluoride intake and adjustment variables, separately for the 251 girls and 230 boys. Mean age at DXA assessment was 11.2 years, mean height was 149.0 cm, and mean weight was 44.7 kg, with similar results for girls and boys.

The mean (SD) numbers of period-specific fluoride intake data points, not including the interpolated endpoints, were 9.1 (1.6) for 0–3 years, 5.8 (1.6) for 3–6 years, 4.1 (1.2) for 6–8.5 years, 3.8 (1.3) for 8.5–11 years, 19.2 (3.1) for 0–8.5 years, and 23.3 (3.7) for 0–11 years (data not shown). Reliability of the selected duplicate water source, dietary fluoride supplements, toothbrushing, and dentifrice assessments were favorable; kappa statistics from birth to 11 years were 0.81 for water filtration status, 0.77 concerning home tap water source, 0.94 concerning use of fluoride supplements, 0.79 (weighted) concerning toothbrushing frequency, and 0.56 (weighted) concerning use of dentifrice.

Average estimated fluoride intake (mg/d, expressed as an AUC) increased from the 0–3 year period to 3–6 years, and then declined slightly for 6–8.5 and 8.5–11 years. Girls consistently had lower mean F intake than boys when expressed in either mg F/d or mg F/kg/d. However, the mean mg F/kg/d declined consistently with increasing age for both girls and boys. Girls had lower mean hip, but greater mean spine and whole body without head BMC. Similarly, mean hip BMD was lower for girls than for boys, while spine BMD was higher.

Estimated daily fluoride intakes during the different time periods were moderately associated, with stronger associations between periods closer to one another (data not shown). For example, correlation coefficients between pairs of 2.5- to 3-year AUC intervals of F intake (mg F/d) ranged from 0.25 for 0–3 years vs. 8.5–11 years to 0.77 for 6–8.5 years vs. 8.5–11 years. In AUC mg F/kg/d units, correlations were similar, but generally slightly lower (ranging from 0.22 for 0–3 vs. 8.5–11 years to 0.77 from 6–8.5 vs. 8.5–11 years).

Tables 2 to 5 show relationships between base outcome measures and daily fluoride intake variables in mg F/d, while Appendices 1 to 4 show them for daily mgF/kg/d. Table 2 presents bivariate associations (Spearman correlation coefficients) between bone outcome measures and fluoride intake variables in mg F/d separately for girls and boys. Gender-specific bivariate patterns were quite different from each other, although all correlations were relatively weak. For girls, correlation coefficients with overall F intake (mg F/d) were consistently lower than for boys (0–8.5 yrs., 0–11 yrs.) and at younger ages (0–3 yrs., 3–6 yrs.), but consistently higher at 6–8.5 and 8.5–11 years, except for spine BMC. All correlation coefficients for girls except spine BMC were ≤ 0.10 for 0–3, 3–6, 0–8.5, and 0–11 years (none statistically significant at $p < 0.01$), and all were 0.08–0.24 for 6–8.5 and 8.5–11 years (4 of 10 statistically significant at $p < 0.01$). For boys, correlation coefficients were 0.10–0.24 for 0–3, 3–6, 0–8.5, and 0–11 years (11 of 20 statistically significant), and were 0.04–0.17 for 6–8.5 years and 8.5–11 years (none significant).

Table 3 presents multivariable linear regression models separately for girls and boys, relating bone outcomes to fluoride intake estimates (mg/d), adjusted for age, height, weight, and Tanner Stage. For girls, all regression coefficients were weak and negative for associations of fluoride intake with hip BMC, whole body BMC, hip BMD, and spine BMD,

and several were negative for spine BMC. No associations were statistically significant ($p < 0.01$). In contrast, for boys, all regression coefficients with all BMC and BMD bone outcomes were positive; however, all were also weak and none were statistically significant ($p < 0.01$).

In order to compare bone measures for children with different levels of fluoride intake, estimated fluoride AUC for 0–3 years, 3–6 years, 0–8.5 years, and 0–11 years were separately divided into tertiles, and separate models were developed for girls and boys, adjusted for height, weight, and Tanner Stage. Results for girls are in Table 4. There were no significant differences in BMC or BMD bone measures (at $p < 0.01$) by tertile of F intake for any of the periods studied. For boys, the associations with fluoride intake were generally positive ones (Table 5), but none were statistically significant.

Parallel analyses relating bone outcome measurements to daily fluoride intake per unit body weight (mg F/kg/d) also found consistently weak associations, with some significant bivariate correlations, but no significant adjusted associations (Appendices 1 to 4.)

Discussion

These results consistently showed weak associations for both boys and girls between all bone outcome measures and all time periods of fluoride intake considered. Boys' associations were consistently positive, while girls' unadjusted associations were generally positive and adjusted ones were generally negative. However, after adjustment, no girls' or boys' bone outcomes were statistically significantly related to any of the period-specific fluoride intake measures.

Relatively few studies have assessed bone development in children, and these studies generally had small numbers of subjects at any specific age and related outcomes to a small number of variables. The results from a normative, cross-sectional study of DXA in children aged 6 to 16 years recently have been published (31).

There are even fewer longitudinal studies of childhood bone development, and the majority have been relatively short-term and/or tested a specific dietary or physical activity intervention (32–34). To our knowledge, there are no longitudinal studies that have looked at associations of estimated total fluoride intake with DXA bone outcomes.

Although fluoride is known to have great affinity for bone and, at high levels, to enhance bone mass and affect bone quality of older adults (35), little is known of fluoride's effects, if any, on normal childhood bone development at lower levels typical in the United States, including from community water fluoridation. As reviewed in the introduction, few studies have related fluoride to childhood bone measures, and the bone assessments were conducted with varied and mostly less refined densitometry machines (7–12). They suggest that fluoride may slightly increase BMD, but the evidence is relatively weak and somewhat conflicting.

Because of the unique longitudinal fluoride intake estimates from the Iowa Bone Development Study, it was possible to estimate mean daily intake for 2.5- to 3.0-year intervals (0–3, 3–6, 6–8.5, and 8.5–11 years), as well as cumulatively (0–8.5 and 0–11 years). Because fluoride intake during any or all of these periods could potentially have been significantly associated with bone outcomes, all these periods were considered in our analysis. Although absolute fluoride AUC in mg/d was primarily emphasized, body weight-adjusted fluoride AUCs were also considered and results are shown in the Appendices, since mg F/kg/d historically has been the most common way for oral health researchers to quantify fluoride intake in young children who often have quite varied body weights.

Because there were multiple ways to consider the correlated fluoride intake estimates, $p < 0.01$ (rather than $p < 0.05$) was the level of significance used in statistical analyses in order to address the multiple hypothesis tests.

Study results generally show weak bivariate associations between fluoride intake and DXA bone outcomes, with relatively few significant relationships ($p < 0.01$). Furthermore, for all significant bivariate correlations, none were significant after adjustment for exam age, body size (height and weight) and Tanner stage.

For both girls and boys, there were consistently weak associations between all 5 bone outcomes and all measures of fluoride intake, and no associations were statistically significant after adjustment for exam age, height, weight, and Tanner stage. However, comparing results for girls vs. boys, we found consistently small positive associations of fluoride with bone outcomes for boys, but more commonly slight negative ones for girls.

The Iowa Bone Development Study analyses have several limitations. The study sample is not fully representative of a defined population, and is from a limited geographic region. It is mostly of relatively high SES, with those remaining in the study of even higher SES than the original study group. The fluoride intake estimates were based on parent reports combined with estimates of fluoride levels of products ingested. The numbers and exact ages of fluoride intake estimates varied across children, as did examination ages. With stratification by gender, there were only about 200 subjects available for most of the individual statistical analyses.

Fluoride plays an important role in the mineralization of body tissues. Numerous professional health organizations endorse fluoridation of public water supplies, an important source of dietary fluoride. For our cohort, water provided the majority of the mean daily intake. Currently, there is insufficient scientific evidence to establish a recommended fluoride intake, so Adequate Intakes (AI) have been established instead (3). The AI is based on observed estimates of average nutrient intake by healthy people within life stage and gender group. The Standing Committee on the Scientific Evaluation of Dietary Reference Intakes has established AIs of fluoride to be 0.01, 0.5, 0.7, 1.0, and 2.0 mg/day for infants and children 0–6 mo, 6–12 mo, 1–3 y, 4–8 y, and 9–13 y, respectively (3). The AI for infants 0–6 mo is based on human breast milk composition. For all other life stage groups, the AI is based on fluoride intakes of 0.05 mg/kgbw/d for a child of a standard reference body weight. This intake level is consistent with documented relationships that demonstrate a high level of protection against dental caries without known unwanted health effects (3,6). Fluoride intakes for our cohort were generally modest and not extreme, with means generally consistent with the level of adequate intake. Specifically, the daily means over the different periods studied ranged from 0.54 to 0.81 mg F (SD ~30–50% of the mean). Thus, it is not possible to conclude that very high fluoride intake during childhood is not associated more strongly with childhood bone development. Therefore, caution should be exercised in generalizing these results to high fluoride intakes. However, the results of this study provide no evidence that fluoride intakes have consequences for bone outcomes at age 11 years in girls or boys within these ranges, which are probably typical for most children in the United States.

Analyses of relationships between fluoride intake and DXA bone outcomes at different ages might yield different results. However, our initial analyses of age 5 bone outcomes with the same cohort also found generally weak relationships (36). Also, it is difficult to think that fluoride intakes early in life would have substantial effects on bone health later in life further removed from the intakes. However, at later ages, with full maturational and hormonal effects, results could be different.

Future directions with the Iowa Bone Development Study concerning fluoride and bone analyses will include analyses with age 13 DXA outcomes (currently being collected), and longitudinal changes in DXA. Different ways to express fluoride intake (i.e., mg, mg/kg, mg/kcal, mg/cm height) should also be explored. Additional analyses also will focus on peripheral quantitative computed tomography (pQCT) of the radius and tibia, allowing fluoride intake to be related to trabecular vs. cortical bone component outcomes.

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Appendix 1

Bivariate Associations Between Bone Measures and Fluoride Intake Variables

Daily Fluoride Intake	N	Hip BMC (g)		Spine BMC (g)		Whole Body BMC-Without Head (g)		HipBMD (g/cm ²)		Spine BMD (g/cm ²)	
AUC		r*	p-value	r*	p-value	r*	p-value	r*	p-value	r*	p-value
Girls											
mg F/kg bw, 0–11 yrs	176	–0.18	0.02	–0.06	0.42	–0.16	0.04	–0.13	0.09	–0.10	0.18
mg F/kg bw, 0–8.5 yrs	184	–0.16	0.04	–0.05	0.48	–0.13	0.09	–0.11	0.14	–0.08	0.28
mg F/kg bw, 0–3 yrs	204	–0.06	0.37	–0.01	0.88	–0.05	0.48	–0.06	0.40	–0.01	0.90
mg F/kg bw, 3–6 yrs	201	– 0.19	0.01	–0.11	0.13	–0.16	0.03	–0.12	0.09	–0.13	0.07
mg F/kg bw, 6–8.5 yrs	217	–0.12	0.07	–0.01	0.83	–0.10	0.16	–0.08	0.27	–0.05	0.49
mg F/kg bw, 8.5–11 yrs	214	–0.10	0.13	–0.01	0.85	–0.11	0.11	–0.11	0.13	–0.06	0.41
Boys											
mg F/kg bw, 0–11 yrs	156	0.01	0.89	0.04	0.61	–0.01	0.95	0.03	0.72	0.08	0.35
mg F/kg bw, 0–8.5 yrs	159	0.03	0.72	0.06	0.46	0.03	0.71	0.04	0.64	0.08	0.32
mg F/kg bw, 0–3 yrs	172	0.11	0.15	0.10	0.18	0.10	0.18	0.10	0.22	0.10	0.18
mg F/kg bw, 3–6 yrs	179	0.06	0.93	0.05	0.54	0.03	0.71	0.03	0.72	0.08	0.27
mg F/kg bw, 6–8.5 yrs	198	–0.09	0.19	–0.05	0.45	–0.12	0.08	–0.07	0.35	–0.01	0.97
mg F/kg bw, 8.5–11 yrs	206	–0.11	0.12	–0.08	0.23	–0.16	0.03	–0.05	0.52	–0.01	0.85

bolded entries are statistically significant.

* Spearman rank correlation coefficients

Appendix 2

Bivariate Associations (Regression Coefficients) Between Bone Measures and Fluoride Intake Variables, Adjusted for Age, Height, Weight, and Tanner Stage

Daily Fluoride Intake	N	Hip BMC (g)		Spine BMC (g)		Whole Body BMC-Without Head (g)		Hip BMD (g/cm ²)		Spine BMD (g/cm ²)	
AUC		β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Girls											
mg F/kg bw 0–11	171	–31.35	0.04	7.80	0.81	–1146.26	0.08	–0.65	0.16	–0.34	0.51
mg F/kg bw 0–8.5	179	–27.21	0.04	3.65	0.89	–886.26	0.10	–0.52	0.19	–0.28	0.51
mg F/kg bw 0–3	199	–5.92	0.53	20.21	0.28	–64.47	0.87	–0.27	0.34	0.14	0.65
mg F/kg bw 3–6	196	–21.46	0.02	–10.98	0.55	–661.74	0.09	–0.29	0.30	–0.32	0.29
mg F/kg bw 6–8.5	212	–21.31	0.11	4.30	0.88	–597.05	0.29	–0.19	0.64	–0.08	0.86
mg F/kg bw 8.5–11	208	–17.41	0.35	26.91	0.49	–1119.89	0.17	–0.38	0.52	0.13	0.84
Boys											
mg F/kg bw 0–11	150	29.85	0.08	32.09	0.14	1070.75	0.09	0.82	0.07	0.61	0.15
mg F/kg bw 0–8.5	153	24.83	0.08	29.56	0.11	1022.89	0.05	0.68	0.08	0.52	0.14
mg F/kg bw 0–3	166	21.19	0.04	21.36	0.11	934.99	0.02	0.43	0.13	0.33	0.20
mg F/kg bw 3–6	172	14.00	0.17	19.55	0.13	570.96	0.14	0.52	0.06	0.39	0.12
mg F/kg bw 6–8.5	191	9.04	0.49	11.48	0.48	146.30	0.77	0.22	0.55	0.38	0.24
mg F/kg bw 8.5–11	199	6.96	0.70	–0.99	0.97	195.07	0.78	0.23	0.65	0.34	0.45

Appendix 3

Least Squares Mean Comparisons for Girls of Outcomes with Daily Fluoride Intake Per unit Body Weight (mg/kg) (adjusted for Age, Tanner stage, Height and Weight)

Dependent Variable	Pr > F	1 Lowest Tertile	2 Middle Tertile	3 Highest Tertile	1 vs. 2 (p-value)	1 vs. 3 (p-value)	2 vs. 3 (p-value)
Fluoride AUC 0–3 yrs, mg/kg/d							
BMC hip (g)	0.81	18.78	18.66	18.50	0.79	0.52	0.72
BMC spine (g)	0.32	31.40	31.62	32.63	0.81	0.16	0.25
BMC whole body without head (g)	0.89	1049.71	1043.36	1052.22	0.74	0.90	0.64
BMD hip (g/cm ²)	0.40	0.75	0.73	0.73	0.23	0.25	0.94
BMD spine (g/cm ²)	0.94	0.72	0.72	0.73	0.81	0.92	0.72
Fluoride AUC 3–6 yrs, mg/kg/d							
BMC hip (g)	0.02	19.21	18.18	18.07	0.02	0.01	0.79
BMC spine (g)	0.63	32.27	31.45	31.71	0.35	0.52	0.77
BMC whole body without head (g)	0.02	1073.80	1022.50	1035.10	0.01	0.04	0.49
BMD hip (g/cm ²)	0.28	0.74	0.72	0.73	0.12	0.29	0.64
BMD spine (g/cm ²)	0.51	0.73	0.72	0.72	0.35	0.29	0.90
Fluoride AUC 0–8.5 yrs, mg/kg/d							
BMC hip (g)	0.04	19.04	18.77	17.89	0.57	0.02	0.06
BMC spine (g)	0.96	31.79	32.00	31.76	0.82	0.98	0.80
BMC whole body without head (g)	0.16	1066.40	1039.49	1030.79	0.16	0.07	0.65
BMD hip (g/cm ²)	0.13	0.75	0.73	0.73	0.08	0.09	0.96
BMD spine (g/cm ²)	0.77	0.73	0.72	0.72	0.59	0.49	0.88
Fluoride AUC 0–11 yrs, mg/kg/d							
BMC hip (g)	0.07	19.11	18.61	18.03	0.29	0.02	0.21
BMC spine (g)	0.99	31.92	31.89	31.87	0.98	0.96	0.99
BMC whole body without head (g)	0.04	1074.15	1031.61	1030.86	0.04	0.03	0.97
BMD hip (g/cm ²)	0.08	0.75	0.72	0.73	0.04	0.10	0.63
BMD spine (g/cm ²)	0.45	0.73	0.72	0.72	0.26	0.30	0.93

Pr>F is the p-value from the overall test of the null hypothesis of no mean difference among the three tertiles.

P-values for pair-wise comparisons are reported without adjustment for multiple comparisons.

Appendix 4

Least Squares Mean Comparisons for Boys of Bone Outcomes with Daily Fluoride Intake Per Unit Body Weight (mg/kg) (adjusted for Age, Tanner stage, Height and Weight)

Dependent Variable	Pr > F	1 Lowest Tertile	2 Middle Tertile	3 Highest Tertile	1 vs. 2 (p-value)	1 vs. 3 (p-value)	2 vs. 3 (p-value)
Fluoride AUC 0–3 yrs, mg/kg/d							
BMC hip (g)	0.02	18.14	18.72	19.64	0.29	0.01	0.09
BMC spine (g)	0.10	29.06	29.78	30.61	0.33	0.04	0.25
BMC whole body without head (g)	0.02	993.96	1008.04	1051.12	0.50	0.01	0.04
BMD hip (g/cm ²)	0.11	0.74	0.76	0.77	0.17	0.04	0.49
BMD spine (g/cm ²)	0.09	0.65	0.66	0.68	0.89	0.05	0.07
Fluoride AUC 3–6 yrs, mg/kg/d							
BMC hip (g)	0.66	18.52	18.99	18.95	0.41	0.46	0.95
BMC spine (g)	0.12	29.14	30.57	30.08	0.04	0.18	0.47
BMC whole body without head (g)	0.28	1004.98	1038.12	1020.49	0.11	0.46	0.39
BMD hip (g/cm ²)	0.47	0.75	0.76	0.76	0.30	0.28	0.96
BMD spine (g/cm ²)	0.17	0.65	0.67	0.67	0.08	0.15	0.78
Fluoride AUC 0–8.5 yrs, mg/kg/d							
BMC hip (g)	0.33	18.30	19.15	18.92	0.15	0.29	0.69
BMC spine (g)	0.60	29.66	29.68	30.32	0.98	0.38	0.39
BMC whole body without head (g)	0.29	996.12	1025.26	1026.60	0.18	0.16	0.95
BMD hip (g/cm ²)	0.32	0.74	0.77	0.76	0.13	0.39	0.50
BMD spine (g/cm ²)	0.34	0.66	0.65	0.67	0.44	0.51	0.14
Fluoride AUC 0–11 yrs, mg/kg/d							
BMC hip (g)	0.28	18.27	19.18	18.97	0.13	0.25	0.71
BMC spine (g)	0.46	29.65	29.64	30.46	0.99	0.29	0.28
BMC whole body without head (g)	0.36	998.47	1027.31	1024.28	0.19	0.24	0.89
BMD hip (g/cm ²)	0.19	0.74	0.77	0.76	0.08	0.20	0.61
BMD spine (g/cm ²)	0.17	0.66	0.65	0.68	0.53	0.24	0.07

Pr>F is the p-value from the overall test of the null hypothesis of no mean difference among the three tertiles.

P-values for pair-wise comparisons are reported without adjustment for multiple comparisons.

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Figure 1.

DXA images. **a** Anterior-posterior (AP) image of the lumbar spine with L1 to L4 region of interest outlined. **b** DXA image of the left hip shows regions of interest of the total hip (femoral neck, greater and lesser trochanteric regions) outlined. **c** Total body scan with sub-regions of interest (whole body without head) outlined. (Adapted from Binkovitz and Henwood (29) with permission from Springer-Verlag.)

Table 1
Descriptive Statistics for Adjustment Variables, Fluoride Intake, and Bone Measures

Variable	N	Girls (N=251)		Boys (N=230)	
		Mean	SD	Mean	SD
Age, years	481	11.24	0.31	11.24	0.32
Height at scan, cm	481	148.93	7.43	148.99	7.62
Weight at scan, kg	481	44.27	12.24	45.21	12.96
Body mass index (BMI), kg/m ²	481	19.76	4.35	20.15	4.56
Total Lean Mass (DXA), kg	481	30.38	6.02	31.92	6.22
Fluoride mg/d AUC 0–3 yrs	385	0.54	0.19	0.59	0.26
Fluoride mg/d AUC 3–6 yrs	384	0.73	0.33	0.81	0.41
Fluoride mg/d AUC 6–8.5 yrs	418	0.64	0.32	0.79	0.41
Fluoride mg/d AUC 8.5–11 yrs	423	0.63	0.32	0.76	0.40
Fluoride mg/d AUC 0–8.5 yrs	353	0.63	0.24	0.72	0.31
Fluoride mg/d AUC 0–11 yrs	344	0.64	0.24	0.73	0.30
Fluoride mg/kg/d AUC 0–3 yrs	376	0.050	0.019	0.052	0.022
Fluoride mg/kg/d AUC 3–6 yrs	380	0.042	0.020	0.044	0.022
Fluoride mg/kg/d AUC 6–8.5 yrs	415	0.026	0.013	0.031	0.017
Fluoride mg/kg/d AUC 8.5–11 yrs	420	0.019	0.009	0.021	0.012
Fluoride mg/kg/d AUC 0–8.5 yrs	343	0.040	0.015	0.042	0.017
Fluoride mg/kg/d AUC 0–11 yrs	332	0.035	0.013	0.037	0.015
BMC hip, g	479	18.57	4.42	19.04	4.40
BMC spine, g	480	31.93	7.75	30.07	5.10
BMC whole body without head, g	481	1045.82	223.79	1032.11	209.71
BMD hip, g/cm ²	479	0.73	0.10	0.76	0.09
BMD spine, g/cm ²	481	0.72	0.11	0.67	0.08

Note: Two children did not have hip data and one child did not have spine data (excluded from the analysis).

Table 2
Bivariate Associations Between Bone Measures and Fluoride Intake Variables

Daily Fluoride Intake	N	Hip BMC (g)		Spine BMC (g)		Whole Body BMC-Without Head(g)		HipBMD (g/cm ²)		Spine BMD (g/cm ²)	
AUC		r*	p-value	r*	p-value	r*	p-value	r*	p-value	r*	p-value
Girls											
mg F, 0–11 yrs	182	0.07	0.33	0.17	0.02	0.10	0.19	0.06	0.40	0.10	0.17
mg F, 0–8.5 yrs	188	0.04	0.58	0.13	0.08	0.08	0.31	0.05	0.50	0.09	0.29
mg F, 0–3 yrs	208	0.03	0.69	0.06	0.43	0.05	0.48	0.01	0.91	0.08	0.46
mg F, 3–6 yrs	202	–0.01	0.90	0.05	0.48	0.02	0.77	0.02	0.74	0.05	0.90
mg F, 6–8.5 yrs	218	0.11	0.11	0.19	0.01	0.16	0.02	0.11	0.10	0.08	0.03
mg F, 8.5–11 yrs	217	0.18	0.01	0.24	0.001	0.19	0.11	0.13	0.06	0.13	0.01
Boys											
mg F, 0–11 yrs	162	0.21	0.01	0.23	0.01	0.23	0.01	0.14	0.07	0.21	0.01
mg F, 0–8.5 yrs	165	0.21	0.01	0.23	0.01	0.24	0.01	0.14	0.09	0.20	0.02
mg F, 0–3 yrs	177	0.22	0.01	0.22	0.01	0.23	0.01	0.15	0.05	0.19	0.02
mg F, 3–6 yrs	182	0.14	0.06	0.18	0.02	0.19	0.01	0.10	0.20	0.18	0.02
mg F, 6–8.5 yrs	200	0.09	0.20	0.12	0.10	0.10	0.15	0.04	0.57	0.13	0.08
mg F, 8.5–11 yrs	206	0.14	0.04	0.14	0.05	0.15	0.04	0.10	0.17	0.17	0.02

bolded entries are statistically significant.

* Spearman rank correlation coefficients

Table 3

Bivariate Associations (Regression Coefficients) Between Bone Measures and Fluoride Intake Variables, Adjusted for Age, Height, Weight, and Tanner Stage

Daily Fluoride Intake	N	Hip BMC (g)		Spine BMC (g)		Whole Body BMC-Without Head (g)		Hip BMD (g/cm ²)		Spine BMD (g/cm ²)	
AUC		β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Girls											
mg F, 0–11 yrs	177	-1.53	0.06	0.41	0.81	-70.55	0.04	-0.03	0.24	-0.01	0.61
mg F, 0–8.5 yrs	183	-1.67	0.04	-0.06	0.97	-65.20	0.05	-0.03	0.23	-0.02	0.50
mg F, 0–3 yrs	203	-0.70	0.47	0.95	0.62	-16.14	0.69	-0.03	0.29	-0.01	0.98
mg F, 3–6 yrs	197	-1.25	0.02	-0.61	0.58	-43.99	0.06	-0.02	0.30	-0.02	0.34
mg F, 6–8.5 yrs	213	-0.77	0.15	0.27	0.80	-28.88	0.21	-0.01	0.69	-0.01	0.90
mg F, 8.5–11 yrs	211	-0.49	0.36	0.60	0.59	-36.97	0.11	-0.01	0.51	-0.01	0.95
Boys											
mg F, 0–11 yrs	156	1.16	0.15	1.52	0.15	36.91	0.23	0.03	0.14	0.04	0.09
mg F, 0–8.5 yrs	159	1.18	0.13	1.66	0.10	45.23	0.13	0.03	0.13	0.03	0.09
mg F, 0–3 yrs	171	1.59	0.07	1.93	0.09	65.86	0.05	0.03	0.18	0.04	0.09
mg F, 3–6 yrs	175	0.89	0.11	1.34	0.06	37.50	0.07	0.03	0.08	0.03	0.06
mg F, 6–8.5 yrs	193	0.48	0.35	0.62	0.35	11.18	0.57	0.01	0.54	0.02	0.16
mg F, 8.5–11 yrs	199	0.30	0.55	0.03	0.97	11.60	0.54	0.01	0.55	0.01	0.33

Table 4

Least Squares Mean Comparisons of Bone Outcomes with Daily Fluoride Intake (mg) (adjusted for Age, Tanner stage, Height and Weight) for Girls

Dependent Variable	Pr > F	1 Lowest Tertile	2 Middle Tertile	3 Highest Tertile	1 vs. 2 (p-value)	1 vs. 3 (p-value)	2 vs. 3 (p-value)
Fluoride AUC 0–3 yrs, mg/d							
BMC hip (g)	0.64	18.86	18.63	18.45	0.60	0.35	0.69
BMC spine (g)	0.37	31.76	31.30	32.50	0.60	0.39	0.16
BMC whole body without head (g)	0.67	1053.75	1038.43	1051.14	0.41	0.89	0.49
BMD hip (g/cm ²)	0.45	0.74	0.74	0.73	0.52	0.21	0.54
BMD spine (g/cm ²)	0.64	0.73	0.71	0.73	0.45	0.94	0.39
Fluoride AUC 3–6 yrs, mg/d							
BMC hip (g)	0.02	19.20	18.20	18.06	0.02	0.01	0.74
BMC spine (g)	0.43	32.47	31.46	31.51	0.25	0.27	0.96
BMC whole body without head (g)	0.03	1072.95	1028.35	1029.65	0.02	0.02	0.95
BMD hip (g/cm ²)	0.04	0.75	0.72	0.73	0.01	0.11	0.31
BMD spine (g/cm ²)	0.26	0.74	0.71	0.72	0.15	0.17	0.92
Fluoride AUC 0–8.5 yrs, mg/d							
BMC hip (g)	0.03	19.17	18.56	17.94	0.18	0.01	0.16
BMC spine (g)	0.85	32.02	31.50	31.86	0.57	0.86	0.70
BMC whole body without head (g)	0.06	1070.20	1038.65	1026.72	0.10	0.02	0.53
BMD hip (g/cm ²)	0.07	0.75	0.72	0.72	0.05	0.04	0.96
BMD spine (g/cm ²)	0.40	0.73	0.71	0.72	0.22	0.27	0.88
Fluoride AUC 0–11 yrs, mg/d							
BMC hip (g)	0.03	18.79	18.96	17.80	0.71	0.03	0.01
BMC spine (g)	0.69	31.32	32.12	31.78	0.39	0.62	0.72
BMC whole body without head (g)	0.05	1061.50	1049.94	1016.46	0.55	0.02	0.08
BMD hip (g/cm ²)	0.29	0.74	0.73	0.72	0.41	0.12	0.46
BMD spine (g/cm ²)	0.89	0.72	0.72	0.72	0.99	0.69	0.68

Pr>F is the p-value from the overall test of the null hypothesis of no mean difference among the three tertiles.

P-values for pair-wise comparisons are reported without adjustment for multiple comparisons.

Table 5

Least Squares Mean Comparisons of Bone Outcomes with Daily Fluoride Intake (mg) (adjusted for Age, Tanner stage, Height and Weight) for Boys

Dependent Variable	Pr > F	1 Lowest Tertile	2 Middle Tertile	3 Highest Tertile	1 vs. 2 (p-value)	1 vs. 3 (p-value)	2 vs. 3 (p-value)
Fluoride AUC 0–3 yrs, mg/d							
BMC hip (g)	0.03	18.05	19.21	19.46	0.04	0.01	0.64
BMC spine (g)	0.07	29.04	29.85	30.72	0.27	0.02	0.22
BMC whole body without head (g)	0.03	989.98	1028.69	1045.67	0.07	0.01	0.40
BMD hip (g/cm ²)	0.05	0.74	0.77	0.77	0.02	0.07	0.60
BMD spine (g/cm ²)	0.05	0.65	0.66	0.68	0.59	0.02	0.08
Fluoride AUC 3–6 yrs, mg/d							
BMC hip (g)	0.31	18.63	18.42	19.25	0.71	0.27	0.14
BMC spine (g)	0.41	29.43	29.85	30.37	0.54	0.19	0.46
BMC whole body without head (g)	0.35	1008.29	1009.82	1035.38	0.94	0.20	0.22
BMD hip (g/cm ²)	0.66	0.75	0.76	0.76	0.54	0.37	0.77
BMD spine (g/cm ²)	0.49	0.66	0.67	0.67	0.50	0.24	0.59
Fluoride AUC 0–8.5 yrs, mg/d							
BMC hip (g)	0.29	18.38	18.77	19.32	0.51	0.12	0.35
BMC spine (g)	0.04	29.45	29.25	31.02	0.79	0.04	0.02
BMC whole body without head (g)	0.15	1003.55	1004.11	1041.32	0.98	0.09	0.09
BMD hip (g/cm ²)	0.32	0.74	0.76	0.77	0.33	0.14	0.58
BMD spine (g/cm ²)	0.02	0.66	0.65	0.69	0.73	0.03	0.01
Fluoride AUC 0–11 yrs, mg/d							
BMC hip (g)	0.28	18.34	18.89	19.28	0.36	0.11	0.50
BMC spine (g)	0.04	29.63	29.10	31.01	0.48	0.07	0.02
BMC whole body without head (g)	0.37	1002.80	1013.64	1033.66	0.63	0.17	0.37
BMD hip (g/cm ²)	0.24	0.74	0.76	0.77	0.22	0.10	0.68
BMD spine (g/cm ²)	0.03	0.66	0.65	0.69	0.55	0.05	0.02

Pr>F is the p-value from the overall test of the null hypothesis of no mean difference among the three tertiles.

P-values for pair-wise comparisons are reported without adjustment for multiple comparisons.