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Effects of Life-long Fluoride Intake on Bone Measures of Adolescents: A Prospective Cohort Study

ABSTRACT

Controversy persists concerning the impact of community water fluoridation on bone health in adults, and few studies have assessed relationships with bone at younger ages. Ecological studies of fluoride's effects showed some increase in bone mineral density of adolescents and young adults in areas with fluoridated water compared with non-fluoridated areas. However, none had individual fluoride exposure measures. To avoid ecological fallacy and reduce bias, we assessed associations of average daily fluoride intake from birth to age 15 yr for Iowa Bone Development Study cohort members with age 15 yr dual-energy x-ray absorptiometry (DXA) bone outcomes (whole body, lumbar spine, and hip), controlling for known determinants (including daily calcium intake, average daily time spent in moderate-to-vigorous intensity physical activity, and physical maturity). Mean (SD) daily fluoride intake was 0.66 mg (0.24) for females and 0.78 mg (0.30) for males. We found no significant relationships between daily fluoride intake and adolescents' bone measures in adjusted models (for 183 females, all p values $\geq .10$ and all partial $R^2 \leq 0.02$; for 175 males, all p values $\geq .34$ and all partial $R^2 \leq 0.01$). The findings suggest that fluoride exposures at the typical levels for most US adolescents in fluoridated areas do not have significant effects on bone mineral measures.

KEY WORDS: skeleton, dual-energy x-ray absorptiometry, observational study, longitudinal, densitometry, children.

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INTRODUCTION

Fluoride is known to have strong affinity for bone, with approximately 99% of body fluoride bound to calcified tissues (Whitford, 1989). At therapeutic doses for the treatment of osteoporosis (≥ 10 mg/day), fluoride has been shown to stimulate the formation of new bone and increase bone mineral density (Vestergaard *et al*., 2008), although there are concerns about atypical mineralization (Fratzl *et al.*, 1994) and decreased mechanical strength (Søgaard *et al*., 1994).

The US Environmental Protection Agency, in a dose-response analysis of the effects of ingested fluoride on severe dental fluorosis and bone structure, estimated that the reference dose (RfD) for fluoride intake from non-therapeutic sources such as diet and drinking water is approximately 6 mg/day for a person weighing 70 kg, based on 0.08 mg fluoride *per* kg *per* day (US Environmental Protection Agency, 2010). While considerably lower than therapeutic doses for osteoporosis, an understanding of the effects of background fluoride levels on bone has public health importance. The majority of studies on the impact of background levels of fluoride on bone have focused on middle-aged and older adults. Studies comparing bone outcomes between adults living in fluoridated and non-fluoridated communities found conflicting results (Jacobsen *et al.*, 1992; Karagas *et al*., 1996; Hillier *et al*., 2000; Phipps *et al*., 2000; Park *et al.*, 2008). Reviews on this topic were inconclusive and recommended more accurately designed studies to reduce the risk of bias and ecological fallacy (Hillier *et al.*, 1996; Allolio and Lehmann, 1999). Another review concluded that optimally fluoridated water was not associated with adverse bone effects in adults (Demos *et al.*, 2001).

Only a few studies have been reported on the effects of fluoride intake on bone in children, at a level typically seen in North America. Dual-energy x-ray absorptiometry (DXA) for assessment of bone outcomes showed that Canadian young adult women and Swedish adolescents residing in optimally fluoridated areas had significantly higher mineral density in lumbar vertebra and the whole body, respectively, than those in non-fluoridated areas (Arnold *et al.*, 1997; Bratteb *et al*., 2002). A recent study compared left radial single-photon absorptiometry bone measures of South African adolescents in residents from areas with 0.19-ppm *vs*. 3.00-ppm water fluoride levels (Grobler *et al.*, 2009). In the 14- to 15-year-old age group only, radial BMD was

significantly higher for children from high- *vs*. low-fluoride areas. We previously reported no association between life-long fluoride intake and DXA-derived BMD in children at age 11(Levy *et al*., 2009).

Given that fluoride can accumulate in the developing skeleton of children at a faster rate than in adults, it is plausible that fluoride's effects on developing bone could be significant (Whitford, 1999). Thus, it is essential that the relationship between fluoride intake and bone development in children be thoroughly investigated. The purpose of this study was to assess the associations between fluoride intake and bone measures in adolescents at age 15 yrs.

MATERIALS & METHODS

Study Participants

Data for this prospective cohort study were collected as part of the longitudinal Iowa Fluoride Study (IFS)/Iowa Bone Development Study (IBDS). Newborns were recruited from 1992 to 1995 through their parents at 8 Iowa hospital postpartum wards to participate in the IFS, with 1,382 retained beyond age 6 mos. From 1998 to 2000, approximately 800 still participating in the IFS were invited to participate also in the IBDS, in which 630 cohort members have received one or more bone densitometry assessments. There were 415 with bone scans at age 15 yrs, and 358 of these also had complete accelerometry data at that time (see Appendix Fig. 1).

The University of Iowa Institutional Review Board approved all aspects of both studies, parents provided informed consent, and participants provided assent. The study was conducted in concordance with STROBE guidelines for observational studies.

Bone Outcomes

DXA scans were acquired for the children at about age 15 yrs by one of three certified technicians using a Hologic QDR 4500A densitometer (Delphi upgrade, Bedford, MA, USA). Scans were analyzed by the three technicians with Hologic software versions 12.3 and 12.4 for the whole-body and spine scans, and by one technician with version 12.6 for the hip scans. Detailed descriptions of analyses of DXA scans have been published previously (Janz *et al*., 2008; Levy *et al.*, 2009). Bone mineral content (BMC, gms) for the whole body (excluding head), left proximal femur (hip), and anteroposterior lumbar spine, and bone mineral density (BMD, gms/cm^2) for left hip and anteroposterior lumbar spine were assessed. These multiple bone outcomes were analyzed because of the substantial morbidity and mortality possible with hip and spine fractures, the possible differential effects of fluoride on different types of bone at the different sites, and differential effects on BMC *vs.* BMD. Whether the participants had a broken bone near the time of the densitometry (*i.e*., from age 14 to 16 yrs) was determined by questionnaire responses.

Cumulative Fluoride Intake

Assessment of daily fluoride intake has been described previously (Levy *et al.*, 2001, 2003, 2009). Parents completed detailed questionnaires regarding cohort members' intake of water, selected foods (including infant foods and other foods with substantial amounts of added water, such as pasta, rice, soup), beverages, and dietary fluoride supplements, as well as ingestion of dentifrice at ages 1.5, 3, 6, 9, and 12 mos, every 4 mos to age 4 yrs, and then every 6 mos to age 15 yrs. Based on measured fluoride levels and intake frequencies, daily fluoride intake (mg/day) was estimated at each age. Cumulative fluoride intake (mg) was calculated as the area-under-the-curve (AUC) from birth to age 15 yrs (independent variable). To compute AUC estimates of cumulative daily fluoride intake for birth to 15 yrs, cohort members needed to have daily fluoride intake estimates for at least: (1) 3 time points up to 12 mos of age; (2) 1 time point every other year from 12 mos to 11 yrs; (3) 1 time-point from 11 to 15 yrs; and (4) 1 time point at age 15 yrs or slightly beyond to interpolate back to age 15 yrs.

Other Covariates

Height and weight were measured at the time of the DXA scan, by means of a Harpenden stadiometer (Holtain, Crosswell, Crymych, Pembs., UK) and a Healthometer physician scale (Continental, Bridgeview, IL, USA), respectively, while participants were wearing indoor clothes with no shoes. Based on height, weight, leg length, and sitting height, as well as gender and age, the number of years since peak height velocity (PHV) was calculated (Mirwald *et al*., 2002). Time since PHV was used in lieu of chronological age in the regression models, since it was expected to be a stronger predictor of bone outcomes. Self-reported pubertal Tanner Stages were collected from standardized drawings for assessment of breast and genitalia development for females and males, respectively (Tanner, 1962).

To assess dietary intake of calcium (mg) and vitamin D (IU) during the previous week, participants completed Block Kids' Food Questionnaires at the clinical examination. Non-dietary (supplemental) calcium and vitamin D intake was assessed by questionnaire. Validity of the questionnaire for assessment of calcium and vitamin D intake was shown previously (Marshall *et al.*, 2008). Total (dietary plus non-dietary) calcium intake and vitamin D intake were used in these analyses.

Beginning at age 5 yrs, accelerometry measurements to assess physical activity were obtained during the fall for cohort members who had completed or were expected to complete DXA scans within ± 6 mos. The accelerometry measurement closest to age 15 yrs was used in these analyses. Cohort members were asked to wear ActiGraph accelerometer model GT1M (Pensacola, FL, USA) during all waking hours for 5 consecutive days, including both weekend days. Detailed descriptions of physical activity assessment have been provided previously (Janz *et al.*, 2008; Kwon *et al*., 2011). The average daily time that each participant spent in moderate- and vigorous-intensity physical activity (MVPA time in min/day; movements with counts above 2,296 *per* min) was estimated.

Statistical Analyses

All statistical analyses were stratified by gender because of differences in growth and maturation and previous evidence of differential fluoride effects by gender (Grobler *et al.*, 2009; Levy *et al*., 2009). Descriptive analyses were conducted for all

Table 1. Descriptive Statistics for Covariates, Daily Fluoride Intake (birth to 15 yrs), and Bone Outcomes for 358 Iowa Bone Development Study Cohort Members, Stratified by Gender

	Females			Males		
Variable	N	Mean	SD	N	Mean	SD
Age (yrs)	183	15.3	0.3	175	15.3	0.3
Maturity (yrs since PHV)	183	3.6	0.7	175	1.6	0.8
Height (cm)	183	164.6	6.6	175	175.3	7.9
Weight (kg)	183	61.5	14.4	175	70.5	16.2
Tanner Stage						
Stages 2-3	29			15		
Stage 4	98			99		
Stage 5	56			61		
Number pre-PHV	0			5		
Cumulative fluoride Intake AUC for birth to 15 yrs (mg/day)	183	0.66	0.24	175	0.78	0.30
Calcium intake at age 15 yrs (mg/day)	174	780.0	386.5	168	1043.2	512.9
Vitamin D intake at age 15 yrs (IU/day)	174	195.5	169.1	168	266.8	163.4
ActiGraph MVPA (min/day)	131	26.2	17.1	140	38.4	19.3
Whole-body BMC without head (g)	182	1686.9	318.3	175	2020.9	423.4
Spine BMC (g)	183	57.4	11.0	175	60.4	12.7
Spine BMD (g/cm^2)	183	1.00	0.13	175	0.95	0.12
Hip BMC (g)	183	28.9	5.8	175	38.0	8.2
Hip BMD (g/cm^2)	183	0.94	0.14	175	0.99	0.14
Any broken bones from ages 14 to 16 yrs, n (%)	16 (8.7%)			28 (16.0%)		

PHV, peak height velocity; AUC, area under the curve; MVPA, moderate- and vigorous-intensity physical activity; BMC, bone mineral content; BMD, bone mineral density.

variables (Table 1). (The Appendix Table presents detailed gender-specific percentiles for fluoride, calcium, and vitamin D intakes and physical activity.) Table 2 shows separate adjusted associations from linear regression analyses between age 15 yrs daily calcium and vitamin D intake and moderate and vigorous physical activity (MVPA) and each of the 5 bone densitometry outcomes. We conducted univariate and multivariable linear regression analyses to investigate unadjusted and adjusted associations between cumulative daily fluoride intake from birth to age 15 yrs as a continuous independent variable, and each bone outcome (Table 3). Adjustment variables included maturity (time in years from age of PHV, and Tanner Stage, with categories 2 and 3 combined *vs*. 4 *vs*. 5) and body size (height and weight). Average daily calcium (or vitamin D) intake and MVPA were included as covariates in additional regression modeling for bone outcomes because they were significant independent predictors from Table 2. Daily calcium intake at age 15 yrs had slightly stronger associations with bone outcomes than did vitamin D intake, and, since these dietary measures were highly correlated (Spearman's correlation coefficients were 0.89 for females and 0.86 for males), only calcium intake was included in the fluoride regression models. We repeated regression analyses with cumulative daily fluoride intake categorized into gender-specific tertiles of the distribution to investigate possible non-linear associations between fluoride intake and bone outcomes (Table 4).

All statistical analyses were performed with the Statistical Analysis System (SAS, version 9.2), with $p < .05$ considered statistically significant. The tables show regression coefficients, standard errors, p values, model R^2 values, and partial R^2 values attributable only to the variable of interest. Sample sizes were reduced when calcium intake and physical activity were included in the models.

RESULTS

Participants were mostly white (98%), non-Hispanic (97%), and middle- to high-SES (49% of mothers with four-year college degrees and 66% with 2007 family income of $60,000 or greater). Table 1 shows gender-stratified descriptive statistics for the 358 cohort members included in the analyses. Although Tanner Stage distributions for females *vs*. males were similar, the number of years since peak height velocity (PHV) for females was substantially higher (mean 3.6 *vs*. 1.6 yrs). Males had substantially higher mean height; weight; daily fluoride, calcium, and vitamin D intakes; PA measures; and most bone outcomes (except mean spine BMD) than females. About 9% of females and 16% of males reported a bone fracture from age 14 to 16 yrs. The mean numbers of available datapoints for estimation of fluoride intake (without interpolated endpoints) were 13.5 for 0 to 5 yrs, 6.7 for 5 to 8.5 yrs, 4.8 for 8.5 to 11 yrs, and 7.4 for 11 to 15 yrs. Concerning the sources of fluoride contributing to the ingested fluoride AUC, water (including water by itself, water added to reconstitute beverages, and water added to selected foods, *e.g*., pasta, rice) contributed approximately 52%,

Table 2. Gender-specific Bivariate Associations (linear regression coefficients) between Calcium Daily Intake (mg/day); Adjusted for Height, Weight, Time since PHV, and Tanner Stage, Without Calcium or MVPA; and Adjusted for Height, Weight, Time since PHV, Tanner Stage, Daily Calcium Intake, and Actigraph-based MVPA.

	Analyses with Calcium									
	Females (n = 174)					Males (n = 168)				
Dependent Variable	β	SE	*p* value	R^2	Partial R^2	β	SE	*p* value	R^2	Partial R^2
Whole-body BMC without head (g)	5.45	3.44	.11	0.73	0.01	16.72	3.55	< .001	0.72	0.12
Spine BMC (g)	0.09	0.16	.56	0.52	<0.01	0.68	0.12	< .001	0.61	0.16
Spine BMD (g/cm^2)	0.001	0.002	.81	0.46	<0.01	0.006	0.001	< .001	0.42	0.08
Hip BMC (g)	0.14	0.08	.07	0.60	0.02	0.30	0.09	< .001	0.54	0.07
Hip BMD (g/cm^2)	0.004	0.002	.09	0.42	0.02	0.007	0.002	< .001	0.35	0.08
	Analyses with Vitamin D									
	Females (n=174)					Males (n = 168)				
Dependent Variable	β	SE	*p* value	R^2	Partial R^2	β	SE	*p* value	R^2	Partial R^2
Whole-body BMC without head (g)	9.38	7.87	.24	0.73	0.01	41.50	11.28	< .001	0.70	0.08
Spine BMC (g)	-0.06	0.36	.88	0.52	<0.01	1.76	0.39	< .001	0.59	0.11
Spine BMD (g/cm^2)	-0.0001	0.004	.98	0.46	<0.01	0.014	0.005	.003	0.40	0.05
Hip BMC (g)	0.32	0.17	.07	0.60	0.02	0.83	0.28	.003	0.53	0.05
Hip BMD (g/cm^2)	0.010	0.005	.05	0.42	0.02	0.016	0.006	.004	0.35	0.05
	Analyses with MVPA									
	Females (n = 131)					Males (n = 140)				
Dependent Variable	β	SE	*p* value	R^2	Partial R^2	β	SE	*p* value	R^2	Partial R^2
Whole-body BMC without head (g)	20.91	9.38	.03	0.70	0.04	41.52	10.71	< .001	0.71	0.10
Spine BMC (g)	0.10	0.45	.82	0.44	<0.01	1.07	0.38	.01	0.59	0.05
Spine BMD (g/cm^2)	0.001	0.006	.90	0.38	<0.01	0.013	0.004	.01	0.45	0.07
Hip BMC (g)	0.36	0.20	.08	0.58	0.02	0.87	0.26	.001	0.56	0.08
Hip BMD (g/cm^2)	0.009	0.006	.11	0.41	0.02	0.018	0.005	< .001	0.36	0.08

MVPA, moderate- and vigorous-intensity physical activity; PHV, peak height velocity; BMC, bone mineral content; BMD, bone mineral density. β coefficients are reported *per* 100 mg increase in calcium intake, 100 IU increase in Vitamin D intake, and 10 min increase in daily MVPA.

other beverages and selected foods contributed approximately 28%, fluoride dentifrices contributed approximately 18%, and dietary fluoride supplements contributed approximately 2%. (Appendix Fig. 2 shows mean percentage contributions from these categories at ages 5, 8, 11, 13, and 15 yrs and for ages 0-15 AUC.)

Separate linear regression models were fit, with adjustment for height, weight, Tanner Stage, and time since PHV, to describe the associations between each of daily calcium and vitamin D intake and physical activity measures and bone densitometry outcomes (Table 2). Estimated daily calcium intake at age 15 yrs was significantly associated with all bone densitometry measures for males ($p < .001$), but not for females ($p > .06$); similarly, relationships with vitamin D intake were much stronger for males than females. There were also significant associations between MVPA time and bone outcomes for males ($p < .007$), but not for females ($p > .08$), with the exception of whole-body BMC ($p = .03$). The adjusted proportions of variance explained by calcium were ~0.01 to 0.02 for females and 0.08 to 0.12 for males; for MVPA, they were ≤ 0.04 (female) *vs*. 0.05 to 0.10 (male).

The associations between bone densitometry outcomes and daily fluoride intake from birth to age 15 yrs are presented in Table 3. Each regression coefficient (β) and related *p* value are the result of a separate fitted linear regression model with one of the 5 bone outcomes as the dependent variable. Unadjusted analyses showed that ≤ 3% of variance was explained by cumulative fluoride intake for both females and males. Two coefficients for females (whole-body BMC and spine BMC) and one for males (spine BMD) were positive and statistically significantly different from zero ($p < .05$, Table 3).

With adjustment for height, weight, time since PHV, and Tanner Stage, none of the associations of daily fluoride intake from birth to age 15 yrs with bone densitometry outcomes was statistically significant (Table 3), and ≤ 2% of variance was

Table 3. Gender-specific Bivariate Associations (linear regression coefficients) between Cumulative Daily Fluoride Intake (mg/day) and Bone Measures at Age 15 yrs: Unadjusted; Adjusted for Height, Weight, Time since PHV, and Tanner Stage, Daily Calcium Intake, and ActiGraph-based MVPA; and Adjusted for Height, Weight, Time since PHV, and Tanner Stage, without Calcium or MVPA

	Unadjusted, Full Sample ¥									
	Females (n = 183)					Males (n = 175)				
Dependent Variable	β	SE	*p* value	R^2	Partial R^2	β	SE	*p* value	R^2	Partial R^2
Whole-body BMC without head (g)	234.01	97.94	.02	0.03	0.03	181.66	106.85	.10	0.02	0.02
Spine BMC (g)	7.32	3.38	.04	0.03	0.03	5.24	3.20	.11	0.02	0.02
Spine BMD (g/cm^2)	0.07	0.04	.08	0.02	0.02	0.06	0.03	.04	0.02	0.02
Hip BMC (g)	2.92	1.79	.11	0.01	0.01	1.79	2.08	.39	< 0.01	< 0.01
Hip BMD (g/cm^2)	0.03	0.04	.53	< 0.01	< 0.01	0.03	0.04	.46	< 0.01	< 0.01
	Partially Adjusted, Full Sample ¥ (no adjustment for calcium or physical activity)									
	Females (n = 183)					Males (n = 175)				
Dependent Variable	β	SE	*p* value	R^2	Partial R^2	β	SE	*p* value	R^2	Partial R^2
Whole-body BMC without head (g)	-50.90	55.42	.36	0.72	< 0.01	-23.77	64.24	.72	0.67	< 0.01
Spine BMC (g)	0.19	2.59	.94	0.49	< 0.01	0.36	2.28	.88	0.54	< 0.01
Spine BMD (g/cm^2)	-0.01	0.03	.78	0.44	< 0.01	0.02	0.03	.47	0.38	< 0.01
Hip BMC (g)	-1.88	1.23	.13	0.58	0.01	-1.49	1.53	.34	0.50	0.01
Hip BMD (g/cm^2)	-0.06	0.03	.10	0.40	0.02	-0.03	0.03	.41	0.29	< 0.01
	Fully Adjusted, Reduced Sample ϕ									
	Females (n = 123)					Males (n = 135)				
Dependent Variable	β	SE	*p* value	R^2	Partial R^2	β	SE	*p* value	R^2	Partial R^2
Whole-body BMC without head (g)	57.65	76.17	.46	0.72	0.01	-55.99	65.17	.40	0.76	0.01
Spine BMC (g)	4.81	3.51	.18	0.49	0.02	0.36	2.30	.88	0.66	< 0.01
Spine BMD (g/cm^2)	0.04	0.05	.43	0.41	0.01	< 0.01	0.03	.97	0.51	< 0.01
Hip BMC (g)	0.32	1.66	.85	0.61	< 0.01	-2.00	1.64	.23	0.60	0.01
Hip BMD (g/cm^2)	-0.01	0.05	.95	0.44	< 0.01	-0.03	0.03	.38	0.43	0.01

PHV, peak height velocity; MVPA, moderate- and vigorous-intensity physical activity; BMC, bone mineral content; BMD, bone mineral density.
¥ Adjusted for: height, weight, time since PHV, and Tanner Stage.
ϕ Adjusted for: height, weight, time since PHV, Tanner Stage, daily calcium intake, and ActiGraph moderate- and vigorous-intensity physical activity (MVPA).

explained by fluoride intake for both females and males. Some of the non-significant associations were positive and some negative. With the addition of daily calcium intake and MVPA time to these models, all *p* values were ≥ .18 and all partial R^2 values due to fluoride intake were ≤ 0.02 (with sample sizes reduced from 183 to 123 for females and from 175 to 135 for males due to missing calcium or MVPA data; Table 3). Again, some of the non-significant associations were positive and some negative. Similar regression analyses with age 14 to 16 yrs occurrence of bone fracture as the outcome showed no significant relationships with fluoride intake (all adjusted *p* values > .34).

There were no statistically significant differences in bone densitometry measures across tertiles of fluoride intake from birth to age 15 yrs (Table 4) in the full sample (adjusted for height, weight, time since PHV, and Tanner Stage) or reduced sample (adjusted for height, weight, time since PHV, Tanner Stage, daily calcium intake, and MVPA time).

DISCUSSION

The results of this prospective cohort study in a group of 15-year-olds showed that the proportions of variance in mineral content or density of lumbar spine, proximal femur, and whole-body skeleton (without head) explained by life-long fluoride intake were very small (consistently ≤ 3%) at the daily intake range considered in this investigation. Based on the unadjusted analyses, all the associations were positive, and 2 of the 5 bone densitometry outcomes for females and 1 for males were significantly associated with fluoride intake. After adjustment for several important determinants of bone outcomes, none of the weak associations with fluoride intakes was statistically significant (all partial $R^2 \leq 0.02$). In addition, the directions of the non-significant associations were inconsistent, with some positive and some negative, and some effect estimates changed direction for the partially adjusted *vs*. fully adjusted samples.

Table 4. Gender-specific Least-squares Means of Bone Outcomes by Tertile of Cumulative Daily Fluoride Intake (mg/day) from Birth to Age 15 yr

	Full Sample ¥							
	Females (n = 183)				Males (n = 175)			
Dependent Variable	Lowest Tertile F (0.26-0.53)	Middle Tertile F (0.54-0.75)	Highest Tertile F (0.76-1.54)	*p* value*	Lowest Tertile F (0.26-0.62)	Middle Tertile F (0.63-0.85)	Highest Tertile F (0.86-1.84)	*p* value*
Whole-body BMC without head (g)	1,669.5	1,701.3	1,651.9	.29	2,003.0	2,042.1	2,015.1	.70
Spine BMC (g)	56.5	57.9	56.8	.62	59.5	60.1	60.4	.86
Spine BMD (g/cm^2)	0.99	1.01	0.99	.49	0.93	0.94	0.95	.57
Hip BMC (g)	28.8	29.5	28.0	.12	37.6	38.5	37.3	.57
Hip BMD (g/cm2)	0.94	0.95	0.92	.26	0.99	1.01	0.98	.51
	Reduced Sample ϕ							
	Females (n = 123)				Males (n = 135)			
Dependent Variable	Lowest Tertile F (0.26-0.53)	Middle Tertile F (0.54-0.75)	Highest Tertile F (0.76-1.54)	*p* value*	Lowest Tertile F (0.26-0.62)	Middle Tertile F (0.63-0.85)	Highest Tertile F (0.86-1.84)	*p* value*
Whole-body BMC without head (g)	1,682.2	1,722.4	1,701.6	.62	2,026.8	2,016.5	2,004.3	.91
Spine BMC (g)	56.4	58.3	58.6	.46	60.3	59.3	60.6	.72
Spine BMD (g/cm^2)	1.00	1.01	1.01	.80	0.95	0.93	0.95	.48
Hip BMC (g)	29.1	29.8	28.9	.55	38.1	37.8	37.3	.80
Hip BMD (g/cm2)	0.95	0.95	0.94	.80	1.00	1.00	0.99	.89

BMC, bone mineral content; BMD, bone mineral density; MVPA, moderate- and vigorous-intensity physical activity.
*From the overall F-test with the null hypothesis of no difference in means of bone outcomes among the 3 tertiles.
¥ Adjusted for: height, weight, time since PHV, and Tanner Stage.
ϕ Adjusted for: height, weight, time since PHV, Tanner Stage, daily calcium intake, and ActiGraph MVPA.

This provides additional evidence that these weak associations are very unlikely to be causal.

These findings are generally comparable with those from other analyses of the IFS/IBDS data from the age 11 yrs DXA scans (Levy *et al.*, 2009). During the intervening 4 yrs, cohort members generally experienced a substantial increase in bone mass accrual. For example, mean whole-body BMC showed mean increases of approximately 61% in females and 96% in males. Despite the acceleration of bone growth near puberty, the associations between fluoride intake and bone outcome measures remained weak, and none was significant after adjustment for other variables. Nevertheless, 2 previous ecological studies of the relationship between community water fluoride and bone measures found significantly higher bone mineral in residents of fluoridated areas compared with those in low-fluoride areas (Arnold *et al.*, 1997; Bratteb *et al*., 2002). Another investigation showed significant differences in left-radius BMD of 14- to 15-year-old adolescents, but not of 10- to 11- or 12- to 13-year-olds, living in an area with 3 ppm water fluoride level *vs.* < 0.2 ppm fluoride (Grobler *et al.*, 2009). These inconsistencies could be attributed to differences in study design. Concerning Grobler *et al*. (2009) specifically, the left-radius single-photon absorptiometry contrasted with our multiple-site DXA, and 3.0 ppm fluoride is very different from our study or nearly all of the United States. A study of the fluoride content of bone specimens in residents who lived in a fluoridated area found considerable variability in the fluoride content of the femoral head (Chachra *et al*., 2010). This emphasizes the importance of more accurate assessment of fluoride intake, as well as bone fluoride content, than of fluoride levels in public water systems alone.

In 2012, approximately 75% of the United States population on community water systems (67% of the total population) benefited from fluoridated water (CDC, 2013). In our study, 66 yrs to 71% of cohort members had access to optimally fluoridated water (≥ 0.7 ppm) at home at each time point of data collection from birth to age 15 yrs. Although water, by itself, was one of the main sources of fluoride intake for IFS/IBDS cohort members, other sources, such as toothpaste and other drinks, provided substantial portions of fluoride intake. For example, 42% of daily fluoride intake at age 15 yrs was attributed to other beverages compared with 39% for water. Based on the time interval from birth to age 15 yrs, the average daily fluoride intakes were 0.66 mg/day and 0.78 mg/day for females and males, respectively. The levels were far below 10 mg/day, the tolerable upper intake levels (UL) or the maximum daily fluoride intake level for individuals aged 14 to 18 yrs that does not generally cause adverse effects (Standing Committee, 1997). However, the levels in the study could be considered typical values for most US adolescents in fluoridated areas.

Several study strengths should be recognized. The data are from a well-defined cohort followed longitudinally. The detailed questionnaires and water fluoride assays allowed detailed age-specific, individual data to be used in estimation of fluoride intake at individual points and cumulatively. Also, key variables

were adjusted for, including body composition, growth/maturity, calcium intake, and physical activity.

There also are several limitations. Because of the longitudinal design, only 183 females and 175 males met the minimum requirement for time points with measured fluoride intake, and 33% of females and 23% of males lacked necessary physical activity and/or calcium data, reducing the power for significant associations to be detected. Fluoride measures were based on parents'/adolescents' reports on the frequency and amount of ingested fluoride-containing products, which were subject to error. The available range of water fluoride levels precludes observation of effects of very high fluoride levels. Participants were selected from families residing in Iowa at the child's birth, and most (> 95%) were non-Hispanic whites with relatively high socio-economic status. Previous studies have acknowledged the important role of genetics in bone density (Videman *et al.*, 2007; Wagner *et al.*, 2013). Therefore, it is important to investigate the reproducibility of study findings in other populations with different genetic backgrounds.

Given the expected continuation of bone mass accrual and additional exposure to fluoride during late adolescence and young adulthood, future steps with regard to the IBDS are to assess relationships between changes in bone outcomes over time and fluoride intakes. Moreover, peripheral quantitative computed tomography (pQCT) is being used for assessment of BMC and BMD in cortical and trabecular compartments of the tibia and radius. Investigations of fluoride's effects on future clinical outcomes, such as fracture, are also of interest.

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