Elevated Serum Fluoride Concentrations in Women Are Not Related to Fractures and Bone Mineral Density[1]

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ABSTRACT Epidemiologic studies of the relations between drinking-water fluoride levels and bone mineral density (BMD) and fracture are characterized by disparate conclusions and an absence of information about individual circulating fluoride levels. This study relates serum fluoride concentrations, which reflect individual fluoride exposures, to BMD and bone fractures. Data are from 1300 female residents of 3 small communities in which the water fluoride concentrations were 52.6 or 210.4 μmol/L. Circulating serum fluoride concentrations were assessed by ion-specific electrode. Fluoride intake was estimated from interviews describing water and water-based beverage consumption and duration of residence in the community. BMD was measured by dual-energy X-ray densitometry and single-photon densitometry. Self-reported fractures were confirmed by medical record abstraction. The mean serum fluoride concentration in the high-fluoride community, 2.11 ± 0.05 μmol/L, was significantly higher than serum fluoride concentrations in the control and high-calcium communities with water fluoridation to 52.6 μmol/L. The mean serum fluoride concentrations in these latter 2 communities were 1.6 ± 0.04 and 1.22 ± 0.05 μmol/L, respectively. Serum fluoride was not significantly related to BMD after adjusting for covariates including age and body size. The mean distal radius BMD, however, was significantly higher in the high-fluoride community. Serum fluoride concentrations were not related to incident osteoporotic fractures with 4 y of observation. Serum fluoride concentrations were not associated with BMD or osteoporotic fractures among female residents of communities with water fluoride concentrations of 52.6 or 210.4 μmol/L. J. Nutr. 135: 2247–2252, 2005.

KEY WORDS: • *water fluoride* • *serum fluoride* • *bone mineral density* • *bone fracture*

Fluoride is a trace element that is ubiquitously distributed throughout the environment in a wide range of concentrations. After its absorption from the gastrointestinal tract, it is rapidly incorporated into calcified tissues, which contain 99% of the body burden (1). Fluoride has the ability to prevent the formation and progression of dental caries and to stimulate the formation of new bone. In pharmacologic doses for the treatment of osteoporosis, fluoride was shown to increase bone mineral density (BMD) (2,3). With such doses, there is concern about atypical mineralization (4,5) and increased risk of fracture (6,7) when steady-state serum fluoride concentrations are chronically above the "therapeutic window" of 5–10 μmol/L (8,9).

There is also uncertainty about the skeletal effects of fluoride at considerably lower intakes from the diet and drinking water. Some epidemiologic studies found no association with BMD or bone fracture among women whose drinking water contained fluoride at <15.8–52.6 μmol/L (10,11) when the fluoride exposures were defined by fluoride concentrations in drinking water at its processing source. In contrast, an increased risk of bone fracture was reported in a community in which the naturally occurring water fluoride concentration was 210.4 μmol/L compared with one in which the water concentration was 52.6 μmol/L (12). None of these studies of BMD and/or fracture, however, included measures of individual circulating fluoride exposure, which is considered the best indicator of body burden and varies widely among residents of the same region. Because these and other studies (10–15) were conducted at the ecologic level with its inherent limitations, there is growing interest in using biomarkers, such as serum fluoride concentrations, to assess individual exposures (16). Serum fluoride concentrations correlate well with long-term levels of intake and with skeletal fluoride concentrations because of the steady-state relation between the concentrations in the exchangeable pool of bone and the extracellular fluids (17–21), although bone biopsies could provide a more direct assessment if they could actually be done in large population-based studies such as these.

Understanding the health effect of relatively low fluoride exposures, from dietary sources and dental products, has substantial public health importance. There are large geographic areas in the United States in which naturally occurring water fluoride levels approach or exceed 105.2 μmol/L, particularly in the southwestern region of the United States but also in

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isolated areas in every state (13). This report describes the relation between individually measured serum fluoride concentrations, BMD, and the 4-y incident fracture frequency among residents of 3 Iowa communities in which the drinking-water fluoride concentrations were 52.6 or 210.4 μmol/L.

SUBJECTS AND METHODS

Data collection was initiated in 1300 women, aged 20–92 y, who lived in 3 communities with diverse mineral content in the water supplies (12); the women were selected to provide an extended intake gradient of 2 minerals (calcium and fluoride) thought to affect bone mineral density levels and facture frequency without the selection factors that occur in studies in which supplements are used to increase the gradient of mineral intake. The communities were similar with respect to size (<2000 residents per community), racial and ethnic mix (>95% Caucasian), mean income, and primary occupations. A community census identified women ≥ 18 y old who were ambulatory (capable of climbing 3 stairs without assistance) and able to provide informed consent. There were no additional selection criteria. Study participation rates among eligible women were 79% in the high-calcium community, 70% in the high-fluoride community, and 81% in the control community. The research was conducted under the auspices of the Institutional Review Boards of the Universities of Iowa and Michigan and the Medical College of Georgia.

Fluoride status measures. Elemental calcium concentrations (means ± SD) in the high-calcium, high-fluoride, and control communities were 9.375 ± 0.200, 0.375 ± 0.075, and 1.500 ± 0.100 mmol/L, respectively. The fluoride concentration in the high-fluoride community water supply (210.4 μmol/L) occurred naturally because of the geology of the area in which the community water wells were drilled. The fluoride concentrations in the high-calcium and control communities were augmented to 52.6 μmol/L by water treatment, which was implemented in 1958. The control community was so designated because calcium and fluoride concentrations in the public drinking water were consistent with nationwide averages. We could not identify a community whose water supply included high calcium content and a comparably high fluoride content that would balance the design. The University of Iowa Hygienic Laboratory, the state public health laboratory, has monitored the calcium and fluoride concentrations in these community water supplies since 1938 as part of the state's ongoing water quality assessment program. The concentrations have varied only slightly over that period. These marked differences in calcium and fluoride levels in the community water supplies exist because the communities are so small that blending and processing of waters from more than one source do not occur.

Serum fluoride was analyzed using an ion-specific electrode (Model 9409, Orion Research) and a miniature calomel reference electrode coupled to a potentiometer after overnight diffusion using a modification of the hexamethyldisiloxane-facilitated diffusion of Taves (21,22). During the diffusion process, fluoride in the 1.00-mL serum samples and the appropriate standards was quantitatively transferred to an alkaline trap of known volume (0.05 mol/L NaOH, 50 μL). Before analysis the next day, the trap was buffered to pH 5.0 with acetic acid (0.20 mol/L, 20 μL) and the final volume was adjusted to 75 μL with deionized water. Compared with the concentration in the serum, this process increased the fluoride concentration in the solution that was analyzed by a factor of 13.3, which improved the reliability and accuracy of the results. The CV was <5%.

Fluoride and calcium intakes were estimated based on the consumption of water and water-based beverages. These sources contributed 75–85% of the total dietary intake of fluoride (21). Total water intake was estimated from inquiry as to the number of glasses of water and water-based beverages consumed each day and multiplying that number by the calcium and fluoride concentrations in the subject's water source. Daily calcium intake from other foods was estimated using the National Cancer Institute FFQ (23). Interviewers used color photographs and food models to enhance the recall of foods and beverages including serving size. Participants reported their supplement use including the quantity of calcium in the supplement. Calcium intake from the 3 sources (water, diet, and supplement) was not included in the final multiple variable models because it did not explain significant variation.

Bone status measures. BMD of the femoral neck and lumbar spine and total body bone calcium were measured by dual X-ray densitometry (DEXA-Lunar; DPX-L™, analysis software version 1.3y). BMD of the distal radius was measured using single-photon densitometry. Calibration was performed daily and a lumbar spine phantom was scanned weekly. The CV for DEXA was <1.5% for the femoral neck site.

Using postal cards mailed every 6 mo for 4 y, the participants were asked to report the site of any bone fracture, its date of occurrence and, if appropriate, the facility where the fracture was treated. Fracture status was confirmed at treatment facilities by abstracting medical records and securing copies of images, if available. This confirmation strategy was successful in 87% of fractures. Fracture frequency was dichotomized into sites more likely to be associated with osteoporotic bone, including hip, spine, wrist, and ribs, compared with other bones.

Serum osteocalcin concentrations were measured using the Incstar™ RIA. The inter- and intra-assay variation was <10%.

Other measures. Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg using a standardized procedure. The data were used to calculate BMI [weight (kg)/height $(m)^2$]. Postmenopause status was based on self-report of at least 12 mo of amenorrhea. Delineation of surgical postmenopause was based on self-report. Medication use was assessed during the in-home interview; enrollees showed the labels of the containers to the interviewer and described why the medication was being used. The interviewer recorded medication names, doses, duration of use, and reason for use. Participants were also asked about the mean time per week (min/wk) spent doing each of 8 activities (walking, jogging, running, bicycling, swimming, aerobics, tennis, and squash or racquet ball). The mean times spent at each activity were summed into a measure of total min/wk of activity, and divided into tertiles, with the tertile of lowest activity as reference for comparison.

Statistical analysis. Continuous variables were evaluated for normality and transformations undertaken when appropriate to improve the error variances and linear fit during regression modeling. Variables were presented as means ± SEM. General linear models were used to estimate group means and test for pair-wise significant differences between groups.

To show the association of serum fluoride with duration of residence in the community, serum fluoride values were categorized into quartiles (because of their skewed distribution) with the lowest (first) quartile acting as the reference category. Duration of residence in the community (y) was highly skewed and classified into tertiles (1–13, 14–2, and 27–77 y) with the 1–13 y category serving as the reference. Then serum fluoride concentrations were calculated for each cell and compared using ANOVA.

Multiple variable regression models were fit, and for those relations that included second-order terms (quadratic terms), terms were centered about their means to minimize multicolinearity. Multiple variable models were built by identifying the relation between fluoride exposure and BMD and then including other variables, such as age, BMI, and menopause status, based on *P*-values for individual terms < 0.05. Logistic regression analyses were used to assess the association between the risk of osteoporotic fractures and important exposures including serum fluoride concentration, BMD, age, body size, and medications. In all multiple variable regression and logistic models, serum fluoride was included as a log-transformed continuous variable; indicator variables represented the high-calcium and high-fluoride communities, with the control community as referent.

RESULTS

Serum fluoride. Serum fluoride concentrations, as expected, were highest in the community with the water concentration of 210.4 μmol/L (**Table 1**). The mean serum fluoride concentration was 32% greater in the high-fluoride community compared with that in the control community and 73% greater than the mean in the high-calcium community. Serum fluoride concentrations increased with greater years of

TABLE 1

Characteristics of women, aged 20–92 y, in communities according to water mineral concentration, with comparisons across community designations[1]

Variable	Community			*P*-value
	Control	High-calcium	High-fluoride	
n	368	406	526	
Age, *y*	55.9 ± 0.96	54.1 ± 0.91	54.8 ± 0.80	NS[2]
Serum fluoride, *μmol/L*	1.60 ± 0.04[b]	1.22 ± 0.05[c]	2.11 ± 0.05[a]	0.0001
Daily calcium intake, *mg*	754 ± 20[b]	1001 ± 25[a]	679 ± 16[c]	<0.0001
Daily water fluoride intake, *μmol/L*	63.65 ± 2.63[b]	40.50 ± 1.58[c]	192.52 ± 6.84[a]	<0.0001
BMD, *g/cm²*				
Lumbar spine	1.179 ± 0.0110	1.197 ± 0.0104	1.195 ± 0.009	NS
Femoral neck	0.914 ± 0.0084	0.912 ± 0.0083	0.912 ± 0.007	NS
Distal radius	0.651 ± 0.0053[b]	0.656 ± 0.0057[ab]	0.667 ± 0.004[a]	0.05
BMI, *kg/m²*	27.85 ± 0.33	28.56 ± 0.31	28.30 ± 0.27	NS
Osteocalcin, *nmol/L*	0.385 ± 0.012[c]	0.446 ± 0.011[a]	0.434 ± 0.010[b]	0.0005
Fracture, *n (%)*				
Osteoporotic	5 (1.4)	14 (2.3)	15 (2.9)	0.01
Nonosteoporotic	11 (3.2)	14 (3.4)	16 (3.1)	NS
Current smoking, *n (%)*	31 (10.8)	51 (16.2)	58 (15.4)	0.02
Thiazide antihypertensive, *n (%)*	22 (7.7)	22 (7.0)	38 (10.1)	0.02
Use in previous 12 mo, *n (%)*	13 (4.5)	16 (5.1)	23 (6.1)	0.01
Hormone replacement, *n (%)*	2 (0.7)	17 (5.4)	16 (4.3)	0.01
Physical activity, *n (%)*				<0.0001
<40 min/wk	98 (27)	79 (19)	248 (47)	
40–150 min/wk	142 (38)	137 (34)	156 (30)	
>150 min/wk	128 (35)	190 (47)	122 (23)	

[1] Values are means ± SE or *n* (%). Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] NS, nonsignificant; $P \geq 0.05$.

residency in the high-calcium community (4th quartile) and the high-fluoride community (3rd quartile) (**Table 2**).

Fluoride, other variables and bone. BMD of the lumbar spine or femoral neck did not differ among the communities (Table 1). The BMD of the distal radius (which is mainly cortical bone), however, was significantly higher in the high-fluoride community compared with the mean value in the control community.

There were no associations of serum fluoride concentrations and BMD measures at the femoral neck, lumbar spine, or radius (**Table 3**). Other variables, however, including age, BMI, osteocalcin concentration, thiazide antihypertensive use, oral contraceptive use, hormone therapy use, and menopausal status, explained 50% of the variation in BMD at the femoral neck and distal radius. Calcium intakes are reported in Table 1, and although there were significantly different mean intakes by community, the combination of water and diet calcium intake did not explain significant variation in the BMD;

TABLE 2

Quartiles of serum fluoride concentration related to years of residence in the community (by tertiles) according to community water supply mineral concentration[1]

Years of residence (tertiles)	Serum fluoride concentration			
	Quartile 1 <25%	Quartile 2 25–50%	Quartile 3 50–75%	Quartile 4 >75%
	μmol/L			
Control community				
T1 0–13 (30%)	0.76 ± 0.04	1.32 ± 0.03	1.68 ± 0.03	2.60 ± 0.18
T2 13–27 (31%)	0.80 ± 0.04	1.29 ± 0.02	1.69 ± 0.02	2.58 ± 0.14
T3 27–77 (38%)	0.79 ± 0.04	1.33 ± 0.02	1.73 ± 0.02	2.54 ± 0.11
High-calcium community				
T1 0–13 (38%)	0.55 ± 0.02	0.92 ± 0.02	1.23 ± 0.02	1.84 ± 0.13*
T2 13–27 (31%)	0.57 ± 0.02	0.89 ± 0.02	1.27 ± 0.02	2.27 ± 0.13
T3 27–79 (30%)	0.55 ± 0.03	0.93 ± 0.02	1.27 ± 0.02	2.24 ± 0.10
High-fluoride community				
T1 0–13 (33%)	0.84 ± 0.03	1.47 ± 0.03	2.09 ± 0.05	3.87 ± 0.33
T2 13–27 (33%)	0.91 ± 0.04	1.43 ± 0.03	2.15 ± 0.04	3.78 ± 0.24
T3 27–78 (33%)	0.91 ± 0.04	1.45 ± 0.03	2.27 ± 0.04**	3.97 ± 0.18

[1] Values are means ± SE, total $n = 1300$. * Different from T2, * $P < 0.01$. ** Different from T2, $P < 0.001$.

TABLE 3

β-Coefficients and variance (R^2) from multiple-variable regression models relating serum fluoride and BMD of the femoral neck, radius, and lumbar spine[1]

	Femoral neck BMD model (R^2 = 50%)		Radius BMD model (R^2 = 51%)		Lumbar spine BMD model (R^2 = 32%)	
Variable	β	*P*-value	β	*P*-value	β	*P*-value
Serum fluoride[2]	0.011 ± 0.0072	0.13	0.005 ± 0.006	0.37	0.019 ± 0.0121	0.12
High calcium community			0.020 ± 0.008	0.011	0.028 ± 0.015	0.07
High fluoride community			0.018 ± 0.007	0.011		
Age	−0.0056 ± 0.0003	0.0001	−0.008 ± 0.003	0.005	0.0001 ± 0.0001	0.004
Age × age quadratic term	0.0001 ± 0.0001	0.0049			−0.014 ± 0.0056	0.01
BMI[2]	0.2742 ± 0.0179	0.0001	0.07 ± 0.014	0.0001	0.256 ± 0.029	0.0001
Osteocalcin[3]	−0.0513 ± 0.0097	0.0001	−0.050 ± 0.008	0.0001	−0.068 ± 0.016	0.0001
Natural postmenopause			−0.07 ± 0.013	0.0001	−0.1237 ± 0.028	0.0001
Surgical menopause			−0.08 ± 0.013	0.0001	−0.1361 ± 0.029	0.0001
Thiazide use					0.048 ± 0.021	0.03
Current hormone use			0.021 ± 0.016	NS[4]	0.072 ± 0.03	0.02
Oral contraceptive use	−0.038 ± 0.0189	0.04			−0.077 ± 0.03	0.01
Moderate physical activity			−0.017 ± 0.007	0.02		
High physical activity			−0.017 ± 0.007	0.02		
Age × postmenopause			−0.005 ± 0.0007	0.0001	−0.008 ± 0.0028	0.004
Age × surgical menopause			−0.004 ± 0.0007	0.0001	−0.005 ± 0.0025	0.05
Age × BMI			0.003 ± 0.0008	0.0002	0.004 ± 0.0016	0.008
Age × osteocalcin			−0.001 ± 0.0004	0.006		

[1] Values are β-coefficients ± SE.
[2] Values were log-transformed.
[3] Expressed in μg/L square-root transformed, where 1 μg/L = 0.171 nmol/L.
[4] NS, nonsignificant; $P \geq 0.05$.

>30% of the variation in the lumbar spine was explained by variables other than fluoride exposure.

Osteocalcin concentrations were 13 and 16% higher in women living in the high-calcium and in the high-fluoride communities, respectively, compared with women in the control community (Table 1, $P < 0.006$, pairwise comparisons). Osteocalcin concentrations in the high-fluoride community did not differ from those in the high-calcium community.

Fluoride and fractures. The frequencies of confirmed osteoporotic and nonosteoporotic fractures that occurred during the 4-y period are listed in **Table 4**. Although there was an association of fracture frequency with type of community (Table 1), this association was no longer significant after adjustment for covariates. Fluoride measures were not associated with a greater risk of osteoporotic fracture after adjustment for BMD levels (**Table 5**). Further, calcium intake was not predictive of osteoporotic fracture risk. Greater femoral neck BMD was associated with a lower fracture risk ($P < 0.006$).

TABLE 4

Site, count, and frequency of confirmed fractures observed in women aged 20–92 y with serum fluoride measures

Type	Site	Count	Frequency
		n	*fractures/1000 person y*
Osteoporotic	Arm/wrist	18	5.68
	Hip/femoral neck	10	3.16
	Neck/spine/back	3	0.95
	Ribs/sternum	3	0.95
Nonosteoporotic	Ankle	5	1.58
	Clavicle	1	0.32
	Foot	10	3.16
	Hand	5	1.58
	Leg	9	2.84
	Other	7	2.21
	Pelvis	3	0.95
	Scapula	1	0.32

DISCUSSION

Several epidemiologic studies found that the risk of bone fracture was marginally increased in communities with higher water fluoride concentrations (12–14), whereas others found no association or a marginally smaller risk (10,11,24). It is unclear whether these contradictory findings are due to complex and poorly understood biological mechanisms, to relatively small or imprecisely documented differences in the community water fluoride concentrations, or to study designs that were primarily ecological and without actual measures of individual fluoride exposures.

In this study, neither serum fluoride concentrations nor the duration of residency in communities with known water fluoride concentrations predicted incident osteoporotic fractures in women 20–92 y old. Serum fluoride concentrations were measured under the assumption that they would serve as meaningful indicators of past fluoride intake and, therefore, the concentration of fluoride in bone, reflecting the contributions from food, water, and dental products as well as that from the turnover of bone. Several reports provided support for this assumption (1,16–19). Taves and Guy (19) estimated that the ratio of the fluoride concentrations in bone and serum in adult humans was 100,000:1, whereas in rats, it was 25,000:1.

We found a substantial fluoride exposure gradient among the communities, as indicated by both the serum concentrations and the time that the women had lived in the 3 com-

TABLE 5

Association of fluoride-based measures and osteoporotic fracture after adjustment for BMD, site, and age (not shown) based on logistic regression modeling

Model	Variable	Reference unit	Risk ratio	Variable *P*-value	Model *P*-value
1	Log of serum fluoride	μmol/L	1.16	0.66	0.001
	Femoral neck BMD	g/cm^2	0.008	0.0024	
	Calcium intake (diet + water)	mg/d	0.999	0.11	
	Age	y	1.01	0.49	
2	Duration of community residence	y	1.03	0.73	0.001
	Femoral neck BMD	g/cm^2	0.005	0.0001	
	High-calcium community	Control community	3.01	0.04	
	High-fluoride community		2.55	0.07	

munities with drinking-water fluoride concentrations of 52.6 or 210.4 μmol/L. The measures of fluoride exposure used in this investigation, and at the amounts identified, were not associated with osteoporotic fractures or with BMD, particularly after adjustment for important covariates including age, body size, thiazide use, hormone use, and menopause status.

We measured BMD at 3 sites: the femoral neck, lumbar spine, and radius. Low BMD increased the risk of fracture at nearly all bone sites (25). There were no independent associations of fluoride exposure with BMD at the 3 measured bone sites that included predominantly cortical (radius) or trabecular bone (lumbar spine). Zipkin (26) reported that bone fluoride concentrations were 20–30% higher in vertebrae than in iliac crest or ribs, probably because fluoride concentrates in areas of bone undergoing active mineralization, where it substitutes for hydroxyl ions in the apatite lattice (27). Measurements of BMD at the various sites allowed us to evaluate whether there was a different effect of fluoride in bone that was more cortical, as in the radius, or more trabecular, as in the lumbar spine. BMD did not differ among the communities in the vertebrae or femoral neck but was significantly higher in the distal radius in the 210.4-μmol/L community. In their study of BMD in residents whose water supplies contained fluoride at 1.58, 36.82, or 131.5 μmol/L, Phipps et al. (28) found significantly higher values in the lumbar spine in both men and women and in the proximal femur in women in the 131.5-μmol/L community, whereas BMD did not differ between the 1.58- and 36.82-μmol/L communities.

This study also included a comparison of BMD and fracture in a community with high calcium intakes driven by high calcium content of the community water supply. In normal adults with reasonable access to an abundant variety of foods, there is a very limited correlation between dietary calcium intake and BMD. Several cross-sectional and prospective studies did not show significant associations between BMD and calcium intake (29)

In summary, this study with individual measures of long-term exposure to fluoride did not demonstrate an association with BMD or the risk of bone fracture. We measured multiple variables including serum fluoride concentration, fluoride exposure, assessment of bone metabolism, and fluoride interactions with other important bone factors including age, body size, menopause status, and medications. We conclude that within the range of these exposures and variables, the risk of deleterious bone-related outcomes was not related to fluoride exposure. The U.S. Environmental Protection Agency has set its primary drinking-water standard (enforceable) at 210.4 μmol/L to protect against the risk of crippling skeletal fluorosis. The present findings agree that at this level, there is little evidence of a bone demineralization defect associated with low BMD or fracture.

LITERATURE CITED

1. Whitford, G. M. (1994) Intake and metabolism of fluoride. Adv. Dent. Res. 8: 5–14.
2. Pak, C. C., Sakhaee, K., Adams-Huet, B., Piziak, V., Peterson, D. R. & Poindexter, J. R. (1995) Treatment of postmenopausal osteoporosis with slow-releasing sodium fluoride. Ann. Med. 123: 401–408.
3. Melton, L. J. (1989) Safe and effective treatment of osteoporosis with intermittent slow release sodium fluoride: augmentation of vertebral bone mass and inhibition of fractures. J. Clin. Endocrinol. Metabol. 68: 150–159.
4. Dambacher, M. A., Ittner, J. & Ruegsegger, P. (1986) Long-term fluoride therapy of postmenopausal osteoporosis. Bone 7: 199–205.
5. Fratzl, P., Raschnger, F., Eschberger, J., Abendroth, B. & Klaushofer, K. (1995) Abnormal bone mineralization after fluoride treatment in osteoporosis: a small angle X-ray scattering study. J. Bone Miner. Res. 9: 1541–1549.
6. Riggs, B. L., Hodgson, S. F., O'Fallon, M., Chao, E., Wahner, H., Cedel, S. L. & Melton, J. (1990) Effect of fluoride treatment on the fracture rate in post-menopausal women with osteoporosis. N. Engl. J. Med. 322: 802–809.
7. Hedlund, L. R. & Gallagher, J. C. (1989) Increased incidence of hip fracture in osteoporotic women treated with sodium fluoride. J. Bone Miner. Res. 4: 233–275.
8. Taves, D. R. (1970) New approach to the treatment of bone disease with fluoride. Fed. Proc. 29: 1185–1189.
9. Pak, C.Y.C. (1989) Fluoride and osteoporosis. Proc. Soc. Exp. Biol. Med. 191: 278–286.
10. Cauley, J. A., Murphy, P. A., Riley, T. J. & Buhari, A. M. (1995) Effects of fluoridated drinking water on bone mass and fractures. The study of osteoporotic fractures. J. Bone Miner. Res. 10: 1076–1086.
11. Lehmann, R., Wapniarz, N., Hofmann, B., Pieper, B., Haubitz, I. & Allolio, B. (1998) Drinking water fluoridation: bone mineral density and hip fracture incidence. Bone 22: 273–278.
12. Sowers, M. F., Wallace, R. B. & Lemke, J. H. (1986) The relationship of bone mass and fracture history to fluoride and calcium intake: a study of three communities. Am. J. Clin. Nutr. 44: 889–898.
13. Jacobsen, S. J., Goldberg, J., Miles, T., Brody, J. A., Stiers, W. & Rimm, A. A. (1990) Regional variation in the incidence of hip fracture—US white women aged 65 years and older. J. Am. Med. Assoc. 264: 500–502.
14. Jacobsen, S. J., Goldberg, J., Cooper, C. & Lockwood, S. A. (1992) The association between water fluoridation and hip fracture among white women and men 65 years and older: a national ecological study. Ann. Epidemiol. 2: 617–626.
15. Cooper, C., Wickham, C., Lacey, R. F. & Barker, D. J. (1990) Water fluoride concentration and fracture of the proximal femur. J. Epidemiol. Community Health 4: 17–19.
16. Clarkson, J. (2000) International collaborative research on fluoride. J. Dent. Res. 79: 893–904.
17. Ericsson, Y., Gydell, K. & Hammarskjold, T. (1973) Blood plasma fluoride: an indicator of skeletal fluoride content. J. Int. Res. Commun. 1: 33–35.
18. Parkins, F. M., Tinanoff, N., Moutinho, M., Anstey, M. B. & Waziri, M. H. (1974) Relationship of human plasma fluoride and bone fluoride to age. Calcif. Tiss. Res. 16: 335–338.
19. Taves, D. R. & Guy, W. S. (1979) Distribution of fluoride among body compartments. In: Continuing Evaluation of the Use of Fluorides (Johansen, E., Taves, D. R. & Olsen, T. O., eds.), pp. 159–185. AAAS Selected Symposium 11. Westview Press, Boulder, CO.
20. Waterhouse, C., Taves, D. R. & Munser, A. (1980) Serum inorganic fluoride: changes related to previous fluoride intake, renal function and bone resorption. Clin. Sci. (Lond.) 58: 145–152.

21. Whitford, G. M. (1996) Absorption and plasma concentrations of fluoride. In: The Metabolism and Toxicity of Fluoride, 2nd ed. Monographs in Oral Science, Vol. 16 (Myers, H. M., ed.), pp. 10–29. Karger, Basel, Switzerland.

22. Taves, D. R. (1969) Determination of submicromolar concentrations of fluoride in biological samples. Talanta 15: 1015–1023.

23. Block, G., Hartman, A. M., Dresser, C. M., Carroll, M. D., Gannon, J. & Gardner, L. (1986) A data-based approach to diet questionnaire design and testing. Am. J. Epidemiol. 124: 453–469.

24. Phipps, K. R., Orwoll, E. S., Mason, J. D. & Cauley, J. A. (2000) Community water fluoridation, bone mineral density, and fractures: prospective study of effects in older women. Br. Med. J. 321: 860–864.

25. Stone, K. L., Seeley, D. G., Lui, L. Y., Cauley, J. A., Ensrud, K., Browner, W. S., Nevi, M. C. & Cummings, S. R. (2003) BMD at multiple sites and risk of fracture of multiple types: long-term results from the Study of Osteoporotic Fractures. J. Bone Miner. Res. 18: 1947–1954.

26. Zipkin, I., McClure, F. J., Leone, N. C. & Lee, W. C. (1958) Fluoride deposition in human bones. Public Health Rep. 73: 732–740.

27. Eanes, E. D. & Hailer, A. W. (1998) The effect of fluoride on the size and morphology of apatite crystals grown from physiologic solutions. Calcif. Tiss. Int. 63: 250–257.

28. Phipps, K. R., Orwoll, E. S. & Bevan, L. (1998) The association between water-borne fluoride and bone mineral density in older adults. J. Dent. Res. 77: 1739–1748.

29. Bainbridge, K., Sowers, M. F., Crutchfield, M., Lin, X., Jannausch, M. & Harlow, S. D. (2002) Natural history of bone loss over 6 years among premenopausal and early postmenopausal women. Am. J. Epidemiol. 156: 410–417.