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# Bone quality in fluoride-exposed populations: A novel application of the ultrasonic method

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ABSTRACT

*Background:* Various studies, mostly with animals, have provided evidence of adverse impacts of fluoride ($F^-$) on bone density, collagen and microstructure, yet its effects on overall bone quality (strength) has not been clearly or extensively characterized in human populations.

*Objective:* In this observational study, we assessed variation in an integrated measures of bone quality in a population exposed to wide-ranging $F^-$ levels (0.3 to 15.5 mg/L) in drinking water, using a novel application of non-ionizing ultrasonic method.

*Method:* We collected 871 speed of sound (SOS) measurements from 341 subjects residing in 25 communities, aged 10–70 years (188 males and 153 females). All subjects received scans of the cortical radius and tibia, and adults over the age of 19 received an additional scan of the phalanx. Associations between $F^-$ in drinking water and 24-h urine samples, and SOS as a measure of bone quality, were evaluated in bivariate and multivariable regressions adjusting for age, sex, BMI, smoking, and toothpaste use.

*Results:* We found negative associations between $F^-$ exposure and bone quality at all three bones. Adult tibial SOS showed the strongest inverse association with $F^-$ exposure, which accounted for 20% of the variance in SOS measures ($r = 0.45$; $n = 199$; $p < 0.0001$). In adjusted analysis, a 1 mg/L increase in $F^-$ in drinking water was related to a reduction of 15.8 m/s (95% CI: −21.3 to −10.3), whereas a 1 mg/L increase in 24-h urinary $F^-$ (range: 0.04–39.5 mg/L) was linked to a reduction of 8.4 m/s (95% CI: −12.7, −4.12) of adult tibial SOS. Among adolescents, in contrast, weaker and non-significant inverse associations between $F^-$ exposure and SOS were found, while age, gender, and BMI were more significant predictors than in adults.

*Conclusions:* These results are indicative of a fluoride-induced deterioration of bone quality in humans, likely reflecting a combination of factors related to SOS: net bone loss, abnormal mineralization and collagen formation, or altered microarchitecture. The portable and low-cost ultrasound technique appears potentially useful for assessment of bone quality, and should be tested in other locations and for other bone-related disorders, to assess the feasibility of its more extensive diagnostic use in hard-to-reach rural regions.

## 1. Introduction

Fluoride ($F^-$) is common in the environment yet is an often overlooked and persistent toxicant that poses risks to an estimated 200 million highly-exposed people globally, primarily via drinking water (Edmunds and Smedley, 2005; US NRC, 2006; WHO, 2016). The skeletal system is particularly susceptible to the adverse effects of $F^-$, bearing roughly 99% of the $F^-$ burden (Everette, 2011; Whitford,

*Abbreviations:* bw, body weight; BMI, Body Mass Index; $F^-$, qFluoride; IRB, Institutional Review Board; ISE, Ion Selective Electrode; mg/L, milligram per liter; mg/kg bw/day, milligram per kilogram body weight per day; MER, Main Ethiopian Rift; NOAEL, No-Observed-Adverse-Effects-Level; SOS, Speed of Sound; TISAB, Total Ionic Strength Adjuster Buffer; U.S. NRC, U.S. National Research Institute; U.S. EPA, U.S. Environmental Protection Agency; WHO, World Health Organization

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1999). The proportion of F that is incorporated by bone tissue depends on the amount ingested, timing of exposure, age of the affected individual, tissue metabolism, and genetic factors (Mousny et al., 2008). The U.S. National Research Council (US NRC, 2006; Barbier et al., 2010) and others (Choi et al., 2012; Everette, 2011; Grandjean and Landrigan, 2014; Tang et al., 2008) have reported multiple health consequences of $F^-$ exposure, including alteration of biochemical (e.g., inhibits enzyme action, including phosphatases) and physiological processes (i.e., bone quality and risk of fracture) and of cardiovascular, reproductive, endocrine, gastrointestinal, and neurological development. Fluoride is readily incorporated into bone and teeth, substituting for hydroxyls in hydroxyapatite crystals and forms fluorapatite (Everette, 2011; Mousny et al., 2008). Several studies in humans and animals have documented the effects of excessive $F^-$ exposure on the physical and chemical properties of enamel cells, bone minerals, bone cells, and bone remodeling processes, via promotion of osteoblast activity and delayed mineralization of new bone (Liu et al., 2016; Lai et al., 2014; Den Besten and Li, 2011; Mousny et al., 2008; Pei et al., 2017; Yao et al., 2019). These various conditions ultimately lead to a disease called chronic skeletal fluorosis (SF), indicated by various bone lesions, including osteosclerosis (i.e., increased bone density), osteoporosis, degenerative joint changes, and ligament calcifications (Cao et al., 2003; Gupta and Chhabra, 2016; Wang et al., 2007). Animal studies have also shown that $F^-$ can alter bone matrix proteins (i.e., collagen and non-collagenous proteins) and thereby affect bone elasticity and biomechanical integrity (Chavassieux et al., 2007; Chen et al., 2017; Fina et al., 2008; Mousny et al., 2008), lowering bone strength (or quality).

Bone is a complex hierarchical structure that includes a network of cells and mineralized fibrils, together with non-collagenous proteins and water, which together determine its mechanical properties (Zioupos, 1998; Natalie et al., 2014). As such, bone quality is difficult to define precisely and depends on multiple parameters, including bone density, characteristics of its microstructure, and collagen composition, which influence bone's resistance to fracture (Hart et al., 2017; Davison et al., 2006; Seeman and Delmas, 2006; Fyhrie, 2005). And though the effects of $F^-$ on bone forming and resorbing cells are well-documented (e.g., Everette, 2011; Mousny 2008), its effect on bone quality in humans has not been clearly characterized and understood. Diagnosis of SF continues to depend on the traditional X-ray imaging used for measuring bone density, without accounting for $F^-$ -related changes to bone microstructure, collagen composition and elasticity.

In view of the growing burden of fluorosis in highly exposed regions, and to allow a quantitative assessment of $F^-$ -associated changes in bone that is both simpler and more complete, we tested a non-invasive and portable ultrasound technology in a rural population, that resides in a hot spot for exposure to $F^-$ : the Rift Valley of Ethiopia. This specific technique measures the speed of sound waves (SOS) in bone, as influenced by a combination of bone parameters (e.g., microstructure, collagen composition, cortical thickness, and bone density) that are related to bone quality (Cortet et al., 2004; Grimal and Laugier, 2019; Hayman et al., 2002; Lee et al., 1997; Mutimura et al., 2016; Prevrhal et al., 2001; Rivas-Ruiz et al., 2016; Sievanen et al., 2001; Weiss et al., 2000; Zadik et al., 2003). While the SOS measure is influenced by the collection of these various bone properties, the contribution of the specific parameters to SOS is unclear, and the relevance of this measure to bone quality therefore requires further study, including in animal studies that would allow a more complete characterization. Nonetheless, the approach appears promising for low-cost screening for bone health including osteoporosis (e.g., Cook et al., 2005; Shenoy et al., 2014; Mutimura et al., 2016; Rivas-Ruiz et al., 2016). Studies in humans have also shown that SOS correlates significantly with bone mineral density (BMD) (Glüer et al., 1994; Vignolo, 2006).

The key feature of the approach is the ability to quantify variation in the SOS, measured in meters per second (m/s) propagated in cortical bone, as caused by changes in the collection of bone parameters that are related to bone quality (Kaufman and Einhorn, 1993; Mehta et al., 1998, 2001; Mutimura et al., 2016; Raum et al., 2014; Sievanen et al., 2001). Cortical bone is dense and solid; it forms about 80% of the skeleton and is thus relevant to the study of bone quality (Augat and Schorlemmer, 2006; Clarke, 2008).

To date, no studies have assessed the utility of this technique for determining the quality (strength) of bone in populations exposed to environmental sources of $F^-$. Our objective in this study was therefore to test the applicability of this technique for determining bone quality among individuals exposed to wide-ranging levels of natural $F^-$ in drinking water (0.3 to 15.5 mg/L). We hypothesized that the SOS would increase with bone density caused by $F^-$ exposure, as increasing bone density is a common diagnostic feature in populations exposed to excessive F (Kaminsky et al., 1990; Tekle-Haimanot, 1990; Kleerekoper and Balena, 1991; Cao et al., 1996; Wu et al., 2015; Wang et al., 2012). We evaluated variation in bone SOS at the radius, tibia, and phalanx and investigated the relationships between F in water sources, and individuals' excreted urine and SOS, controlling for potential modifiers (such as age, gender, BMI, current toothpaste use, and smoking habit).

## 2. Materials and methods

### 2.1. Study population

During two sampling periods (between 2018 and 2019), a total of 341 individuals (188 males and 153 females) were enrolled in a cross-sectional study conducted in 25 rural communities in the Main Ethiopian Rift (MER), each of which were primarily dependent on a single groundwater well. The selection of sampling sites was informed by prior research conducted to characterize $F^-$ levels in water sources in the same study region, which has identified wide variation in exposures to $F^-$ (ranging from 0.3 to 15.5 mg/L) from water extracted from groundwater wells that are used for drinking and cooking (Rango et al., 2012, 2013, 2019). Study participants are primarily engaged in cereal-based agriculture as their primary livelihood activity and are generally low income and rural. In order to better select populations with known exposures to $F^-$, we recruited participants collecting water for domestic uses, from the community sources that had been tested. Study inclusion criteria were: Consent to participate, permanent residence in the community, age between 10 and 70 years, and duration of residency that was at least as long as the age of the sampled well from which community members were consuming. In each community, we further attempted to select individuals to obtain an equal representation across the different ages and sex (10–70 years). Survey data were collected to record the sex, age, place of birth, water intake per day, current toothpaste use, and smoking habits of enrolled individuals, as well as the drilling date for the water source being used. Only a few individuals meeting the study eligibility criteria detailed above were excluded from the sample, based on a judgment that they would be incapable of undergoing detailed health examinations.

We also collected 24-h urine samples from 193 of the 341 individuals associated with 17 of the 25 community wells. These 17 wells had $F^-$ concentrations ranging between 0.3 and 10.7 mg/L and were selected for complementary urine sampling because they were active during our sampling period, which is necessary to obtain an appropriate exposure-biomarker relationship. The other 8 communities were found to use well water only intermittently because of malfunctions (3 wells), including during sampling, or used their wells only for cooking purposes (5 wells). Households in the latter communities primarily obtained drinking water that was piped from nearby towns low in $F^-$ ($< 2$ mg/L).

Six individuals from these sites did not provide urine samples as they were unable or being unwilling to collect a 24-h sample. We also included anthropometric measures of weight and height using an electronic scale for weight and measuring tapes for height. These

measures allowed calculation of the body mass index (BMI: weight(kg)/height($m^2$)).

Field enumerators (graduate students and nurses/medical doctors) were trained on the content of the questionnaire. The study received ethical approval from the Institutional Review Board (IRB) at Tulane University (Protocol No. 2018–043) and locally from the National Research Ethics Review Committee (NRERC; reference no. MoSHE/144/1096/19). All subjects provided consent, and parents/guardians gave permission for children to participate in addition to children giving their own assent.

### 2.2. Sampling and analysis of water and 24-h urine

All sample collection materials for water and urine were pre-cleaned sequentially with trace metal grade 1 N $HNO_3$, and 1 N HCl (for at least three hours in each step) and finally rinsed three times with deionized water of resistivity > 18 MΩ/cm. Water samples were then filtered in the field using disposable 60 mL volume luer lock syringes and 0.45 μm Mixed Cellulose Ester membrane filters directly into 60 mL polyethylene bottles.

Urine samples were collected in disposable plastic urine collection containers with a closing cap (each with a capacity of 1 gal) along with transferring plastic beakers (capacity of 1 l) that were provided to each individual. The volume of each collected urine sample was registered, and the sample was immediately transferred into a 60 mL polyethylene bottle. Participants were instructed to carefully collect urine samples and shown how to avoid contamination. Water and urine samples were then kept in a zero-degree freezer, and properly packed, stored, and transported to the testing laboratory at Tulane University.

Water and urine $F^-$ content was determined using the ion selective electrode and the hexamethyldisiloxane (HMDS)-facilitated diffusion method. Details on this diffusion technique are described in Rango et al. (2017). Given that $F^-$ levels in urine were typically well above the limit of detection of the $F^-$ electrode (0.02 mg/L), we measured urinary $F^-$ using a direct method, buffering the standards and urine samples using equal volume ratios (1 mL each) with a total ionic strength adjustment buffer (TISAB II). This allows optimal analysis of $F^-$ ion by adjusting the pH of the solution between 5 and 5.5, and the ionic strength of the standards and samples to the same values. Calibration standards were prepared from 100 mg/L stock solution. The mean electrode calibration slope for a 10-fold change in F concentration was within the acceptable theoretical slope range (from −57 to −58 mV). For validation, we also retested a sub-set of samples with the diffusion method. In brief, diffused F standards and samples were captured quantitatively in 0.05 N NaOH drops on the interior of a nonwettable sealed petri dish cover. The diffused $F^-$ were then buffered with 0.20 N acetic acid, and the $F^-$ ions in this solution were measured using the $F^-$ combination ion specific electrode (ISE; Orion Research, model 9609BNWP) coupled to a potentiometer (Orion, model A324). The result with the direct method matched well with those from the diffusion method (r = 0.95). Quality control was conducted using freeze-dried urine reference material (SERO210705; LGC Standards). The accuracy of ISE $F^-$ measurements for both urine and water standards ranged from 98% to 102.5% relative to the standard, in analyses with both the diffusion and the direct methods.

### 2.3. Quantitative measurement of bone status

A total of 871 ultrasound scan measurements were done on 341 individuals in all: 194 adults above the age of 19 years and 147 children and adolescents (aged 10 to 19 years). The measurements were made at three skeletal sites (radius, tibia, and phalanx) for the former; and on at two sites (radius and tibia) for the latter, using the adult (> 19 years old) and pediatric (< 19 years old) software version 3.2 of the Sunlight MiniOmni ultrasound device (Sunlight, Tel Aviv, Israel). The scans were taken using the hand-held probe of the ultrasound device at 1) the radius at the point halfway between the edge of the elbow to the tip of the distal phalanx of the middle finger of the left hand, 2) the mid-tibia, at the point halfway between the edge of the heel and the proximal edge of the knee, and 3) at the middle proximal phalanx. The hand-held probe contains sets of transducers that produce pulsed acoustic waves at a mean frequency of 1.25 MHz, inaudible to human ears. These waves traverse the soft tissue and enter the bone at a critical angle. The shortest propagation time of the signal between the transmitter and the receiver is used to calculate the SOS (e.g., Prevrhal et al., 2001), which is expressed in meters per second. The device was calibrated daily using the manufacturer's verification phantom. The transducer was placed on the marked site of measurement and rotated without lifting the transducer from the skin, and the speed of sound measurements were performed three times at the pre-marked location, and the measurement was recorded. The intra-individual coefficients of variation (CVs) for SOS values for 15 individuals at the radius and tibia was between 0.8 and 1%.

### 2.4. X-ray SF examination

For validation purposes, we enrolled a subset of thirty-nine subjects from three communities with higher water $F^-$ concentrations of 7, 9.8, and 13.7 mg/L. The subjects received standard digital X-ray radiographs of the forearm in a frontal projection (on the wrist, fingers, and forearm bones), and of the lower leg in a frontal projection (on the knee, tibia and fibula). All radiographs were analyzed off-site by a radiologist, a co-author specialized in SF diagnosis. SF severity was classified into three groups: 1) mild, 2) moderate, or 3) severe SF as described in the diagnostic criteria detailed in Wu et al. (2015). In brief, bones were evaluated for increased bone density, and other features including osteoporosis, osteomalacia, bone turnover, bowing, arthritis, ligamental or interosseous membrane calcification and ossification. The diagnostic features of 1) *mild* SF are cortical thickening, sand-like or granular bone pattern, and scattered coarse trabeculations with blurry trabecular outlines, 2) *moderate* SF are condensed and coalescing coarse trabeculations with overall increased bone density, cortical thickening, and medullary narrowing with interosseous ossification or calcifications, and 3) *severe* SF are a marble white appearance and wooly blurring of the medullary bone, where the trabecular details cannot be distinguished as there is widespread fusion of the trabeculae associated with interosseous membrane ossification.

### 2.5. Statistical analysis

Demographic, anthropometric, $F^-$ exposure, and cortical bone SOS variables were first described by their quartiles and means ± standard deviation. The distribution of SOS was tested for normality using a Shapiro-Wilk test and by fitting a normal model examining plots of the residuals against fitted values, and residuals against normal scores. The $F^-$ level in water was analyzed as a continuous variable and was also categorized into four groups (< 2, > 2–6, > 6–10, and > 10–15.5 mg/L). As age and SOS are known to be related, we next conducted preliminary analyses by fitting a regression line to evaluate the associations between these variables. Comparisons of means in the different $F^-$ exposure groups, and by sex were carried out with one-way ANOVA. We then utilized a linear regression model to examine the effects of $F^-$ exposure on the relative SOS values at three bone sites (radius, tibia, phalanx), adjusting for sex, age, BMI, current toothpaste use, and smoking. We also tested the interaction effect of $F^-$ exposure and gender on relative SOS levels. The results are presented as regression coefficients $\beta$ with their 95% confidence intervals (95% CIs). Finally, a prediction model for SOS was developed using a mixed effects model with bone type (cluster) as a random effect and BMI, sex, $F^-$ levels in drinking water, toothpaste use and smoking as fixed effects. All study hypotheses were tested at the 5% level of significance. All analyses, summaries, and graphs were performed using the Statistical Analysis System (SAS 9.4,

**Table 1**
Statistical descriptions of the characteristics, $F^-$ exposures, SOS measures in study participants.

| | N | Min | Percentiles | | | Max | Mean ± SD |
|---|---|---|---|---|---|---|---|
| | | | 25th | 50th | 75th | | |
| Anthropometric measures | | | | | | | |
| Age | 341 | 10 | 15 | 22 | 38 | 70 | 27.8 ± 14.9 |
| Weight (kg) | 341 | 15.5 | 44.2 | 52.6 | 59.0 | 88.9 | 51.4 ± 12.0 |
| Height (m) | 341 | 1.26 | 1.54 | 1.62 | 1.70 | 1.88 | 1.61 ± 0.12 |
| BMI ($kg/m^2$) | 341 | 9.8 | 18.6 | 20.0 | 20.4 | 25.2 | 19.7 ± 3.4 |
| Biomarkers of exposures | | | | | | | |
| Water $F^-$ concentrations | | | | | | | |
| Water intake (liter/day) | 341 | 0.3 | 0.9 | 1.2 | 1.80 | 4.5 | 1.3 ± 0.63 |
| $F^-$ in groundwater (mg/L) | 25 | 0.3 | 3.0 | 6.5 | 10.2 | 15.5 | 6.8 ± 4.30 |
| $F^-$ intake (mg/day) | 341 | 0.17 | 3.53 | 7.8 | 12.6 | 50.4 | 9.13 ± 7.30 |
| Dose (mg/kg bw/day) | 341 | 0.003 | 0.08 | 0.15 | 0.25 | 0.70 | 0.18 ± 0.15 |
| Urinary $F^-$ concentrations | | | | | | | |
| $F^-$ in 24-h urine (mg/L) | 193 | 0.035 | 2.63 | 5.53 | 11.4 | 39.5 | 8.2 ± 7.6 |
| $F^-$ excretion (mg) | 193 | 0.022 | 1.57 | 3.99 | 6.97 | 25.5 | 5.01 ± 4.5 |
| Biomarker of effect | | | | | | | |
| SOS measures | | | | | | | |
| SOS in all bone sites (m/s) | 871 | 2881 | 3620 | 3736 | 3880 | 4246 | 3742 ± 198 |
| SOS on radius (m/s) | 341 | 3251 | 3755 | 3903 | 3994 | 4246 | 3875 ± 171 |
| SOS on tibia (m/s) | 336 | 3074 | 3598 | 3704 | 3798 | 4026 | 3687 ± 151 |
| SOS on phalanx (m/s) | 194 | 2881 | 3509 | 3622 | 3716 | 3986 | 3605 ± 172 |
| All SOS measures by sex and bone sites | | | | | | | |
| Male | | | | | | | |
| SOS in all bone sites (m/s) | 491 | 2881 | 3624 | 3728 | 3872 | 4211 | 3741 ± 191 |
| SOS on radius (m/s) | 189 | 3251 | 3712 | 3886 | 3982 | 4211 | 3855 ± 178 |
| SOS on tibia (m/s) | 189 | 3074 | 3611 | 3707 | 3802 | 3976 | 3696 ± 150 |
| SOS on phalanx (m/s) | 115 | 2881 | 3546 | 3644 | 3730 | 3986 | 3626 ± 174 |
| Female | | | | | | | |
| SOS in all bone sites (m/s) | 380 | 3139 | 3609 | 3744 | 3894 | 4246 | 3745 ± 206 |
| SOS on radius (m/s) | 153 | 3393 | 3803 | 3925 | 4011 | 4246 | 3901 ± 159 |
| SOS on tibia (m/s) | 147 | 3193 | 3574 | 3693 | 3794 | 4026 | 3676 ± 153 |
| SOS on phalanx (m/s) | 80 | 3139 | 3474 | 3604 | 3707 | 3882 | 3575 ± 166 |
| All SOS measures by age | | | | | | | |
| 10–25 years | 424 | 3074 | 3624 | 3727 | 3840 | 4214 | 3729 ± 170 |
| > 25–35 years | 122 | 3410 | 3663 | 3802 | 3959 | 4246 | 3806 ± 188 |
| > 35–50 years | 234 | 3132 | 3624 | 3749 | 3947 | 4234 | 3768 ± 216 |
| > 50–70 years | 91 | 2881 | 3513 | 3676 | 3842 | 4074 | 3651 ± 239 |
| Lifestyle factors | Yes | No | Yes (%) | No (%) | | | |
| Current smoking habit | 16 | 325 | 4.7 | 95.3 | | | |
| Current toothpaste use | 15 | 326 | 4.4 | 95.6 | | | |

SAS Institute, Cary, NC, and GraphPad Prism 8.02).

## 3. Results

### 3.1. Characteristics of the study population

Demographic, anthropometric data, $F^-$ concentrations in drinking water and urine, ultrasound measures (as SOS) categorized by skeletal sites (radius, tibia and phalanx), sex and age groups, toothpaste and smoking habit of the studied population are presented in Table 1. The range and mean (± SD) of SOS in study subjects was 3074–4026 (3687 ± 151) for tibia, 3225–4246 (3875 ± 178) for radius and 2881–3986 (3605 ± 172) for phalanx, and the SOS across the three bone sites was different ($p < 0.0001$), with the radius having the highest mean value. The average age of the study participants was 27.8 ± 14.9 years and approximately 44% (n = 140) were male with no significant differences in age among the sex subgroups. The age of the groundwater wells the communities use ranged between 12 and 65 years old (mean: 34.9 ± 17.1 years). The population in the study region is primarily of Oromo ethnicity. Study subjects had a mean BMI of 19.7 ± 3.3 $kg/m^2$ with a 25th percentile of 18.6 $kg/m^2$ and 75th percentile of 20.4 $kg/m^2$. Most participants had BMI between 18.5 and 24.9 $kg/m^2$ and were categorized as having normal weight (56%; n = 192), followed by 38.4% (n = 131) with BMI below 18.5 $kg/m^2$, indicating underweight. A small percentage (5.3%; n = 18) of subjects were overweight or obese (≥25.5 $kg/m^2$). More than 95% of the participants reported not smoking or using toothpaste; as noted later this lack of variation makes detection of associations between SOS measures and these factors unlikely.

### 3.2. Fluoride exposure from drinking water

Epidemiological studies have shown that mild and advanced SF can occur at intake levels of at least 10 mg/day $F^-$ for exposures of 10 or more years (ATSDR, 2003; Cao et al., 2003; IOM, 1997). Participants in this study reported consuming between 0.3 and 4.5 l of water per day (mean: 1.3 ± 0.66 l). The $F^-$ concentrations in groundwater ranged between 0.3 and 15.5 mg/L. The interquartile ranges of the estimated daily $F^-$ intake per person and dose was 3.53–12.6 mg/day, and 0.08–0.25 mg/kg bw/day, respectively. In terms of per-person exposure from drinking water sources, study participants are exposed to $F^-$ intake ranging from 0.17 to 50.4 (mean: 9.13 ± 7.3) mg/person/day. Approximately 65.7% (n = 224) of the individuals had F intake from drinking water that exceeded 5.0 mg/person/day, whereas 34.3% (n = 117) and 8.8% (n = 30) had $F^-$ intake exceeding 10 and 20 mg/person/day, respectively. The mean ± SD dose value across all ages was 0.18 ± 0.15 mg/kg bw/day, and the means and ranges across different age sub-groups were generally similar. These daily $F^-$ doses were compared with the No-Observed-Adverse-Effects-Level (NOAEL) for $F^-$ (0.06 mg/kg bw/day) published by US EPA (2002), and showed

that the majority (79%; n = 269) of individuals ingested a daily $F^-$ dose from water alone that exceeded this NOAEL value.

### 3.3. Fluoride in 24-h urine samples

The 24-h urinary $F^-$ concentration serves as an ideal biomarker for current exposure. The urinary $F^-$ concentration in individuals providing urine samples ranged from 0.035 to 39.5 mg/L (mean: 8.15 ± 7.6 mg/L). The corresponding range of urinary $F^-$ excretion was 0.022 to 25.5 mg (mean: 5.0 ± 4.5 mg). About 82% (n = 154) of urine samples contained $F^-$ at levels higher than the Biological Exposure Index (BEI) of 2 mg/L, which is used by industrial hygienists as a guideline for occupational exposure to $F^-$ (ACGIH, 2012). In our study, participants consuming lower F (< 2 mg/L) in drinking water from four community wells had 24-h urinary $F^-$ concentrations ranged from 0.66 to 3.9 (mean: 1.7 ± 1.1 mg/L).

### 3.4. Age and sex influence on the SOS

The relationship between age and SOS values displays a distinct pattern at each bone site that varies by sex, particularly at the radius and tibia (Fig. 1). Females had faster SOS than males at earlier ages (between 10 and 40 and 10–28 years at the radius and tibia, respectively). Males in contrast had faster SOS at later ages (Fig. 1A and B). At the phalanx, men had faster SOS than females over most ages (~25 to 70 years); it is difficult to draw conclusions at younger ages (< 19 years) due to limited data points (Fig. 1C). At the radius, we observed a gradual increase in mean SOS with age from around 3700 m/s to a peak value of 4050 m/s at age 29 for women, and from 3550 to a peak of 4000 m/s at age 33 for men. At the tibia, a sharper increase in mean SOS was observed, starting at 3520 and 3470 m/s and increasing to a peak value of 3825 m/s at age 23 for women and at 3785 m/s at age 29 for men.

### 3.5. Association of $F^-$ in drinking water with bone SOS

We observed a significant inverse association between $F^-$ exposure in drinking water and bone quality across all ages, as quantified using individuals' SOS at the three skeletal sites (Fig. 2). In categorical analysis (Table 2), the water $F^-$ exposure, adjusted for age, gender, BMI, toothpaste and smoking, explained the largest variability of tibial SOS (9%) as compared to the other bone sites. While all sites show decreasing SOS with higher $F^-$ exposure groups, the tibia SOS showed the most significant negative association with the $F^-$ exposure in Group 3 and 4 as compared to Group 1 (< 2 mg/L), whereas the $F^-$ exposure in Group 2 showed negative association but was not significant. The tibia had adjusted $\beta$ of −108 m/s (95% CI: −153, −63) for individuals exposed to > 10–15.5 mg/L water $F^-$ (Group 4), and − 61.5 (95% CI: −108, −16.3) for individuals exposed to > 6–10 mg/L water F (Group 3), when compared to the individuals exposed to < 2 mg/L water $F^-$. For the radius and phalanx, $F^-$ exposure Group 4 (> 10–15.5 mg/L) was only significantly different as compared to the reference F exposure (< 2 mg/L) ($p < 0.05$). For instance, the adult tibial SOS values of participants in the high-F group (> 10–15.5 mg/L) were significantly lower than in the low-$F^-$ (< 2 mg/L) group (3809 ± 112 m/s vs. 3640 ± 139 m/s) ($p < 0.0001$).

Unlike the adults (Figs. 3 and S1), $F^-$ and SOS were poorly correlated at three bone sites in adolescents (Fig. S2). In Figs. 2, 3 and S3, the participants in each community have similar age and sex distributions, such that sex- and age-related effects on bone weakening cannot explain the variation in observed outcomes across $F^-$ exposures.

To better characterize the effect of $F^-$ on SOS, and resolve age-related bone development dynamics, specifically that peak bone density is reached at the end of adolescence, with only a slight increase thereafter (e.g., Van der Sluis et al., 2002; Lu et al., 1994), we conducted linear regression analysis in two age groups (Tables 3 and 4): 1) 10 to 19 year old adolescents (the period with high bone growth) and 2) adults over the age of 19 years (the period during which bone remodeling predominates). In adults, significant inverse relationships between F and SOS were.

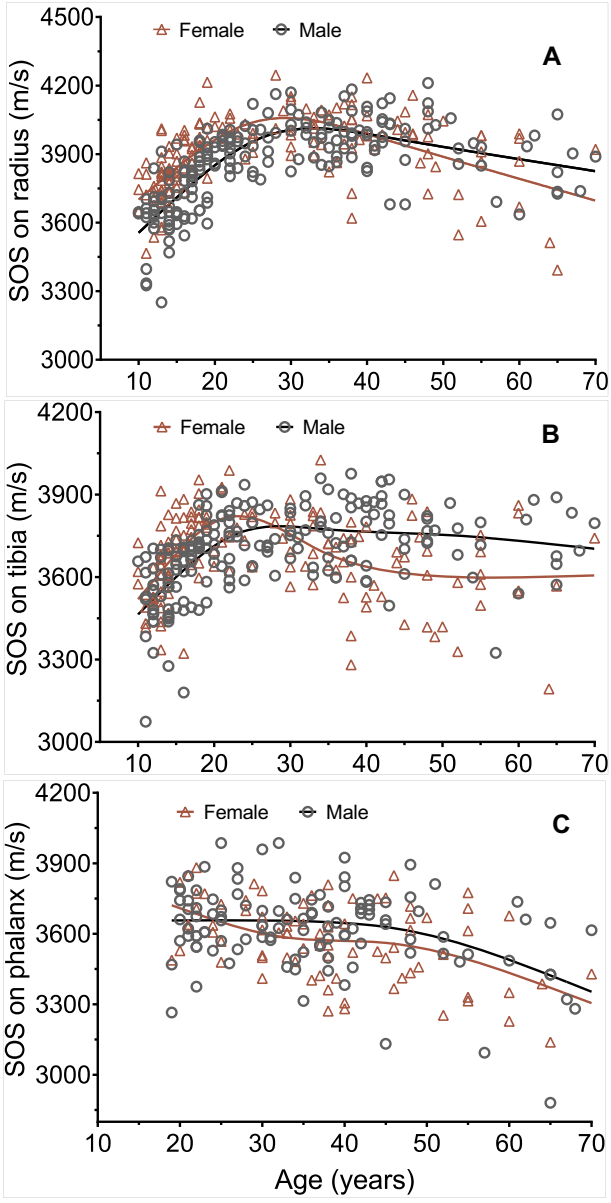


**Fig. 1.** Plots of age versus SOS for tibia (A), radius (B), and phalanx (C) with curve fit obtained using a smoothing spline regression analyses in 10 to 70 years adolescents and adults in all communities (0.3–15.5 mg/L of $F^-$) from the Ethiopian Rift Population.

observed, and were strongest for the tibia (Table 3, Fig. 3A–C); In crude analysis, $F^-$ exposure accounted for 21, 8.5 and 5.6% of the individual variability in SOS at the adult tibial, radial, and phalanx SOS, respectively ($p < 0.001$). In adjusted multivariable models, $F^-$ exposure and age were significant predicters of adult SOS, whereas BMI, current toothpaste use, and smoking status were not significantly associated with SOS. Gender was a significant predictor for tibial SOS, but not significant for other bone sites. At each bone site, the interaction of female sex and $F^-$ level in water was associated with a larger increase in SOS (Table 3).

Adjusting for all covariates, a 1 mg/L increase in $F^-$ in drinking water is linked to on average 15.8 m/s (95% CI: −21.3 to −10.3), 7.2 m/s (95% CI: −13.6 to −1.8), and 11.2 m/s (95% CI: −18.4 to

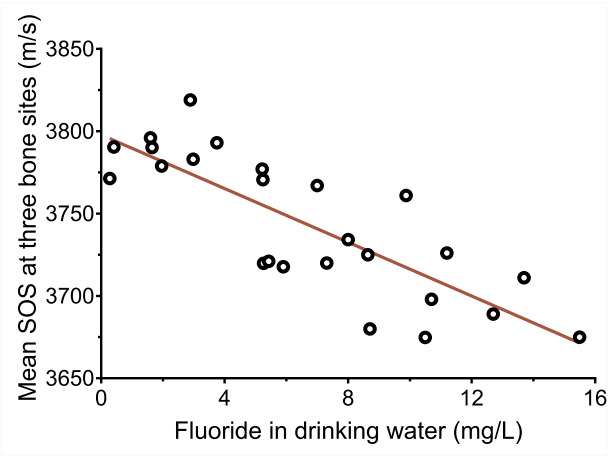


**Fig. 2.** Mean community-level SOS (n = 25), determined using compiled skeletal measurements from all bone sites (radius, tibia, and phalanx), as a function of the $F^-$ concentrations in community wells (r = 0.81, p < 0.0001), among the 341 study individuals.

−3.87) lower SOS in the adult tibia, radius, and phalanx, respectively. All covariates accounted for 32%, 19% and 21% of the variability in SOS in the respective bone sites. Females had about 77 and 65 m/s lower SOS than males on average at adult tibia and phalanx, respectively. Unlike the adults, non-significant and weak inverse association between F exposure and SOS were found in adolescent tibiae and radii, whereas age, gender and BMI were significant predictors of the SOS in both bone sites of adolescents (Table 4, Fig. S2). Adolescent females had about 141 and 84 m/s higher SOS than males at the radius and tibia, respectively (Table 4). In adolescents, gender and water $F^-$ level interactions showed a more strongly decreasing SOS in females than males, though the interaction remained insignificant.

### 3.6. Association of 24-h urine $F^-$ with bone SOS

A significant positive association was observed between $F^-$ concentration in drinking water and individuals' 24-h urine concentration (r = 0.75, p < 0.0001; Fig. 4A) and excretion (r = 0.64, p < 0.0001; Fig. S4). Adjusting all covariates, $F^-$ in water was the most significant contributing factor to the urinary $F^-$ concentration (p < 0.0001). This supposition was supported in our study by the observed correlation between higher urinary F and lower ultrasound conduction in the tibia, radius and phalanx and by the X-ray findings (Fig. 5). Among cortical bone sites, the tibia showed more significantly decreasing SOS with increase in 24-h urinary $F^-$ (r = 0.64, p < 0.0001) (Fig. 4B). Inverse associations were also observed in the radius and phalanx, though these were not statistically significant (Fig. S5).

After adjusting for confounding factors (Table 5), a significant reduction of 8.4 m/s (95% CI: −12.7, −4.12) and 8 m/s (95% CI: −12.4, −3.62) was observed with every 1 mg/L increase in urinary $F^-$ concentrations for tibial and radial SOS, respectively, and a non-significant reduction of 0.76 m/s SOS was observed in phalanx. The urinary F and other covariates contributed 20%, 28% and 17% SOS variance in adult tibia, radius, and phalanx, respectively. As the $F^-$ in water, the urinary $F^-$ contributed significantly to SOS variability on tibial bone site in particular.

### 3.7. Radiological X-ray findings

A subset of study subjects (n = 39) were screened for X-ray radiography from three communities with $F^-$ concentrations of 7, 9.8, and 13.7 mg/L in drinking water. The bones of these individuals were evaluated primarily for osteosclerosis (i.e., increased bone density), and other features including osteoporosis, osteomalacia, bone turnover, bowing, arthritis, ligamental or interosseous membrane calcification and ossification. The diagnosis was classified into three grades: 1) *mild*, *2) moderate*, *and 3) severe SF*. Accordingly, 74% (n = 29) of the subjects had mild SF, which showed cortical thickening, sand-like granular bone spots, and scattered coarse trabeculations with blurry trabecular outlines. About 18% (n = 7) of the subjects in the subsample had *moderate* SF showing condensed and coalescing coarse trabeculations with overall increased bone density, cortical thickening, and medullary narrowing with interosseous ossification or calcifications. In *moderate* SF, 13% (n = 5) subjects showed interosseous ossifications. Three subjects (7.8%) had severe SF showing marble white and wooly blurring of the medullary bone, and the trabecular details cannot be

**Table 2**
Associations between $F^-$ concentrations and relative SOS values in 10–70 years subjects.

| Fluoride concentration (mg/L) | Relative SOS values | | | |
|---|---|---|---|---|
| | Crude, $\beta$ (95% CI) | p value | Adjusted, $\beta$ (95% CI) | p value |
| For tibia | | | | |
| Water $F^-$ exposure group | $R^2$ = 0.089 | | $R^2$ = 0.089 | |
| Group 1 (< 2) | Reference | | Reference | |
| Group 2 (> 2–6) | −5.4(−51.1, 40.2) | < 0.82 | 0.76(−42.9, 44.5) | 0.97 |
| Group 3 (> 6–10) | −62.3(−109.4, −15.1) | 0.01 | −61.5(−108, −16.3) | < 0.05 |
| Group 4 (> 10–15.5) | −109.7(−156.7, −62.8) | < 0.0001 | −108(−153, −63) | < 0.0001 |
| Linear trend | | < 0.001 | | < 0.0001 |
| For radius | | | | |
| Water $F^-$ exposure group | $R^2$ = 0.017 | | $R^2$ = 0.022 | |
| Group 1 (< 2) | Reference | | Reference | |
| Group 2 (> 2–6) | −24.1(−77.3, 29.2) | 0.38 | −14.8(−61.3, 31.7) | 0.53 |
| Group 3 (> 6–10) | −48.1(−102.9, 6.8) | 0.09 | −45.4(−93.5, 2.63) | 0.06 |
| Group 4 (> 10–15.5) | −60.8(−115.4, −6.2) | 0.03 | −61.2(−109.1, −13.4) | 0.01 |
| Linear trend | | 0.02 | | 0.02 |
| For phalanx | | | | |
| Water $F^-$ exposure group | $R^2$ = 0.059 | | $R^2$ = 0.036 | |
| Group 1 (< 2) | Reference | | Reference | |
| Group 2 (> 2–6) | −31(−101.7, 39.6) | 0.39 | −4.4(−6.24, −2.46) | 0.26 |
| Group 3 (> 6–10) | −37.6(−109.5, −15.1) | 0.30 | −43(−110.2, −24.1) | 0.21 |
| Group 4 (> 10–15.5) | −116.3(−188.3, −44.2) | 0.002 | −102.6(−171.9, −33.3) | 0.004 |
| Linear trend | | 0.001 | | 0.001 |

$\beta$, regression coefficient; *CI*, confidence interval.

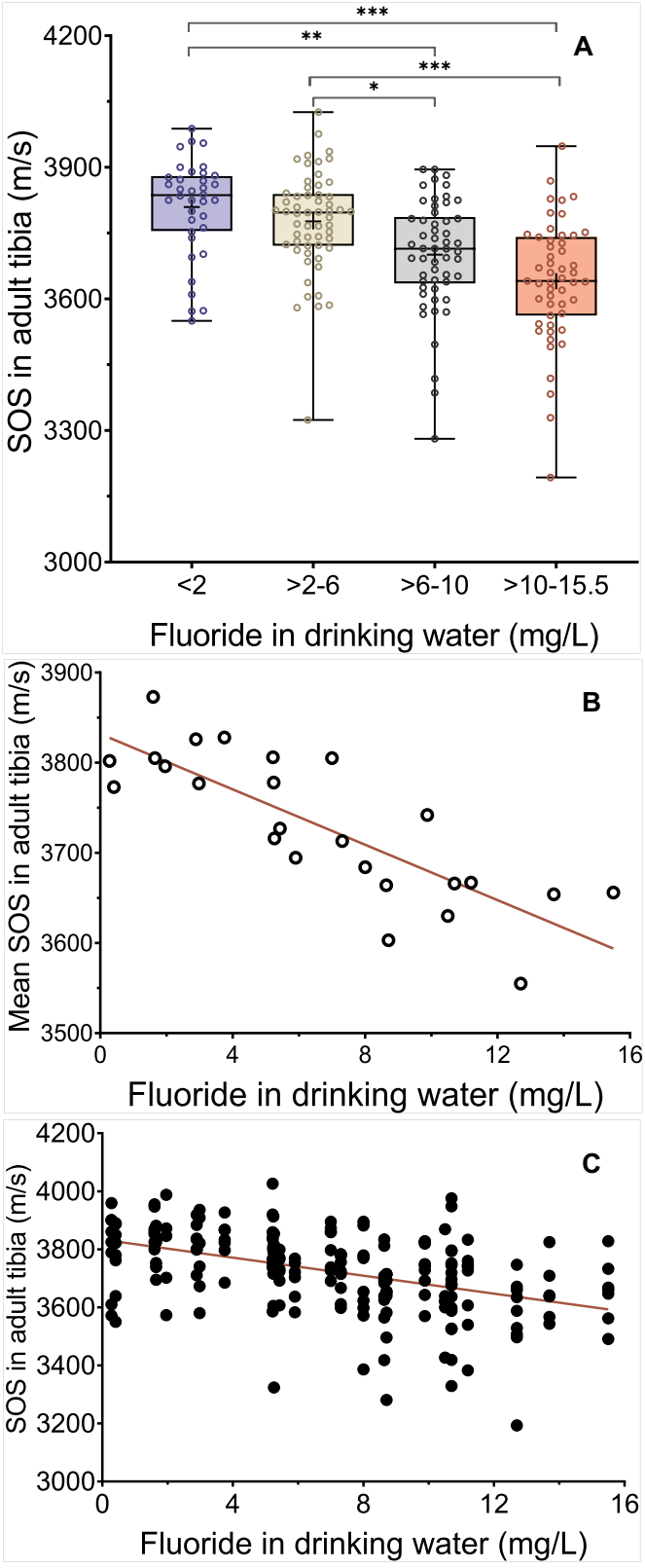


**Fig. 3.** A) Box and whiskers plot showing association between the speed of sound (SOS) measured at the tibia in adults (> 19 to 70 years old), and the four exposure categories of $F^-$ concentrations in drinking water (< 2, > 2–6, > 6–10, and > 10–15.5 mg/L), *p < 0.01; **p < 0.001; ***p < 0.0001, and B) Mean community-level SOS among adults and $F^-$ concentrations in drinking water from the 25 sample communities (r = 0.81, p < 0.0001), C) SOS in all adults and $F^-$ concentrations in corresponding community water sources (r = 0.45, p < 0.0001).

distinguished as there is widespread fusion of the trabeculae associated with interosseous membrane ossification (e.g., Fig. 5).

Twenty-two subjects (56.4%) showed osteoporosis with absence of bony trabeculation, as seen in the *peri*articular region of the wrist and all of these also showed sclerotic *mild* SF, observed in the diaphyseal region. Fifteen percent (n = 6) of the study subjects showed osteoarthritic changes which presented with marginal and intercondylar eminence osteophytes, peri articular sclerosis, and narrowed joints spaces. Bowing of the long bones was seen in ulna or radius in 15% (n = 6) of the subjects, all of whom had associated peri articular osteoporosis and a bowing deformity, suggesting osteomalacia.

## 4. Discussion and conclusion

In the first observational study of this type, we used a quantitative ultrasound method to measure the SOS at multiple bone sites in a population that is chronically exposed to naturally contaminated level of $FF^-$ in drinking water wells (0.3 to 15.5 mg/L of $F^-$). The high $F^-$ concentrations in urine (up to 39.5 mg/L) indicate that a significant proportion of $F^-$ is retained in bone of the study population, and we expect that this would lead to a range of $F^-$ -related disorders in bones, joints, and cartilage. Further, we initially hypothesized that increased SOS would reflect bone density/mineralization and directly correlate with increasing $F^-$ exposure. This is because SF studies in many endemic countries including in our study site (e.g., Cao et al., 1996; Tekle-Haimanot, 1990; Wu et al., 2015; Wang et al., 2012), primarily employ X-ray radiography to detect increased bone density in SF patients. Contrary to our hypothesis, we discovered that $F^-$ exposure is associated with lower SOS in all three cortical bone sites that were tested (Figs. 2 and 3), and thus appears to indicate a deterioration of bone quality. Given that ultrasound characterizes the overall material property of bone (e.g., Grimal and Laugier, 2019; Sievanen et al., 2001; Mutimura et al., 2016; Rivas-Ruiz et al., 2016; Lee et al., 1997; Weiss et al., 2000; Zadik et al., 2003), these observations serve to highlight the inherent complexity and diverse manifestations of $F^-$ effects in bone that go beyond changes in bone density alone. For instance, our evaluations of X-rays from selected subjects also showed a co-presence of osteosclerosis and osteoporosis (see Fig. 5). In addition, we observed the occurrence of $F^-$ associated osteoarthritis in the study sample. Other studies have demonstrated increased prevalence of osteoarthritis in $F^-$ exposed populations (e.g., Cao et al., 2013; Petrone et al., 2011; Savas et al., 2001).

In adults' tibiae, radii, and phalanx, significant inverse associations were found between $F^-$ in drinking water or urine and mean SOS in bones of individuals in the communities. From theses correlations, the lower bone SOS value indicates a higher bodily burden of $F^-$ in bone. In adolescents, we found weak and non-significant associations in the tibia and radius (Fig. S2), suggesting that other factors such as high bone growth rate at younger ages mask the effect of $F^-$ exposure in their bones. In fact, the regression model show that age, gender and BMI are significant predictors of SOS measures in adolescents (Table 4).

The age-SOS trend in Fig. 1 mimics the general age-BMD (bone mineral density) pattern observed in a healthy population (e.g., Weaver et al., 2016); however, age-SOS in our results showed females had higher SOS values than males at an earlier age before adolescence. A higher SOS in females may be due to the earlier onset of puberty in females (2 years prior to males). This highlights potential sex differences in bone growth (areal BMD), which is greater at younger ages in females than in males (Baroncelli et al., 2006; Maggioli and Stagi, 2017). The declining SOS in females is then expected as a consequence of estrogen deficiency in women at older ages, which leads to increased osteoclastic formation, expanded remodeling space, increased cortical porosity, and thus net bone loss (Falahati- Nini et al., 2000; Ho-Pham and Nguyen, 2013; McKane et al., 1997; Riggs et al., 2002). SOS partly accounts for BMD, as other studies have shown (Glüer et al., 1994; Lee et al., 1997; Shenoy et al., 2014; Vignolo, 2006).

**Table 3**
Multivariable linear regression between SOS values at the cortical bone sites of adults and $F^-$ exposure including other covariates.

| | > 19–70 years old | | | | |
|---|---|---|---|---|---|
| | Unadjusted | | | Adjusted | |
| | $\beta$ (95% CI) | $R^2$ | p-Value | $\beta^a$ (95% CI) | p-Value |
| Tibia | | | | | $R^2$ = 0.318 |
| Water $F^-$ | −15.8(−20.1, −11.4) | 0.21 | < 0.0001 | −15.8 (−21.3, −10.3) | < 0.0001 |
| Age | −2.5(−4.0, −0.97) | 0.047 | 0.0015 | −1.9 (−3.3, −0.48) | 0.0093 |
| Female | −74.7(−114, −35.7) | 0.07 | 0.0002 | −77.2 (−146.3, −8.13) | 0.03 |
| BMI | −0.67(−6.9, 5.5) | 0.0001 | 0.83 | −0.27 (−5.6, 5.1) | 0.95 |
| Toothpaste use | 121.0(7.35, 234.7) | 0.017 | 0.04 | 65.5 (−33, 163) | 0.19 |
| Tobacco use | 119.5 (6.82, 232) | 0.0086 | 0.038 | 74.5 (−42.7, 192.2) | 0.22 |
| Water $F^-$ *Female | | | | 2.83 (−5.58, 11.3) | 0.51 |
| Radius | | | | | $R^2$ = 0.191 |
| Water $F^-$ | −9.73 (−14.2, −5.32) | 0.085 | < 0.0001 | −7.2 (−13.6, −0.8) | 0.028 |
| Age | −3.0 (−4.5, −1.6) | 0.08 | < 0.0001 | −3.2 (−4.7, −1.8) | < 0.0001 |
| Female | 8.0 (−29.8, 45.7) | 0.0009 | 0.68 | −7.68 (−62.4, 77.8) | 0.83 |
| BMI | 2.34 (−3.4, 8.1) | 0.0034 | 0.42 | 4.5 (−0.96, 9.93) | 0.11 |
| Toothpaste use | 70.3 (−46.7, 187.3) | 0.002 | 0.24 | 19.6 (−89.1, 128) | 0.72 |
| Tobacco use | 27.8 (−89.5, 145.2) | 0.011 | 0.64 | 11.2 (−97, −119.4) | 0.84 |
| Water $F^-$ *Female | | | | 4.3 (−4.3, 6.32) | 0.32 |
| Phalanx | | | | | $R^2$ = 0.212 |
| Water $F^-$ | −10.3 (−16.1, −4.5) | 0.056 | 0.0006 | −11.2 (−18.4, −3.87) | 0.003 |
| Age | −5.1 (−6.9, −3.3) | 0.14 | < 0.0001 | −4.47 (−6.33, −8.3) | < 0.0001 |
| Female | −51.0 (−100, −1.9) | 0.016 | 0.04 | −65.3 (−155.6, 25) | 0.16 |
| BMI | −4.8 (−12.3, 2.7) | 0.003 | 0.21 | −1.86 (−8.92, 5.2) | 0.61 |
| Toothpaste use | 136.0 (−3.7, 275.8) | 0.014 | 0.06 | 51.8 (−77.7, 181.2) | 0.43 |
| Tobacco use | −84.0 (−237.7, 69.7) | 0.0008 | 0.28 | −112 (−252, 28.2) | 0.12 |
| Water $F^-$ *Female | | | | 4.4 (−6.7, 15.4) | 0.11 |

Abbreviation: $\beta$, regression coefficient; *CI*, confidence interval.

In a general population, the peak of BMD is reached at the end of adolescence and can extend to ages 20–35 after which it decreases after approximately age 40 (e.g., Lu et al., 1994; Van der Sluis et al., 2002; Weaver et al., 2016). In our subjects, peak tibial mean SOS (3825 m/s) was found at age 23 in females and age 29 in males (3795 m/s). This peak was followed in females by a sharply decreasing SOS from 23 to 40 years and stabilization thereafter; whereas in men the SOS gently decreased after 29 years (Fig. 1B). A bone study in a healthy Mexican population reported a peak mean SOS for tibia at the age 28 (3835 m/s) and 35 (3896 m/s) years in females and males, respectively (Rivas-Ruiz et al., 2015). Despite the differences in origin between our study and the Mexican populations, the early and lower peak SOS values in the Ethiopian Rift population can be attributed to the adverse effects of $F^-$ on bone mineralization, which compromises its quality. We also noted that a follow-up study by Rivas-Ruiz et al. (2016) showed that the Mexican population had significantly lower SOS than that found in other countries, among healthy populations (Israel, Turkey, Greece, Portugal, and Canada), where the latter group had similar SOS differences.

We reemphasize here that bone quality is complex and a function of several properties; the way that $F^-$ alters specific bone parameters and overall bone quality in humans remains unclear. Yet many animal studies strongly suggest that $F^-$ alters bone remodeling (balance of bone formation and resorption processes) and other bone properties (density, microstructure, collagen content, and elasticity). A study in mice found increased osteoid formation and decreased mineralization

**Table 4**
Multivariable linear regression between SOS outcomes at the cortical bone sites of adolescents and $F^-$ exposure including other covariates.

| | 10–19 years old | | | |
|---|---|---|---|---|
| | Unadjusted | | Adjusted | |
| | $\beta$ (95% CI) | p-Value | $\beta^a$ (95% CI) | p-Value |
| Tibia | | | | $R^2$ = 0.36 |
| Water $F^-$ | *−3.78* (−9.16, 1.60) | 0.17 | *−3.47* (−9.7, 2.76) | 0.277 |
| Age | *30.5* (22.0, 39.0) | < 0.0001 | *27.5* (16.6, 38.4) | < 0.0001 |
| Female | 73.1 (24.5, 121.7) | 0.004 | 83.7 (9.29, 158.2) | 0.0292 |
| BMI | *23.2* (14.6, 31.8) | < 0.0001 | *5.76* (−4.62, 16.14) | 0.2788 |
| Toothpaste use | *45.14* (−57.5, 147.7) | 0.39 | −9.29 (−99.7, 81.16) | 0.8408 |
| Tobacco use | *159.2* (−139.4, 457.7) | 0.29 | 29.34 (−235.4, 294.1) | 0.8284 |
| Water $F^-$ *Female | | | −0.91 (−10.9, 8.15) | 0.8449 |
| Radius | | | | $R^2$ = 0.51 |
| Water $F^-$ | −2.04 (−7.62, 3.55) | 0.4728 | −1.6 (−7.37, 4.2) | 0.2103 |
| Age | 32.4 (23.9, 40.9) | < 0.0001 | 23.8 (14.5, 33.2) | < 0.0001 |
| Female | 131.5 (85.0, 178.0) | < 0.0001 | 141.4, (75.2, 207.6) | < 0.0001 |
| BMI | 29.7 (21.6, 37.9) | < 0.0001 | 13.5 (4.50, 22.5) | 0.0038 |
| Toothpaste use | 99.2 (−6.53, 205.0) | 0.0657 | 11.4 (−70.5, 93.3) | 0.7851 |
| Tobacco use | 250.2 (−59.2, 559.6) | 0.1122 | 68.3 (−167.2, 303.8) | 0.5705 |
| Water $F^-$ *Female | | | −2.0 (−10.0, 6.1) | 0.6266 |

Abbreviation: $\beta$, regression coefficient; *CI*, confidence interval.

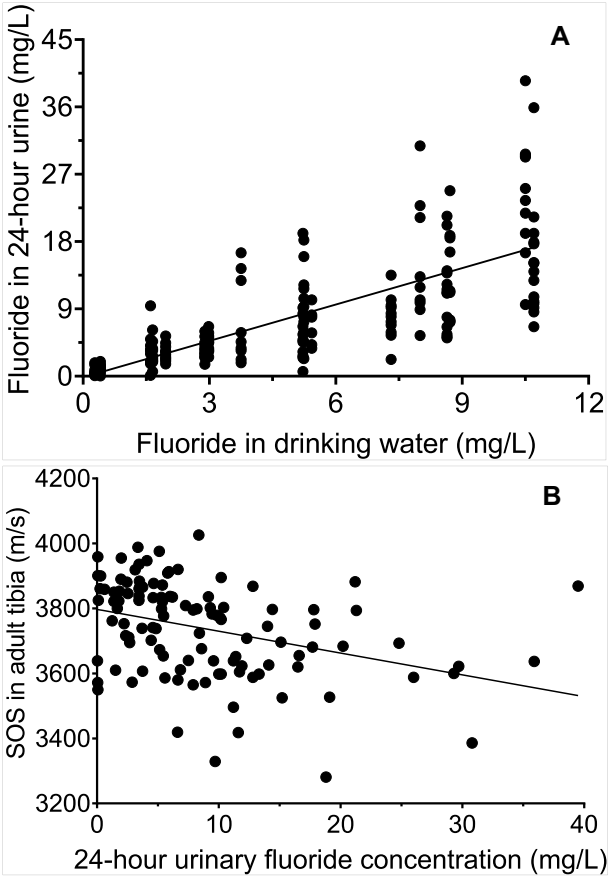


**Fig. 4.** Associations between $F^-$ concentrations in drinking water and A) 24-h urine concentrations for the individuals (n = 193) from 17 communities with such monitoring (r = 0.75; p < 0.0001), and B) tibia SOS measures (r = 0.36; p < 0.0001).

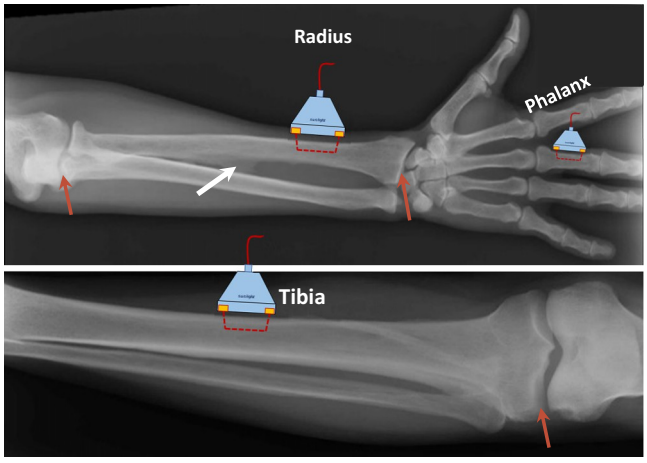


**Fig. 5.** Diagram of ultrasound device probes, used to assess bone quality at radius, phalanx, and tibial. The X-ray images of a 60-year old adult exposed to 13.7 mg/L of $F^-$ in drinking water from our study area. The orange squares are the transducers; indicate the principal pathways of the ultrasound waves in cortical bones from the emitter to receiving transducer parallel to the bone axis. The X-ray at the radius showed advanced (Severe) SF with diffuse sclerosis coarse trabeculations and ossification interosseous (white arrow at radius) and osteoarthritic changes of the radiocarpal and humero-ulnar joints (red arrows). A leg frontal X-ray showed a band of the intra medullary ossification and coarse trabeculation and osteoarthtic changes (red arrow) of the knee evidenced by irregularity and sclerosis of the articular surface, and intercondylar eminence osteophytes with joint space narrowing.

heterogeneity, suggesting that F stimulates osteoblastic activity and delays mineralization of new bone (Mousey et al. 2008). A recent study on rats exposed to $F^-$ reported changes in bone elasticity (due to altered collagen composition) but no change in BMD (Fina et al., 2008). Other animal studies of the effects of $F^-$ on different tissues have shown disrupted collagen metabolism and synthesis, resulting in imperfect collagen and/or non-collagenous protein structure and a breakdown of collagen in tissues (Fina et al., 2008; Gupta et al., 2013; Gupta et al., 2015; Yan et al., 2007). In our study, the inverse association between $F^-$ exposure and SOS suggests a strong effect of $F^-$ on an aggregated measure of bone quality, presumably due to various abnormalities or defects that affect mineralization, and alter BMD and bone microarchitecture (e.g., collagen and organic matrix). Although we observed increased osteosclerosis as well as bone loss in our SF subjects, both processes likely lead to compromised bone quality (strength).

It should be noted that exposure to environmental toxicants such as cadmium (Cd), lead (Pb), and uranium (U) are also associated with higher risks for bone loss (Engström et al., 2011; Kurttio et al., 2005; Khalil et al., 2008). Our recent biomonitoring study (Rango et al., 2019) found very low levels of Cd, Pb, and U in drinking water and in study subject's urine. The mean and 95th percentiles of urinary concentration of Cd, Pb, and U were 0.76 (2.28), 0.88 (0.26), and 0.05 (0.03) μg/L, and all ranges were within the level reported in uncontaminated, healthy reference populations. This strengthens the argument that chronic exposure to high levels of $F^-$ plays a central role in changing the properties of bone and contributes to deterioration of bone quality, in our specific study region.

Despite the strong correlations between $F^-$ exposure and reduced bone quality, the specific $F^-$ -altered bone parameters that significantly contribute to SOS variance in the studied cortical bone sites remain unclear. While the majority of the communities continue to rely on $F^-$ contaminated wells, a few use other sources of lower $F^-$ water owing to a gradual shifting of water-sourcing behavior that stems from the increasing awareness of $F^-$ problems in the study region. Furthermore, we only considered a few low $F^-$ sites (< 2 mg/L), and thus larger reference datasets are needed from healthy populations to generate T-scores and *Z*-scores for SOS and to better characterize bone health over time. Future work could focus on establishing such reference values, so that more bone-related health issues can be studied using this low-cost, radiation-free, and highly portable method. Another question is related to whether the SOS data obtained from cortical bones can be translated to characterize the mechanical competence and quality of other clinically-relevant bone sites (such as femur neck, and spine), which are more prone to fracture. While we expect that similar correlations may be present for these other bone sites, further study is required to confirm this hypothesis.

We therefore conclude that this study, and the ultrasound method we deployed in a novel application and setting, provide new evidence in humans that excessive $F^-$ affects many bone characteristics beyond their mineral content. Given our observed findings of $F^-$ related bone quality decline in the study population, we expect that high prevalence of SF will lead to an increased rate of skeletal fractures in high-$F^-$ exposure areas. Our results also have implications for the millions of people who are highly exposed to $F^-$ globally, and who suffer from fluorosis and its other potential effects on the endocrine and neurological systems (Choi et al., 2012; Grandjean and Landrigan, 2014; US NRC, 2006). To be sure, more attention needs to be paid to the public health significance of elevated $F^-$ exposure, especially in countries where mitigation efforts so far have been limited. While this study underscores the importance of $F^-$ in affecting bone quality in SF subjects, the specific role of various abnormalities in material (mineral and matrix) and structure of bone as detected using SOS measures, requires additional research and investigation. Similarly, applications of this technique in the context of other bone-related disorders and causes merit further attention, in order to assess the potential for application of the method beyond $F^-$-induced bone disorders.

**Table 5**
Association between the SOS values at the cortical bone sites across all ages with urinary $F^-$ and covariates.

| Variables | Crude model | | | Adjusted model | |
|---|---|---|---|---|---|
| | $\beta$, 95% CI | $R^2$ | p-Value | $\beta^a$, 95% CI | p-Value |
| Tibia | | | | | $R^2$ = 0.203 |
| Urinary $F^-$ | −7.20 (−10.22, −4.19) | 0.1 | < 0.0001 | −8.4 (−12.7, −4.12) | 0.0002 |
| Age | 2.49 (0.97, 4.01) | 0.05 | 0.0015 | 2.73 (0.95, 4.50) | 0.003 |
| Female | −31.5 (−78.6, 15.6) | 0.004 | 0.1889 | −44.06 (108, −20) | 0.1791 |
| BMI | 9.41 (3.10, 15.7) | 0.04 | 0.0037 | 1.94 (−5.26, 9.13) | 0.5984 |
| Toothpaste | 77.5 (−33.4, 188.4) | 0.005 | 0.1698 | 90.6 (−13.1, 194.3) | 0.0884 |
| Urinary $F^-$ *Female | | | | 2.1 (−3.7, 7.89) | 0.482 |
| Radius | | | | | $R^2$ = 0.28 |
| Urinary $F^-$ | −3.22 (−6.58, 0.13) | 0.01 | 0.06 | −8.0 (−12.4, −3.62) | 0. 0004 |
| Age | 4.73 (3.23, 6.24) | 0.16 | < 0.0001 | 3.98 (2.18, 5.79) | < 0.0001 |
| Female | 46.3 (−3.31, 95.8) | 0.01 | 0.067 | −18.10 (−83.3, 47.0) | 0.5872 |
| BMI | 18.3 (12.03, 24.6) | 0.14 | < 0.0001 | 7.99 (0.66, 15.3) | 0.0339 |
| Toothpaste | 27.3 (−91.8, 146.3) | 0 | 0.652 | 47.9 (−58.1, 154) | 0.3768 |
| Urinary $F^-$ *Female | | | | 8.39 (2.54, 14.2) | 0.006 |
| Phalanx | | | | | $R^2$ = 0.17 |
| Urinary $F^-$ | −3.03 (−6.86, 0.80) | 0.01 | 0.1202 | −0.76 (−6.22, 4.70) | 0.7849 |
| Age | −3.45 (−5.69, −1.20) | 0.07 | 0.0029 | −2.83 (−5.29, −0.37) | 0.0261 |
| Female | −70.9 (−129.4, −12.4) | 0.04 | 0.018 | −31.8 (−120, 56.2) | 0.4803 |
| BMI | −7.30 (−15.3, 0.70) | 0.02 | 0.0734 | −4.43 (−12.6, 3.76) | 0.2912 |
| Toothpaste | 165.7 (−12.8, 344.1) | 0.02 | 0.0685 | 107.0 (−66.3, 280.3) | 0.2289 |
| Urinary $F^-$ * Female | | | | −3.29 (−10.7, 4.14) | 0.3871 |

Abbreviation: $\beta$, regression coefficient; *CI*, confidence interval.

## Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the funding sources.

## Author roles

Tewodros Rango Godebo: Design of study concept, conducted the field work, analytical data analysis and interpretation, wrote the manuscript.

Marc Jeuland, Redda Tekl-Haimanot, and Biniyam Alemayehu: Design of study concept, field work assistance, critical revision of the manuscript for important intellectual content.

Arti Shankar: Analytical data analysis and interpretation.

Getachew Assefa: Field work assistance, readings of X-ray findings, and manuscript writing.

Gary Whitford, and Amy Wolfe: Study design, and critical revision of the manuscript for important intellectual content.

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## Declaration of competing interest

The authors declare they have no actual or potential competing financial interests.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bonr.2019.100235.

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