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Measurements of Strontium Levels in Human Bone In Vivo Using Portable X-ray Fluorescence (XRF)

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Abstract

Measurement of bone strontium (Sr) is vital to determining the effectiveness of Sr supplementation, which is commonly used for the treatment of osteoporosis. Previous technology uses radioisotope sources and bulky equipment to measure bone Sr. This study demonstrates the effectiveness of portable X-ray fluorescence (XRF) for bone Sr measurement and validates it using data from a population of 238 children. We identified correlations between bone Sr and age in our participants.

Keywords

X-ray fluorescence; XRF; bone; strontium; in vivo

Introduction

Strontium (Sr) is a metal that is closely related to calcium (Ca) in the body, and has beneficial or toxic effects on bone formation. Ninety-nine percent of strontium is stored in the teeth and bones after uptake in the body.[1,2] Strontium treatments have been extensively studied and have been found to have a net positive effect in bone formation, especially in reducing the risks brought on by osteoporosis.[3–5] During the Sr supplementation in these studies, the storage of Sr in bone was shown to have little effect on bone apatite crystal characteristics in comparison to normal bone structure.[6] Sr supplementation has been shown to primarily accumulate Sr in newly formed bone.[3,6,7] High levels of Sr have been correlated to skeletal abnormalities in animals and children.[8,9] Children have a significant amount of

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Conflict of Interest

The authors report there are no conflicts of interest.

newly formed bone during development, which puts them at particular risk when considering Sr overexposure. Measuring bone Sr is traditionally done using a bone biopsy, which is a painful procedure unlikely to be completed multiple times.[7] Blood Sr can be used to measure exposure levels, but it is unlikely to correlate with long-term exposures.[10]

X-ray fluorescence (XRF) has been used for decades to measure bone lead (Pb) levels.[11,12] A similar device has been used to measure bone Sr, but this device uses a radiosotope source, has bulky equipment using liquid nitrogen cooling, and requires a 30-min measurement time to achieve its detection limit.[13] Another technique utilizing the differences in absorption of photon energies called dual photon absorptiometry (DPA) was able to non-invasively measure bone Sr, but this device was also fairly large, required 10 min measurement times, and immersion in water.[14] More recently, a portable XRF using a low energy X-ray tube has been used to measure bone Pb.[15,16] This same technology can be used similarly to measure Sr in bone, but with the additional advantages of being portable, using an X-ray tube source, and a 2 min measurement time. This study demonstrates a calibration of Sr using the portable XRF and uses the data collected in Specht et al. 2016 to quantify bone Sr for a group of children in Shanghai, China, using in vivo measurements.[16]

Materials and Methods

Strontium and Soft-Tissue Phantoms

Strontium phantoms were made using Mowiol 4-88 and bone meal. The Mowiol 4-88 was prepared as described by Mostafaei et al. using a combination of glycerol and distilled water. It has been proven to be effective in making calibration phantoms.[17] Mowiol 4-88 is traditionally used as a preservative for stained cell dishes for anti-fading purposes. For our phantom preparation, it acts as a binding agent for the bone meal and is Sr-free. The bone meal was derived from ground cattle bone and has the same elemental properties of natural bone as a result of this. Bone meal easily replicates the bone matrix, since it is ground cow bone. From the calibration line in Figure 1, the bone meal was determined to have a Sr contamination level of around 74 parts per million (ppm). Sr contamination was a significant problem when using traditional methods that utilized plaster-of-paris, which had Sr contamination of greater than 1000 ppm. Our Mowiol 4-88 phantoms had added concentrations of Sr of 0, 5, 10, 15, 20, 30, 50, 75, 100, and 200 ppm in order to get a calibration line and accurately determine the detection limit of Sr. Lucite slabs were then used as a soft-tissue equivalent phantom and measurement of the Sr phantoms at different Lucite thickness was used in the calibration and detection limit calculations. Lucite slabs have attenuation properties that differ from human soft tissue by less than 10% multiplicatively and 5% absolutely for the average energy of 20 keV for our portable XRF.[15] The spectra between soft tissue and Lucite were found to be nearly identical for similar thicknesses of each in our previous work.[15] Measurements were made by using the flat bottom portion of the Sr phantom with lucite slabs placed between the phantom and the portable XRF.

Study Population

The study participants were 238 Pb-exposed children and controls recruited through Xinhua Hospital, Shanghai Jiaotong University, China. The parents of the participants completed a questionnaire, and the participants had their blood samples taken, and their bone measured for Pb and Sr concentrations using a portable XRF system.

The study received IRB approval from Purdue University and Xinhua Hospital. When recruited, a trained research assistant would present the participants and their parents with the details of the study and the consent forms. Signed consent forms were received from the parents of each participant, as well as an assent form from any child aged seven years or older.

Portable X-ray Fluorescence Device

The customized portable XRF used in this study was obtained from Thermo Fisher (XL3t GOLDD+, Thermo Fisher Scientific Inc.). The device specifications were discussed in a previous study.[15] The X-ray tube used 50 kV and a 40 μA setting with a silver and iron filter. The detector is a large area silicon drift detector with resolution <185 eV at 60 000 cps. We took our measurements from the middle of each participant's tibia. This measurement site is used for many studies using XRF bone measurements and is chosen for its cortical bone with low bone resorption rate to get a better cumulative estimate of bone Sr. Before each measurement, the participants' legs were cleaned using alcohol and EDTA cotton swabs to remove any contamination. In placing the device on the participant's leg, we used non-metal ink to make a dot on the measurement site. This dot could then be found using a camera mounted in the head of the portable XRF, which ensured a consistent measurement location. The participant's leg was held horizontally with their foot resting in the operator's lap during the measurement. In this study, we used a measurement time of 2 min. The average dead time for the measurements was 38 ± 6%. The energy resolution of the detector was calculated from measurements of the highest concentration phantom and found to be about 2% at 14.2 keV. Based on our previous study, which used TLD dosimeters and Monte Carlo simulations, we calculated the entrance skin dose of the system to be 21 μSv to a 1 cm^2 area and the whole-body effective dose was 2.4 μSv.[18] Because the X-ray beam is focused and of low energy, the whole-body dose almost exclusively derives itself from the dose to the skin and bone of the leg during measurement. The whole-body dose for this measurement is 40 times less than the whole body effective dose for a standard AP chest X-ray, which is about 100 μSv.

Spectral Analysis

The spectra were analyzed using traditional peak fitting, which was done for the Pb measurements, and is described in detail in our previous work, but we will describe briefly here.[15] This analysis used a Gaussian with an exponential background to extract net counts. Then, we used the Compton scattering peak to normalize counts to irradiated bone, correct for bone composition, and to estimate soft-tissue thickness. The normalization was based on a calibration line from Lucite and plaster-of-paris phantoms to reflect the changes with soft tissue and bone, respectively. The Compton scattering peak was shown to correlate with soft-tissue thickness, verified by ultrasound in our previous study, and had strong relations

with inverse square degradation and attenuation of the signal from observed fluorescence.[15,18] As well as accounting for inverse square degradation and attenuation from soft tissue, the Compton scattering peak would also take into account bone shape and size by averaging skin thickness over the exposed area. Soft tissue would be more prevalent in a participant with smaller bone or different bone shapes, which would then be accounted for in our calibration.[15] The Compton scattering peaks from the Ag X-ray tube was fitted using a second order polynomial function relating to an increasing Lucite thickness with an R-squared value of 0.99. This function then could be used to estimate soft-tissue thickness from measurements on participants. The soft-tissue thickness could then be used to identify the relationship between soft-tissue corrected net counts and concentration. With this process, we used the peak energy of Sr at 14.2 keV instead of lead peak energies, which was the only part of the procedure that differed from our previous work. The other Sr peak at 15.8 keV has a significant amount of background from the characteristic X-rays produced by the Ag target in the X-ray tube that underwent Compton scattering in the sample. The 15.8 keV peak also only represents 14% of the total characteristic production with the majority being from the 14.2 keV peak. Thus, the 14.2 keV was the only peak we used in our analysis and calibration procedures.

The uncertainty (σ) of each measurement was calculated using Eq. 1,

$$\sigma = \frac{c \times \sqrt{\frac{BKG + Gross}{t}}}{Net} \quad (1)$$

where c is the concentration, BKG is the background counts as estimated by the exponential function in our fitting, Gross is gross counts under the fitted peak, t is measurement time, and *Net* is the net Sr counts from the Gaussian function in our fitting. Negative values for bone Sr are left as such because they represent the point estimate of bone Sr with uncertainty in the measurement. If an individual measurement is close to zero, then the point estimate from that individual can be negative with the associated uncertainty.

Statistical Methods

Linear regressions were used to determine correlation values and levels of significance for the relationships between age, sex, and portable XRF bone Sr. The portable XRF data were excluded if the measurement time was less than 2 min. Participants moving during the measurement became a problem if the device was not properly held to the skin, and these measurements were ended early and discarded with the measurement time being less than 2 min. For the difference measures between sex and age, we first used a related sample Friedman's two-way analysis of variance (ANOVA) to determine whether significant differences existed between the groups, and then did individual related-sample Wilcoxon signed rank tests to determine the differences of each group individually.

Results

Calibration Curve for Bare Phantoms

The portable XRF was calibrated for in vivo bone Sr measurement by measuring Sr phantoms of varying concentration at different Lucite thicknesses. Figure 1 shows the results of the calibration curve obtained at 0 mm Lucite thickness. As we increased to 5 mm of Lucite thickness the R-squared of this calibration curve was in the range of 0.996–0.976. To account for the contamination, which increased the highest concentration phantom to 274 ppm, we used a function for calibration derived from signal increase versus actual concentration of Sr phantoms, which included both contamination and added concentrations.

Calibration Function for Measurement of Bone Sr at Different Soft-Tissue Thicknesses

The function in Figure 2 below shows the calibration of the net Sr counts change with increasing Lucite for our highest concentration phantom of 274 ppm. This relation takes into account the attenuation of soft tissue and inverse square degradation of the signal.[15]

Minimum Detection Limit

The minimum detection limit (MDL) was found by measuring our phantoms of varying concentrations of Sr with different Lucite thicknesses and using Eq. 2:

$$MDL = \frac{2 \times \sqrt{BKG}}{Slope} \quad (2)$$

where BKG is the background under the 14.2 keV Sr peak calculated using the exponential function from our fitting program in units of counts, and the slope is taken from the calibration curve of all ten phantoms at a particular Lucite thickness, which is in units of counts per ppm.[19] The resultant MDL is displayed in Figure 3 in units of ppm.

Portable X-ray Fluorescence In Vivo Sr measurements

Figure 4 shows a sample in vivo spectrum obtained with the portable XRF. Table 1 shows summary statistics for the population measured for in vivo bone Sr. The average age of the children was 5.7 ± 3.1 years. The difference between male and female participants for average bone Sr level was found to be non-significant with a P value of 0.25.

Correlations with Age—Figures 5–7 show correlations of bone Sr concentrations with ages. Figures 5 and 6 show significant correlations between age and bone Sr for a combined group and then just girls, respectively, with P value <0.05. Figure 7 shows a non-significant correlation between age and bone Sr for boys. The slope of the correlations demonstrates the estimated increased storage of Sr in bone per year in ppm.

Age and Sex Differences—We then investigated differences when splitting age groups depending on an estimation of puberty status. A previous study estimated the ages in which puberty began in boys and girls as 8–14.9 and 9.7–14.1, respectively.[20] We separated our results based on the minimum age and determined if there were further significant

differences between these groups. Table 2 summarized statistics for the separated groups. Our Friedman's two-way ANOVA showed significant differences between the groups in Table 2 with a P value <0.05. Then Wilcoxon signed rank tests were done to determine the individual difference levels between groups. The female groups had significant differences of average bone Sr between the age groups with P value <0.05. The male groups had a significant difference of average bone Sr level between age groups with P value <0.05.

Discussion

This study demonstrated the ability of a portable XRF for measurement of in vivo bone Sr levels with a 2-min measurement. The device was calibrated using Sr doped phantoms and Lucite and used to measure bone Sr in a group of lead poisoned children. We found that bone serves as a major storage site for Sr and bone Sr has a significant relation with age in children, which indicates that bone Sr may increase over time. Accumulation of bone Sr can be accurately monitored with the portable XRF. This technique can then be used to monitor health effects associated with Sr exposure as well as monitoring patients using Sr supplementation to treat osteoporosis with a much more convenient approach than currently available methods.

Bones in fingers or ankles have been used for similar studies in the past; however, the ankle and finger have a variation of trabecular and cortical bone which would not serve as the most appropriate biomarker for cumulative Sr uptake and bone formation in this study, and finger bone would be much more difficult to measure on young children due to its small size.[11,21] Therefore, we measured tibia bone, which is primarily cortical bone, to reflect the cumulative biomarker of bone Sr.

Our calibration line demonstrated the linearity of the device with respect to Sr levels. The contamination of the phantoms was evenly distributed throughout the bone matrix, and with this level of contamination, our calibration line was accurate to the amount of Sr added to our phantoms. The contamination did increase the uncertainty of the bone Sr concentrations which results from the increased uncertainties of the parameters for the calibration line we obtained using our phantoms. In the future, we can improve upon this calibration by using standards with less Sr contamination. We determined that using background obtained from the exponential function in the peak fitting was the most acceptable means to estimate the MDL, since we were not able to produce a 0 ppm phantom. The calculated MDL was at a level low enough to detect Sr in most participants with less than 5 mm skin thickness, as the average bone Sr level was found to be 43.2 ± 57.6 ppm. Soft-tissue thickness does have a significant effect on the detection limit, as seen in Figure 3. The soft-tissue thickness increases uncertainty in the measurement by decreasing our signal to background ratio as shown in Eq. 2. This is where the negative values in our study arise; bone Sr levels close to zero with high uncertainty due to soft tissue thickness have a greater possibility to be negative. Children in general will have thicker overlying soft tissue than adults, and will have higher uncertainty in their measurements.[22,23]

The correlations with age likely reflect the natural development of the children's bones. There was no correlation between BMI and age, nor BMI with bone Sr. Our Compton

scattering normalization should account for variations in bone growth by correcting for the loss of signal in smaller bones, thus, the effect of higher bone Sr levels with age should not be an artifact of the measurement itself. There are several other environmental factors here that could affect the correlation between age and Sr. We did not have any information on the Sr exposure for each participant, so the possibility of someone being more or lesser exposed could be involved. Since diet plays a large role in Sr accumulation, there could be variations between the participants. It is also probable that some of the children had a higher metabolism of Sr due to less vitamin D and protein in their diets.

Studies from Popovic et al.[24] and Mostafaei et al.,[25] on lead and fluorine, demonstrated that differences in bone metabolism between sexes could influence bone metal concentrations. Our study demonstrates similar sex differences in storage of bone Sr. The female participants were more likely to have higher bone Sr at older age than the male participants. Both male and female participants showed an increase in storage of Sr after puberty age. It should also be noted that there is a significant difference in the number of girls (n =72) versus boys (n =165) throughout the results of our study, and the group sizes for older participants were small (23 for girls and 18 for boys). In addition, since this was an estimation of puberty status based on age, a better group distinction could be made using a questionnaire for puberty status. Along with this, the relation of puberty and age was derived from a differing population, which may influence these results as well. A better test for these differences would be to compare the bone Sr relation with age before and after puberty age or over time, but due to the small sample size of older participants and cross-sectional study design, we were unable to do this type of analysis. A pilot study in adults with wider age ranges would help to further illuminate the differences seen in this study.

Conclusion

Strontium in bone is a valid biomarker for cumulative Sr exposure and can be used to assess the effectiveness of treatments or health effects associated with Sr intake or exposure. Our data show that the portable XRF can be used to measure Sr in bone and accurately determine bone Sr concentration for most participants with a 2-min measurement time, which makes for a much more convenient measurement of bone Sr than previous in vivo systems. The results also suggest possible sex differences in Sr storage and metabolism of Sr in bone.

Acknowledgments

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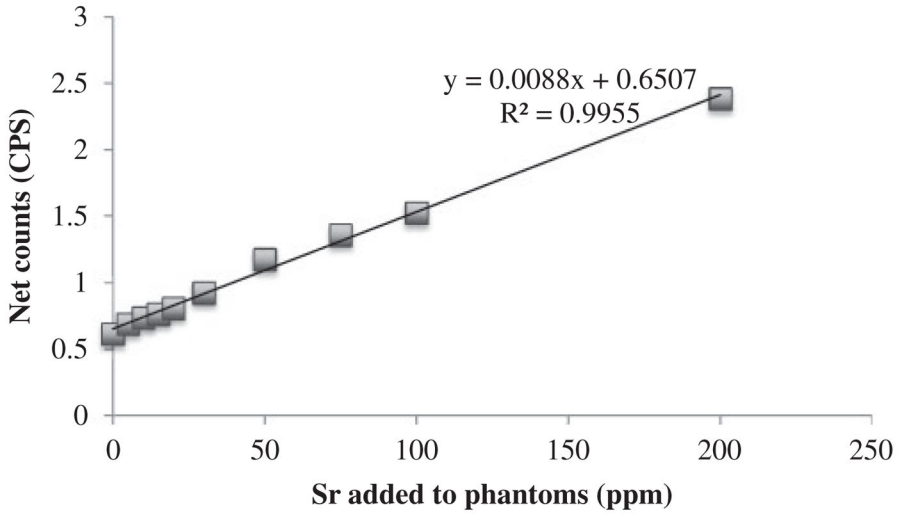

Figure 1.
Calibration curve for Sr at 0 mm Lucite thickness.

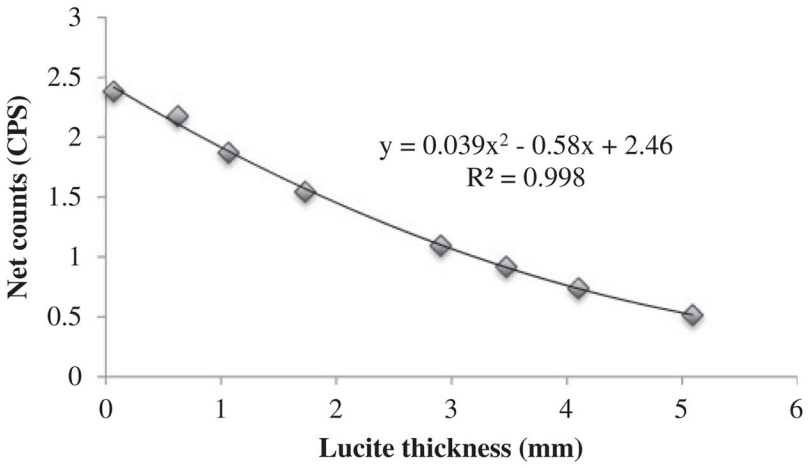

Figure 2.
Calibration function for Sr counts at different Lucite/ soft-tissue thicknesses with 274 ppm phantom.

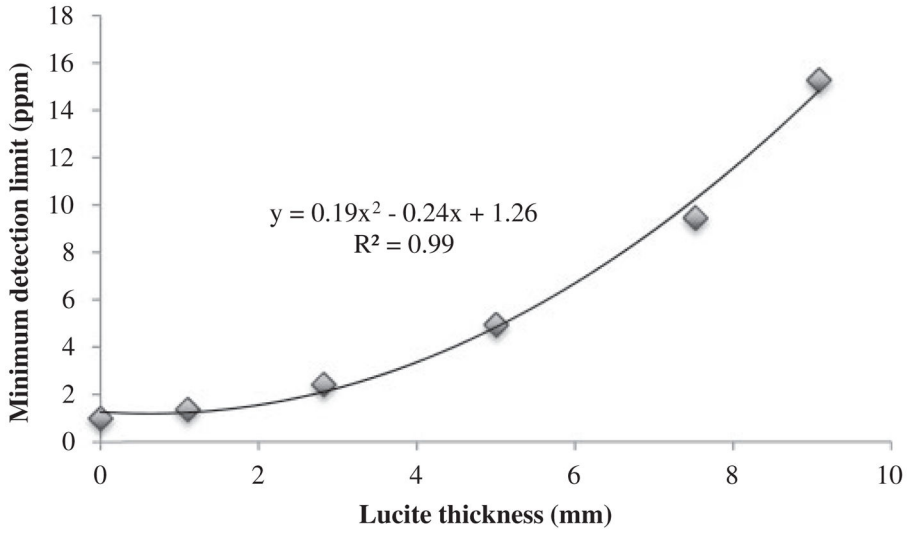

Figure 3.
Minimum detection limit change over increasing Lucite thickness.

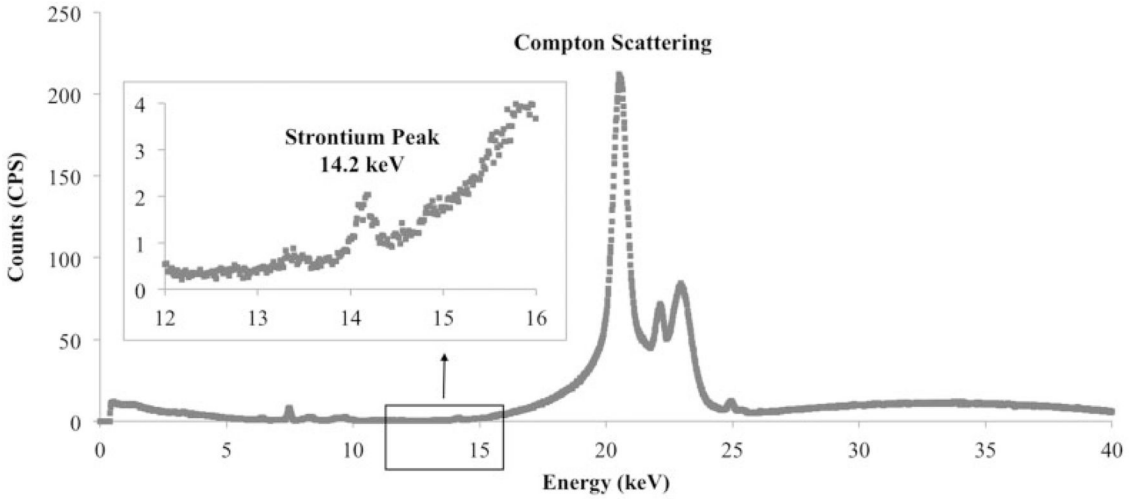

Figure 4.
Sample in vivo spectrum for bone Sr measurement.

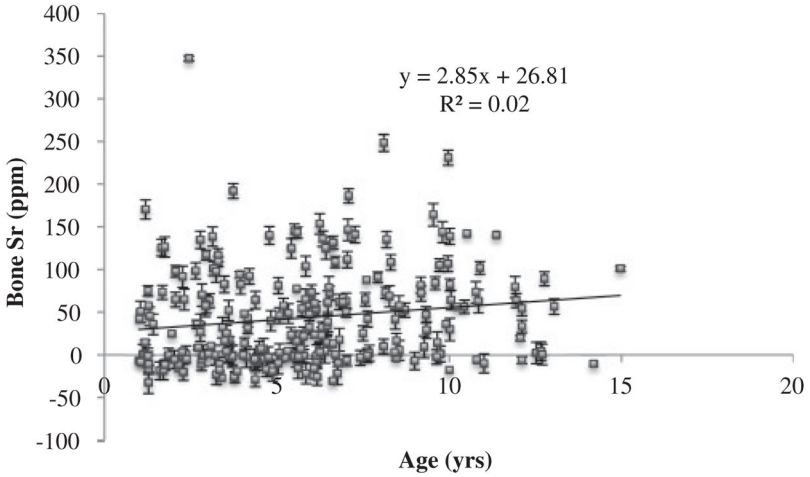

Figure 5.
Significant correlation between bone Sr and age with combined male and female participants.

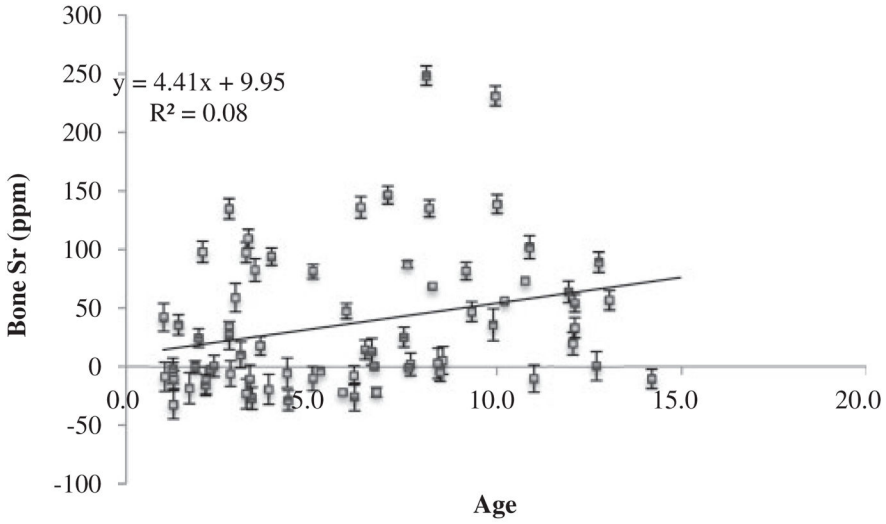

Figure 6.
Significant correlation between bone Sr and age for female participants only.

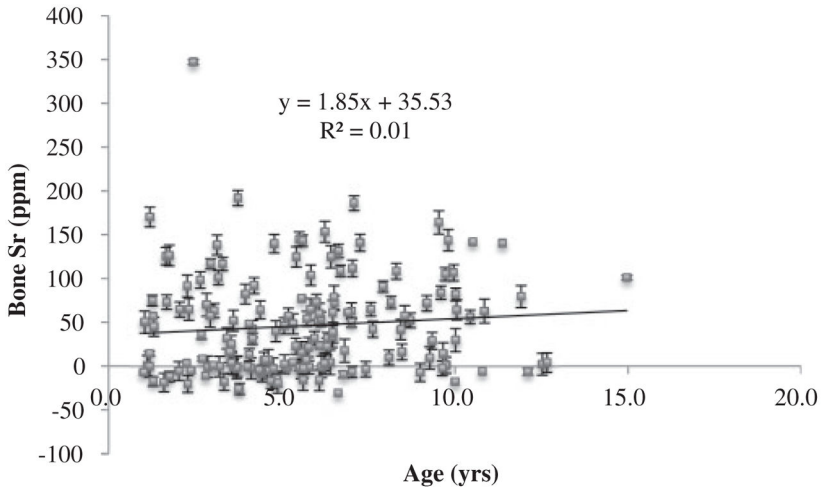

Figure 7.
Non-significant correlation between bone Sr and age for male participants only.

Table 1

Statistics of bone strontium and age for the population.

Bone strontium (ppm)					
	N	**Average**	**Minimum**	**Maximum**	**Standard deviation**
Male	165	45.9	−30.4	347.6	56.9
Female	72	36.6	−32.3	248.6	59.3
Total	237	43.2	−32.3	347.6	57.6
Sigma	237	9.7	2.0	14.1	2.6
Age (years)	237	5.7	1.0	15.0	3.1
Age male	165	5.6	1.0	15.0	2.8
Age female	72	6.0	1.0	14.2	3.7

Table 2

Statistics of bone strontium and age for the population.

Bone Sr (ppm)						
	N	**Average**	**Min**	**Max**	**Standard deviation**	**Average Sigma**
Girls >8 years	23	66.2	−10.3	248.6	69.4	8.3
Girls <8 years	49	22.6	−32.3	146.7	48.6	9.8
Boys >9.7 years	18	60.8	−17.7	144.0	55.6	10.0
Boys <9.7 years	147	44.1	−30.4	347.6	57.0	9.7