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The Abundance of Trace Elements in Human Bone Relative to Bone Type and Bone Pathology

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Abstract As the global population ages and the proportion of individuals afflicted with musculoskeletal disease spirals upward, there is an increasing interest in understanding and preventing bone-related diseases. Bone diseases, such as osteoporosis and osteoarthritis, are known to be influenced by a variety of factors including age, gender, nutrition, and genetics, but are also inherently linked to the human body's ability to produce biominerals of suitable quality. Because the crystal lattice structure and mineralogy of bone hydroxyapatite is surprisingly analogous to geological hydroxyapatite, trace element levels and exposure have long been proposed to influence the structure of biominerals as they do geological minerals (e.g., strontium substitution changes the crystal lattice of bone minerals, while toxic lead disrupt bone cellular processes leading to bone disease). Here, we explore the distribution of trace elements in human bones to evaluate the distribution of these elements with respect to bone type (cortical vs. trabecular) and bone disease (osteoarthritis vs. osteoporosis). We find higher concentrations of many metabolically active transition metals, as well as lead, in cortical bone compared to trabecular bone. When compared to patients who have osteoarthritis, and thus presumably normal bone minerals, osteoporosis patients have higher concentrations of scandium and chromium (Cr) in trabecular bone, and Cr and lead in cortical bone. Lower concentrations of barium and titanium are associated with osteoporotic trabecular bone. This survey is an exploratory cross-sectional geochemical examination of several trace element concentrations previously understudied in human bone minerals.

Plain Language Summary Bone-related diseases, like osteoporosis, are a growing concern as the global population ages. There are many factors which can influence bone health, including age, sex, and genetics, but ultimately osteoporosis is a disease of bone mass and bone strength. Bone minerals are like minerals found in nature, which contain trace element impurities. When these impurities occur in bones, they affect bone strength, with some trace elements increasing bone strength, and others decreasing it. The amount and kind of trace elements found in bone can be affected by environmental exposures or from the body's own processes which may be different between healthy and diseased individuals. This study compares the concentrations of 16 trace elements in bones from people with osteoporosis, a disease where bones themselves are diseased, and osteoarthritis, as disease of joint cartilage that is not expected to impact the trace element chemistry of bone minerals. We find that bones from osteoporosis patients have higher concentrations of some elements, including toxic lead, but lower concentrations of other elements. The location of these element deficiencies or excesses in the bones may suggest if their concentrations are more likely to be a cause of osteoporosis or a symptom of it.

1. Introduction

As the global population ages and the proportion of elderly people continues to rise, the number of individuals affected by bone diseases increases each year (Briggs et al., 2016); these trends are especially prevalent in developed countries. Aging of the global population is also shifting the burden of global diseases from an array of predominantly communicable/infectious, fetal/maternal health, and nutritional health diseases to predominantly noncommunicable, chronic conditions. For example, musculoskeletal diseases (MSD) are now some of the most prevalent (Briggs et al., 2018) noncommunicable diseases. While there is a broad spectrum of disorders that fall under the umbrella of MSD, osteoporosis (OP) and osteoarthritis (OA) are two of the most prevalent in elderly patients, specifically in the US (Briggs et al., 2016).

Osteoarthritis is the clinical and pathological outcome of a range of disorders that results in degeneration of joint cartilage and many of its surrounding tissues (Palazzo et al., 2016). Osteoarthritis is a leading cause of disability

among older adults, and the most common types of osteoarthritis occur in the knee, hip, and hands. An estimated 30 million Americans (~14%) have been diagnosed with OA and the lifetime risk for developing OA is between 25% and 40% (Mendy et al., 2018; Park et al., 2018). The prevalence and incidence of OA increase with age and there are several other established risk factors, including obesity, traumatic injury, and chronic inflammation (Mendy et al., 2018; Palazzo et al., 2016).

Osteoporosis, on the other hand, is thought to be strictly a bone disease caused by the loss of bone mass and bone mechanical integrity with age, which results in a marked increase in the risk for fractures. Osteoporosis begins with an approximate 0.2%–1.0% loss of bone per year, typically starting after the age of 40–45 (Lowe et al., 2002), and commonly increasing (up to 2%–5%) in the years surrounding and following menopause in women (Beck et al., 1993; Cauley et al., 2012; Gossiel et al., 2018; Matkovic et al., 1994; Recker et al., 2000; Vilaca et al., 2017). An estimated 10 million (~10%) older adults (>50 years) in the US have osteoporosis (Wright et al., 2014) and the lifetime risk for osteoporotic fracture is as high as 40% for a patient with osteoporosis (Rachner et al., 2011). Patients with osteoporotic fractures are also at risk for a 12-month excess mortality rate between 5% and 40% due to hospital stays and reduced mobility (Abrahamsen et al., 2009; Center et al., 1999; van den Bergh et al., 2012), with an overall increased mortality risk in the first 5 years following osteoporotic-related fractures (up to 10 years in patients that have experienced hip fractures) (Bliuc et al., 2009). There are a variety of current therapies (e.g., vitamin D and calcium supplementation, strontium ranelate, and parathyroid hormone) that work to either slow down bone resorption or stimulate bone formation as reviewed elsewhere (Rachner et al., 2011).

Bone diseases (e.g., osteoporosis, osteoarthritis, and osteopenia [pre-osteoporosis]) and bone pathology (e.g., fracture, necrosis) result from a complex interaction of genetics, metabolism, exercise, environmental exposure, and nutrition (Aaseth et al., 2012; Dermience et al., 2015; Karasik & Kiel, 2016). Among these factors, only nutrition, exercise, and, to some extent, environmental exposure, can be easily controlled by the populations at the greatest risk for specific types of bone disease. Previous research has found links between the levels of various trace elements in the exposome (e.g., diet, drinking water, and air particulates) and their corresponding levels in bone minerals. The variations of specific trace element concentrations have also been associated with a variety of issues related to bone quality and health (Allen & Golightly, 2015; Hendrickx et al., 2015; Palazzo et al., 2016). Bone health ultimately depends on the quality of bone minerals (Bigi et al., 2016; Ferretti et al., 2003; Ilich & Kerstetter, 2000). Incorporation of trace elements into bone hydroxyapatite can have either deleterious (Bigi et al., 2016; Brzóska & Moniuszko-Jakoniuk, 2004; Dermience et al., 2015; Levy et al., 2018; Milgram et al., 2008; Puzas, 2000; Schrooten et al., 2003; Zofkova et al., 2017) or beneficial (Almeida et al., 2016; Dermience et al., 2015; Marie, 2005; Meunier et al., 2004; Mousny et al., 2006; Sierpinska et al., 2014; Verberckmoes et al., 2004; Zofkova et al., 2017) effects on bone health, which depend upon the element, the chemical species of that element, and the concentration of each elemental species.

Human bone mineral crystal lattices are analogous to their geological counterparts (Glimcher, 2006; Rey & Combes, 2016; Rey et al., 2009). Trace elements can incorporate into geological minerals by substituting for major elements (e.g., calcium [Ca], magnesium [Mg], sodium [Na]) in the crystal lattice structure (e.g., Bell & Rossman, 1992; Bigi et al., 2007, 2016). Importantly, the substitution of a trace element with a different charge or ionic radius than those major elements can cause quantifiable changes in the mineral composition and bone mineral crystal lattice structure (Gose et al., 2008; Panero & Caracas, 2017) leading to microscopic structural defects and vacancies in minerals. These structural defects can lead to mechanical weakness in geological materials and an increased susceptibility to fracturing and faulting of rocks in nature. Prior work suggests that bone mineral crystal lattices, best approximated as carbonated hydroxyapatite minerals on an organic collagen matrix, are susceptible to the same processes that affect geological hydroxyapatite (Bigi et al., 2016; Riedel et al., 2017; Verberckmoes et al., 2004).

One critical difference between geological and biological hydroxyapatite, is the dynamic nature of hydroxyapatite crystal formation and dissolution in human bone. While bone minerals can easily incorporate trace elements into their mineral structure, bone minerals do not permanently store and sequester those trace elements. Instead, throughout an individual's lifetime, parathyroid hormone (PTH) levels and osteoclast activity continuously regulate Ca homeostasis, and release Ca and other essential trace elements (e.g., zinc [Zn], iron [Fe]) stored in bone tissue to maintain biologically relevant blood levels (Berglund et al., 2000). In fact, the entire human skeleton of a healthy adult is replaced in ~5–10 years, which reflects a combination of bone remodeling processes that occur

in the more metabolically active trabecular (or spongy) bone tissues (3–5 years) and the rigid, dense cortical (or cancellous) bone tissues (20+ years). The estimated rates of bone remodeling are also influenced by an individual's age, sex, and metabolic condition (Boskey & Camacho, 2007; Glimcher, 2006; Mann, 2001); these processes of bone formation and resorption are lumped together and termed bone remodeling.

The bone remodeling process is essential for bone formation, maintaining calcium homeostasis, and rejuvenating the skeleton. However, the process also allows bone to *both* mitigate metal toxicity when the body is exposed to acute doses of toxic metals (e.g., from a metallodrug therapy) (Darrah et al., 2009) and act as a potentially important source of endogenous toxic metal burden to the body (Darrah et al., 2009; Gulson et al., 2016). The former is accomplished by the bone mineral effectively removing toxic elements from the circulatory system (Rey & Combes, 2016) and thus minimizing their short-term toxic impact to the body. While this has some obvious evolutionary advantages to the short-term viability of an individual exposed to toxic metals, it creates a repository of toxic elements that may influence bone health. When osteoclast cells resorb bone minerals, trace elements previously incorporated into bone structures can be released back into the extracellular fluid. As a result, bone resorption can release both beneficial elements (e.g., Ca, Na, and Zn) and elements that are either toxic or have little biological function (e.g., lead [Pb], gadolinium [Gd]). For example, bone resorption can account for up to 70% of Pb in blood plasma (Gulson et al., 1995). In populations with high rates of bone resorption (individuals affected by osteoporosis or women who are either in the second trimester of pregnancy or are lactating), there have been observable increases in Pb remobilization and higher blood Pb levels (Gulson et al., 2003, 2016; Mendola et al., 2013).

Recently, intake of strontium (Sr), and fluoride (F), either through routine diet or a treatment regimen, has been associated with bone strength (Briançon, 1997; Levy et al., 2018; Querido et al., 2016; Riedel et al., 2017). Similarly, studies taking a variety of approaches have found association between osteoporotic fracture and either exposure to, or higher bone concentrations of, manganese (Mn), Fe, Pb, cadmium (Cd), aluminum (Al), and vanadium (V) (Åkesson et al., 2014; Beier et al., 2016; Bondy & Campbell, 2017; Budis et al., 2014; Carmouche et al., 2005; Facchini et al., 2006; Gruzewska et al., 2014; Karaaslan et al., 2014; Li et al., 2011; Ngwa et al., 2017; Nishijo et al., 2017; Puzas, 2000; Sadeghi et al., 2014). Nonetheless, it has been difficult to understand the role of these trace elements and specifically determine any potential causative effects because bone tissue type, age, sex, locality, and exposure sources have also been shown to affect the accumulation of many trace elements (Zn, Sr, Pb, Cd, Fe, cobalt [Co], copper [Cu], and rare earth elements (REEs)) (Budis et al., 2014; Harkness & Darrah, 2019; Karaaslan et al., 2014; Lanocha et al., 2013; Roczniak et al., 2017; S. Zaichick, Zaichick, Karandashev, & Moskvina, 2011; S. Zaichick, Zaichick, Karandashev, & Nosenko, 2011; Zioła-Frankowska et al., 2015).

Because the substitution of trace elements and molecules in bone crystal lattice structures may induce structural defects in bone minerals or interfere with bone cellular function, both of which potentially increase the risk for bone disease, there is an evident need to better understand the roles of a more comprehensive suite of trace elements in bones. To this end, the current cross-sectional study investigates trace element concentrations in trabecular and cortical bone tissues from the femoral heads of patients affected by osteoarthritis and osteoporotic fracture to identify potential relationships with bone pathological conditions. As part of this investigation, we also compare trace element compositions in bone tissues between male and female patients and according to patient age, which ranges from 41 to 100 years.

2. Methods

2.1. Sample Selection

Bone tissue samples were selected from the femoral heads of 58 patients resident to upstate New York, USA who underwent hip replacement surgery at the University of Rochester Medical Center (RSRB approval #0019010) (Prutsman-Pfeiffer, 2008). Bone samples were collected post-surgery following total hip arthroplasty (total hip replacement surgery). The overwhelming majority of bones that become available for analysis consist of patients diagnosed with some type of bone pathology, of which osteoporosis-related fracture and osteoarthritis were two of the most common causes for surgery (Prutsman-Pfeiffer, 2008). The decision to use only bone tissue that exhibits medical pathology is recognized as a limitation in this and prior work (Darrah et al., 2009; Harkness & Darrah, 2019; Prutsman-Pfeiffer, 2008). The reason for using surgically resected femoral heads is that some of
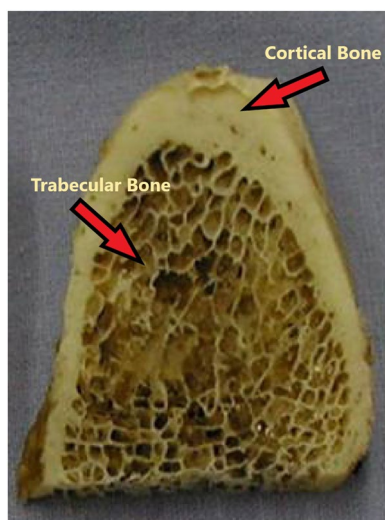
Figure 1. Cross section of a femoral head used in this study; the spongy trabecular bone is encased by cortical bone as shown by the arrows. Adapted from Darrah 2009.

the limitations usually encountered using bones removed from cadavers, such as difficulty in obtaining detailed medical histories, having the opportunity to select suitable samples for geochemical analysis of bones, and/or knowledge of the geographic history of individuals are circumvented. Despite this, we do recognize the lack of data on nutrition, lifestyle, occupation, and other possible factors that may affect bone metal concentrations as a limitation of this study.

The minimum requirements for inclusion in this study were biographical data: date of birth, sex, and characterization of bone pathology. From the total population of human femoral heads that became available during the study period, 58 patients were chosen to provide 29 samples for each type of bone pathology (i.e., osteoarthritis vs. osteoporotic fracture) (Darrah et al., 2009; Prutsman-Pfeiffer, 2008). Osteoarthritis pathology for these samples was confirmed following surgical removal of the femoral head (Prutsman-Pfeiffer, 2008). The remaining 29 hip replacement surgeries occurred following hip fractures, which were post-surgically diagnosed as resulting from osteoporosis (Prutsman-Pfeiffer, 2008). The fracture group includes 12 patients who were also diagnosed with OA prior to fracture. We include these patients in the fracture group as we are assuming that the interior of OA bones is similar to normal bone in terms of mineralogy and elemental abundance. The sample set consisted of 42 women and 16 men with ages ranging between 41 and 100 years at the time of surgery. More complete lists of available biographical and medical records of the 58 patients included in the study are documented in Prutsman-Pfeiffer (2008).

2.2. Sample Analysis

Sample preparation and analytical methods were documented in Darrah et al. (2009); Harkness and Darrah (2019) and summarized here. Samples were dried for 48 hr at 80°C in a vacuum oven to remove water. Samples of cortical and trabecular bone tissue (Figure 1) types were then separated for each patient. Separation of trabecular and cortical bone tissue samples was conducted in a class 100 trace metal free clean laboratory. Trabecular and cortical bone tissues were extracted from cross-sectioned samples using a manually operated precision Dremel® with graphite carbide tip to provide approximately 300 mg of each bone tissue type per patient. Care was taken to avoid anomalous sections of bone, such as areas near fracture zones, areas of necrosis, zones of hemorrhage, or arthritic nodules. Aliquots of between 50 and 100 mg were then separated to individual sample containers for chemical analyses and stored at room temperature in the trace metal free clean lab.

Approximately 50 mg each of cortical and trabecular tissues from each patient were dried and then crushed with a ceramic mortar and pestle in a trace metal free clean laboratory. After crushing, sample weights were recorded to ± 0.001 mg, and the dried bone material was digested in trace element free vessels using concentrated (15.9 N) ultra-pure (trace element free) nitric acid (HNO_3) at 90°C for approximately 5 hr or until digestion was complete. Following digestion, the remaining solution was baked for approximately 3 hr (or until dry) at 90°C to remove excess nitric acid. The residual material remaining after drying was then redigested with ultra-pure nitric acid (HNO_3). Internal standards consisting of known quantities of indium (In) and bismuth (Bi), used to correct for instrumental drift, were added to the digested bone material. The solutions were then diluted using trace element pure 18MΩ water to result in a final concentration of 2% nitric acid (by volume) and known concentrations of the internal standards.

Samples were analyzed for trace elements, using a Thermo X7 ICP-MS. Solution based ICP-MS analyses were conducted with modifications to the EPA 6020A methodologies (US EPA, 1991; US EPA, 1994), the approved ICP-MS analytical procedure for inorganic trace elements in soils and sediments. This method requires the use of both internal and external standards and blanks. Internal standards are necessary to correct for variations associated with instrumentation drift associated with variations in the behavior of analytes interacting with the plasma and to correct for physical interference effects. In this study, all samples, standards, and blanks were spiked with an internal standard comprised of known quantities of indium and bismuth. Indium and bismuth were chosen

because of their atomic masses reflect the range of analyte masses included in the study, have low natural abundances, and were not elements of interest in the study.

Chemical analyses by ICP-MS were conducted in two batches with varying total dilution factors representing the ranges of trace metal concentrations expected in human bone inferred from the NIST 1486 SRM and Trueman et al. (2006a). Rare earth elements (REEs) and low concentration trace elements (<1 ppb in solution) were diluted approximately 200 times (~100 mg into 20 mL of water). Analyses of higher concentration trace elements and minor elements were performed on aliquots with dilution factors of approximately 500 times.

To clean sample lines and reduce memory effects, sample lines were washed for 2 minutes between analysis of each sample with 2% nitric acid solution. Procedural blanks were prepared in an analogous fashion to samples and standards and were analyzed within each block of approximately eight samples, to monitor and correct for instrumentational and procedural backgrounds.

All data are available free from an online repository as described in the Data Availability Statement below (Coyte et al., 2021).

2.3. Statistical Treatment of Data

Statistical evaluations using nonparametric Mann-Whitney-Wilcoxon tests (at 95% confidence interval) to determine statistical significance of differences between distributions of values in groups (OA vs. fracture; male vs. female) and Spearman's rank-order correlations for nonparametric correlation analysis used the Pingouin statistical package for Python 3 (Vallat, 2018). We report both raw p-values and p-values adjusted to control for the family wise error rate using the Holm-Bonferroni method. For comparisons between trabecular and cortical bone data, a sign test was performed in Python 3 using the SciPy package (Virtanen et al., 2020). PCA was performed on data which had been centered to the mean and scaled to unit variance using the scikit-learn package (Pedregosa et al., 2011).

3. Results

In addition to element concentrations, total metals (TM), a summation of all measured trace elements, and total rare earth elements (TREE), a summation of all measured rare earth elements except Gd are reported. TREE were reported as a group due to their similar chemistry, but separate from Gd because of known exposures in some patients to Gd-based contrast agents for MRI procedures (Darrah et al., 2009). These tests were also performed on data normalized to Ca, but this did not impact any results, so only non-normalized data was reported.

3.1. Comparing Trace Element Concentrations in Cortical and Trabecular Bone

The concentrations of many trace elements vary significantly between cortical and trabecular bone. Cortical bone has significantly higher concentrations of Ti (Titanium), Mn, Co, Cu, Zn, Sr, Molybdenum (Mo), and Pb (Table 1) (Figure 2). Trabecular bone has significantly higher concentrations of Fe, Al, Gd, and TREE (Table 1) (Figure 2). Trabecular bones tended to have a larger range in concentrations than cortical bones; this was the case for Gd, Al, scandium (Sc), Ti, V, chromium (Cr), Fe, Co, Zn, barium (Ba), Pb, TREE, and TM (Figure 2).

3.2. Age and Sex

Age and sex data were recorded for all patients. Patients' ages at time of surgery ranged from 41 to 100, with the median age being 77.5. The median fracture patient was older than the median OA patient (86 vs. 65), and the distribution of ages between the two groups was significantly different (p-value <0.001; Figure S1 in Supporting Information S1), which is recognized as a limitation of this study. Coefficients (Spearman's rho) between age and trace elements concentrations are universally weak (Table S1 in Supporting Information S1). Before adjustment for family wise error, the p-value for correlations between Pb and age in cortical bone, and Sc, Ti, Cr, Al, and TM and age in trabecular bone were significant, but none of these correlations remained significant after adjustment.

As trabecular bone is remodeled faster than cortical bone, we calculate the ratio of trabecular to cortical abundances of trace elements to identify changes in relative incorporation between the two tissue types with age.

Table 1
Comparison of Element Concentrations in Trabecular and Cortical Femoral Heads

Transition metals

	Sc (ppb)	Ti (ppb)	V (ppb)	Cr (ppb)	Mn (ppb)	Fe (ppm)	Co (ppb)	Cu (ppm)	Zn (ppm)
Mean cortical	174.3	387.3	18.6	645.4	103.7	105.1	303.8	2.3	126.8
Median cortical	176.3	392.5	16.5	631.7	88.5	108.5	312.3	1.4	125.7
SD	28.6	64.1	8.4	139.0	52.4	17.6	51.1	4.7	21.7
Mean trabecular	162.2	316.6	17.7	700.2	54.7	317.5	225.4	1.1	93.8
Median trabecular	172.0	315.3	15.2	812.0	36.0	123.4	227.1	1.0	96.9
SD	54.3	71.5	13.7	413.7	46.5	347.4	77.9	0.9	26.0
p-value	0.51	<0.001***	0.36	0.24	<0.001***	0.02*	<0.001***	<0.001***	<0.001***
Adjusted *p*-value	1	<0.001***	1	0.95	<0.001***	0.17	<0.001***	0.001***	<0.001***

Other metals

	Al (ppb)	Sr (ppm)	Mo (ppb)	Cd (ppb)	Ba (ppm)	Pb (ppb)	Gd (ppb)	TREE	TM
Mean cortical	849.5	62.9	23.5	45.1	3.0	3578.7	413.5	364.8	306967.2
Median cortical	709.5	59.3	19.2	26.0	2.7	2960.5	7.5	100.9	307600.8
SD	526.3	19.5	23.2	103.3	1.7	2286.7	929.9	1201.0	53804.5
Mean trabecular	11556.7	40.2	16.0	29.2	3.4	2490.5	767.8	730.2	476044.0
Median trabecular	4584.5	37.6	13.1	19.2	3.0	1796.0	18.3	209.9	303380.5
SD	15160.2	17.2	9.3	25.7	2.5	2207.3	1550.1	2252.2	352968.3
p-value	<0.001***	<0.001***	<0.001***	0.09	0.05*	<0.001***	<0.001***	<0.001***	0.60
Adjusted p-value	<0.001***	<0.001***	<0.001***	0.43	0.29	<0.001***	<0.001***	<0.001***	1.00

Note. p-values refer to a sign test for the difference between cortical and trabecular data.
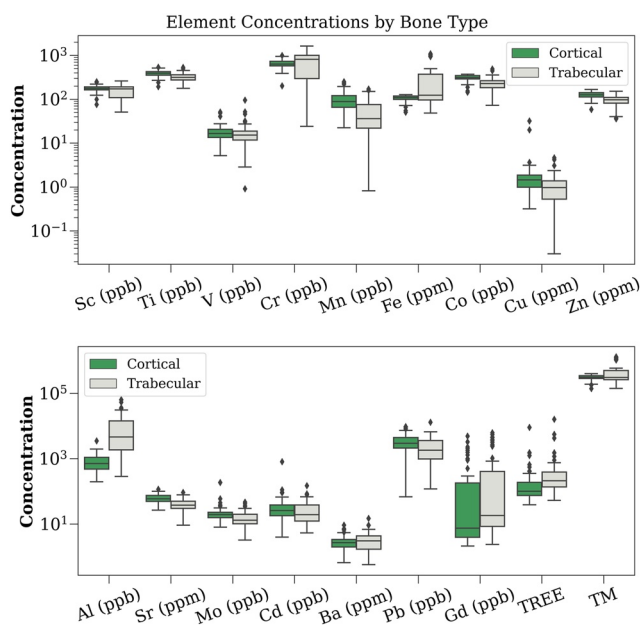

Figure 2. Boxplots showing concentrations of various trace elements in cortical and trabecular bone. Differences between the two types of bone were significant for Ti, Mn, Fe, Co, Cu, Zn, Al, Sr, Mo, Ba, Pb, Gd, and TREE, though the differences for Fe and Ba did not remain significant after *p*-value adjustment.

Before family wise error adjustment, correlations between T/C and age were significant for Sc, Cr, Al, and Gd. Correlation coefficients between age and T/C were weak, and not statistically significant following adjustment (Table S1 in Supporting Information S1).

There were more females than males in the study population (42 vs. 16). Of the men, 6 were fracture patients and 10 were OA patients. Of the women, 23 were fracture patients and 19 were OA patients. Median Mn concentrations were higher in female patients compared to male patients in cortical bone (Table S2 in Supporting Information S1), and Zn, Ba, and Pb concentrations were higher in male patients compared to female patients in trabecular bone (Table S3 in Supporting Information S1). Following *p*-value adjustment for family wise error, we do not find differences in element distributions between the sexes for any element.

3.3. Relation of Trace Element Concentrations to Bone Pathology

Because osteoarthritis is primarily a disease that affects the joints rather than affecting the bone structure itself, we assume that OA bone minerals are like normal bone in terms of elemental abundance. Therefore, we compare the elemental composition of osteoporosis-related fractured bone to bone from patients with osteoarthritis when evaluating trace element risk factors that may increase the susceptibility of bone to fracture. In cortical bone median concentrations of Cr and Pb are both higher in fracture patients than in OA patients (Figure 3) (Table 2). In trabecular bone, median concentrations of Sc
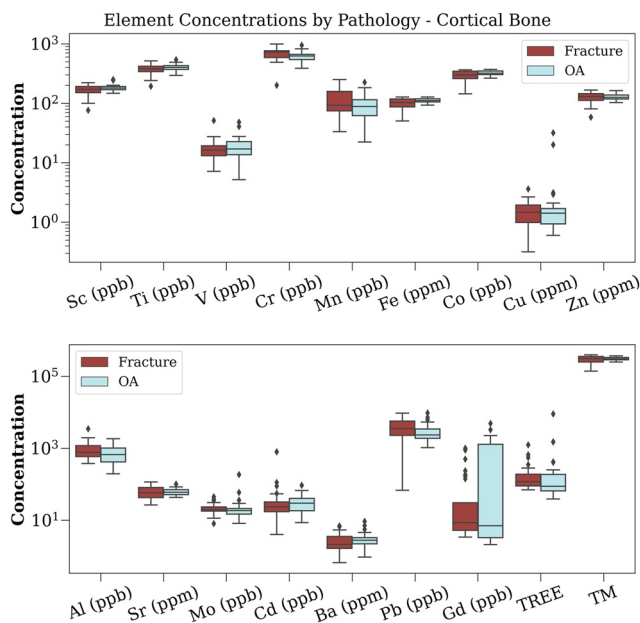
Figure 3. Boxplots comparing the concentrations of trace elements between fracture and OA patients in cortical bone. Differences between the two pathologies were significant for Cr and Pb, but these differences did not remain significant after *p*-value adjustment.

and Cr were higher in fracture patients than OA patients, and median concentrations of Ti were lower in fracture patients than OA patients (Figure 4) (Table 3). Of these differences, only the distribution of Cr concentrations in trabecular tissue was found to be significantly different between OA and fracture patients after *p*-value adjustment for family wise error. The range of concentrations in OA patients was larger than in fracture patients for several transition metals in trabecular bone, including V, Cr, Mn, and Cu (Figure 4).

Principal component analysis (PCA) was performed on both the cortical and trabecular data, which included all trace element data and data for patient sex and age to see if there was any disaggregation between the OA and fracture patients. Separation can be seen along the PC3 axis for cortical bone (Figure 5). PC3 for cortical bone has high loadings on patient age at surgery (−0.58) and Gd concentration (0.50) (Table 4). While Gd concentrations are not significantly correlated with pathology in this study, the median fracture patient is older than the median OA patient. In trabecular tissue, there may be some separation between fracture and OA patients along the PC2 axis. Ba (0.40), Cr (−0.37), Ti (0.34), Mn (−0.34), and Co (0.33) have the highest loadings on PC2 (Table 5).

4. Discussion

4.1. Differences Between Trabecular and Cortical Bone

Trabecular bone tissue, which is more metabolically active than cortical bone tissue, contains significantly higher concentrations of trivalent nonmetabolic trace elements (i.e., Al, Gd, and TREE) and significantly lower concentrations of metabolically regulated trace elements (e.g., Cu, Sr, Ti, Co, and Zn). Our results for metabolically regulated Sr and Zn are consistent with prior literature (Brodziak-Dopierala et al., 2006; Takata et al., 2005; V. Zaichick, 2006; Zioła-Frankowska et al., 2015). For the metabolically regulated trace elements, these differences are potentially the result of increased levels of bone resorption of metabolically active trabecular bone tissues, with more trace elements lost from the bone mineral to the extracellular fluid.

In contrast to most metabolically regulated elements (e.g., Cu, Sr, Ti, Co, and Zn), Fe shows the opposite trend with distinctly higher concentrations in trabecular bone tissue. One hypothesis for this observations is that the higher concentrations of Fe observed in trabecular bone may reflect the degree of trabecular bone interaction with blood, as Fe is concentrated in hemoglobin and myoglobin (e.g., Eguchi & Saltman, 1984). Similar to the distribution observed for Fe between cortical and trabecular tissue, the nonmetabolically Mn and Mo (Harkness & Darrah, 2019) are also significantly higher in trabecular bone tissue. Although these results for Mn and Mo are consistent with prior work (Brodziak-Dopierala et al., 2006; Takata et al., 2005; V. Zaichick, 2006; Zioła-Frankowska et al., 2015), the explanation for this is unclear.

Lead shows significantly higher concentrations in cortical compared to trabecular bone. Because Pb is toxic and has no known biological role, it is unlikely that bone tissue concentrations are related to increased loss during the more frequent remodeling of the trabecular bone. One hypothesis for the observed Pb data relates to the changing abundance of this element in the environment over the last several decades in the United States. The phasing out of Pb paint in US gasoline starting in the 1970s, as well as other efforts to limit human exposure to lead in the environment have produced decreases in blood Pb levels over the last few decades (Johnson et al., 1995; Kennedy et al., 2014; Magavern, 2018). Studies on occupational exposure have also shown that bone Pb concentrations decrease slowly after exposure is ceased (Christoffersson et al., 1986; Nilsson et al., 1991). Although we anticipate that cortical and trabecular bone would incorporate Pb at similar levels from the same extracellular fluid at any given time, the significantly longer remodeling rates of Pb in cortical bones (∼25–30 years) (Christoffersson et al., 1984; Gerhardsson et al., 1993; Weisskopf & Myers, 2006) compared to trabecular bones (2–5 years) (Boonen et al., 1997; Dorsey et al., 2006; Glimcher, 1998) suggests that the Pb incorporated into trabecular bone would have been incorporated over the last five years when ambient Pb levels are significantly lower than were observed in past decades. In comparison, significant portions of Pb in cortical bone would have been incorporated

Table 2
Statistical Summaries and Comparison of Element Abundances in Cortical Tissue Samples of Osteoarthritic (OA) and Fractured Bones

Transition metals

Name	Sc (ppb)	Ti (ppb)	V (ppb)	Cr (ppb)	Mn (ppb)	Fe (ppm)	Co (ppb)	Cu (ppm)	Zn (ppm)
Mean OA	181	402	19.2	612	94.1	111	320	3.08	128
Median OA	180	399	17.0	622	87.8	111	319	1.42	125
SD OA	22.6	54.2	8.76	113	42.4	9.20	28.6	6.54	16.0
Mean fracture	167	373	17.9	678	113	100	287	1.51	126
Median fracture	171	377	16.3	726	92.4	103	299	1.46	130
SD fracture	32.4	70.7	8.19	156	60.0	21.9	62.8	0.72	26.5
U	517	522	469	280	357	533	542	393	405
p-value	0.14	0.12	0.46	0.03*	0.33	0.08	0.06	0.67	0.82
Adjusted *p*-value	1.00	1.00	1.00	0.53	1.00	1.00	0.92	1.00	1.00

Other Metals

Name	Al (ppb)	Sr (ppm)	Mo (ppb)	Cd (ppb)	Ba (ppm)	Pb (ppb)	Gd (ppb)	TREE	TM
Mean OA	741	62.0	25.5	33.4	3.21	3040	713	523	313000
Median OA	672	59.6	18.5	29.7	2.79	2330	6.99	88.0	306000
SD OA	421	13.1	32.0	20.2	1.69	1930	1230	1680	32000
Mean fracture	958	63.7	21.5	56.7	2.73	4120	114	207	301000
Median fracture	764	57.6	19.6	23.5	2.13	3540	8.53	117	309000
SD fracture	601	24.6	7.92	145	1.62	2510	255	244	69000
U	309	414	343	464	527	296	385	298	429
p-value	0.08	0.93	0.23	0.50	0.10	0.05*	0.59	0.06	0.90
Adjusted *p*-value	1.00	1.00	1.00	1.00	1.00	0.91	1.00	0.92	1.00

Note. U and *p*-values refer to the U statistic and p-value of a Mann-Whitney comparison between OA and fracture patients.

during past decades with much higher ambient levels of Pb in the environment. For these reasons, we suggest that the lower levels of Pb observed in trabecular bone could be the result of ongoing environmental efforts to reduce Pb exposure.

The cause of significantly higher concentrations of non-metabolic trivalent cations (i.e., Al and REE) in trabecular tissue is also unclear. Al and REEs both coprecipitate with Fe in flocculation processes observed in seawater (Sholkovitz, 1976; Valente et al., 2006), and Al has been shown to coprecipitate with Fe in bone during instances of kidney failure (Miyoshi et al., 2006), which may explain the association between higher Fe, Al, and REEs in trabecular bone. Alternatively, the high field strengths (i.e., higher ratios of ionic charge to ionic radius) of Al and REEs may cause them to be preferentially retained during bone tissue resorption either by slower rates of dissolution from the crystal lattice or lower solubilities in the extracellular fluid, increasing their concentrations in more frequently remodeled trabecular tissues. If the latter is validated in future work, this hypothesis would further confirm that bone resorption processes behave similar to dissolution processes in solution chemistry.

4.2. Trace Elements and Their Relation to Bone Pathology

Albright Fuller once described osteoporosis as "a disease in which there is too little bone in the bone, but what bone is there is normal" (Fuller et al., 1941), suggesting that there are no chemical compositional differences between osteoporotic and normal bone. However, subsequent research has shown a link between osteoporosis and deficiency in several elements, usually measured in either serum or diet, including Zn (Jamieson et al., 2006; Suzuki et al., 2015; Zheng et al., 2014), Cu (Aaseth et al., 2012; Beattie & Avenell, 1992), and Sr deficiency (Demontiero et al., 2002; Grynpas et al., 1996; Marie, 2007), as well as increased Fe (Jeney, 2017), Pb (Beier
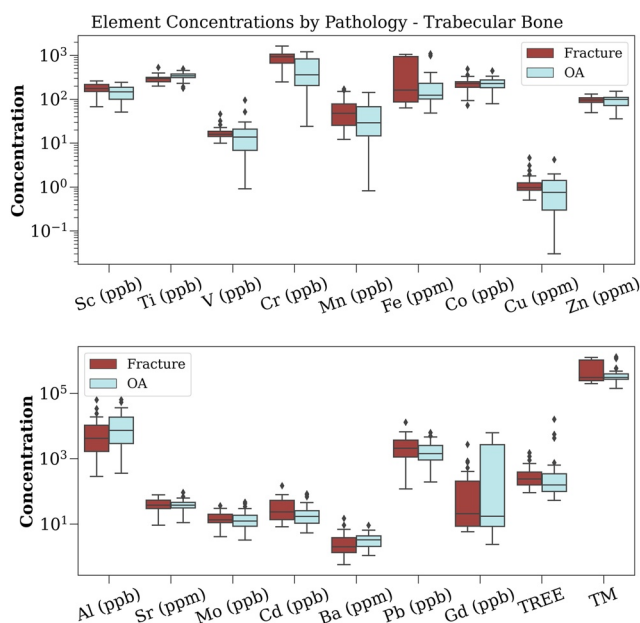
Figure 4. Boxplots comparing the concentrations of various trace elements between fracture and OA patients in trabecular bone. Differences between the two pathologies were significant for Sc, Ti, and Cr, though differences for Sc and Ti did not remain significant after *p*-value adjustment.

et al., 2016; Carmouche et al., 2005; Puzas et al., 2004), and Cd concentrations (Berglund et al., 2000; James & Meliker, 2013; Youness et al., 2012) in bone minerals. However, in this cross-sectional study, we did not observe significant differences between OA and fracture patients for Zn, Pb, Sr, Fe, Cu, or Cd in trabecular bone tissues.

Of these elements, only Pb showed significant differences between pathologies in our study for cortical bones tissues. In the current study, median Pb concentrations are higher in fracture patients for cortical bone tissues. As this is seen in cortical tissue, it likely suggests a difference in lifetime Pb exposure, rather than recent changes in bone Pb concentrations. Bone Pb incorporation alters the metabolism of osteoblast and osteoclast cells responsible for bone formation and resorption (Beier et al., 2016; Birnbaum et al., 1995; Dowd et al., 2001; Hamilton & O'Flaherty, 1994; Klein & Wiren, 1993; Puzas et al., 1992), decreases osteoblast stem cell production (Carmouche et al., 2005), disrupts calcium homeostasis (Berglund et al., 2000; Dowd et al., 2001), limits essential growth factors (Jamieson et al., 2006; Milgram et al., 2008), and alters bone maturation signaling and remodeling (Zuscik et al., 2007). In rodents, Pb causes decreased bone mineral density, produces defective bone minerals (Campbell et al., 2004; Gruber et al., 1997; Potula & Kaye, 2005), induces abnormal mineralization even at low concentrations (Gonzalez-Riola et al., 1997), and reduces the rate of fracture healing (Carmouche et al., 2005). However, it is important to note that the differences in Pb were not significant following correction for family wise error suggesting larger datasets are required to evaluate the role of Pb.

We also observed significantly higher concentrations of Cr, Sc, and Ti in trabecular bone tissue of patients with osteoporosis and Cr concentrations in cortical bone tissue. However, only Cr in trabecular tissue remained statistically significant after correcting for family wise error. Higher concentrations of Cr in fracture patients for both trabecular and cortical bone is particularly interesting because there have been conflicting reports on its role in bone health. Cr has been suggested to be both protective against osteoporosis due to enhanced insulin sensitivity decreasing bone resorption (McCarty, 1995) and harmful to bone formation because of its role in inducing oxidative stress (Dermience et al., 2015). Presently, the "healthy" range of Cr in bone remains undefined. One study on Cr levels in quail found that higher dietary levels of this element weakened bones when dietary Cu concentrations were low, but strengthens them when dietary Cu concentrations are high, suggesting the potential importance of synergistic and antagonistic interactions between trace elements (Hermann et al., 1997). We note a positive correlation between Cr and Cu in trabecular bone in our data ($\rho = 0.68$, *p*-value <0.001), but do not observe any statistically significant difference between the fracture and OA patients (Tables 2–3) (Figure S2 in Supporting Information S1). Metal alloys used for surgical implants often contain Cr, and in vitro studies have shown that the chromium particles resulting from the wearing down of the implants can induce bone resorption and reduce bone formation (Sansone et al., 2013). It is unknown if any patients in our study had been previously outfitted with a Cr containing implant. In our database, there is a weak correlation with Cr and age, which may be related to the much older fracture patient population (Figure S1 in Supporting Information S1), but other studies with wider population age ranges have not reported correlations between Cr concentrations and age (García et al., 2001; Kuo et al., 2000).

There are multiple interpretations for the observed differences in the trace element data between trabecular and cortical bone, as they relate to pathology. As mentioned above, cortical bone trace element concentrations represent long-term (decadal) accumulation, while elements in trabecular bone tissues have been deposited (and are typically remodeled) within the last few (<5) years, though different elements may have different residence times (Gerhardsson et al., 1993; Nilsson et al., 1991; O'Neal et al., 2014; Rodríguez & Mandalunis, 2018). Therefore, the higher transition metal concentrations in the trabecular bone may be the result of recent changes in incorporation and regulation mechanisms due to age, disease (e.g., osteoporosis), or changing exposure or incorporation of trace elements over the lifetime of the patient. Given transition metals are typically highly biologically regulated (Harkness & Darrah, 2019), this mechanism seems an unlikely explanation for the summation of data. In addition

Table 3
Statistical Summaries and Comparison of Element Abundances in Trabecular Tissue Samples of Osteoarthritic (OA) and Fractured Bones

Transition metals								
Name	Sc (ppb)	Ti (ppb)	V (ppb)	Cr (ppb)	Mn (ppb)	Fe (ppm)	Co (ppb)	Cu (ppm)	Zn (ppm)
Mean OA	144	338	17.7	513	42.8	253	223	0.94	92.5
Median OA	146	343	13.7	359	29.0	123	227	0.75	99.4
SD OA	55.5	69.9	18.2	371	38.2	290	76.6	0.88	30.5
Mean fracture	180	295	17.6	888	64.9	382	228	1.25	95.0
Median fracture	176	296	15.9	927	48.1	162	227	0.97	96.3
SD fracture	47.3	67.7	7.10	372	51.1	391	80.5	0.85	20.9
U	271	601	321	198	260	421	420	310	408
p-value	0.02*	0.01*	0.12	0.001**	0.08	1.00	0.99	0.13	0.85
Adjusted *p*-value	0.33	0.09	1.00	0.01*	1.00	1.00	1.00	1.00	1.00

Other metals								
Name	Al (ppb)	Sr (ppm)	Mo (ppb)	Cd (ppb)	Ba (ppm)	Pb (ppb)	Gd (ppb)	TREE	TM
Mean OA	14400	39.2	15.8	24.0	3.66	2090	1330	1110	413300
Median OA	7290	37.5	12.4	17.0	3.29	1430	17.2	156	303000
SD OA	16800	16.8	11.2	20.8	1.87	1670	2011	3180	305000
Mean fracture	8750	41.1	16.2	34.3	3.23	2890	222	361	537000
Median fracture	4150	37.7	13.4	23.4	2.00	2070	20.6	238	303000
SD fracture	13000	17.8	7.09	29.2	3.04	2600	527	329	390000
U	506	399	342	306	532	340	435	299	379
p-value	0.19	0.74	0.23	0.08	0.08	0.21	0.65	0.09	0.67
djusted *p*-value	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Note. U and *p*-values refer to the U statistic and *p*-value of a Mann-Whitney comparison between OA and fracture patients.

to the comparisons above, difference between the chemistry of trabecular and cortical bone as they relate to pathology can also be seen in the results of the PCA. Fractured and OA trabecular bone are most separate on PC2, which has high loadings primarily in Ba and transition metals Cr, Mn, Ti, V, and Co, with fracture patients having a lower loading on PC2 and thus being associated with lower Ba, Ti, and Co and higher Cr and Mn concentrations. These observations are broadly consistent with the statistical comparison that shows differences between the incorporation of transition elements related to osteoporosis. Separation between fracture and OA bone in the PCA of cortical bone on the other hand, was primarily along PC3, which appears to be heavily influenced by age.

Some trace elements are known to play vital roles in bone formation and structure, and their depletion, rather than excess, in bone likely pose health risks. Both Sr and Zn directly promote osteoblast activity and bone formation (Almeida et al., 2016; Hadley et al., 2010; Hyun et al., 2004; Jamieson et al., 2006; Lymperi et al., 2008), and increase bone quality with dietary increases of these elements (Brzóska et al., 2008; Gur et al., 2005; Hyun et al., 2004; Jamieson et al., 2006; Nielsen, 2004; Querido et al., 2016), and are commonly used to treat osteoporosis and prevent fracture (Gur et al., 2005; Kawamura et al., 2003; Marie, 2005; Yamaguchi, 2010). Average Sr concentrations (~40 ppm) in this study are consistent with the average value reported by Demontiero et al. (2002) (~35 ppm) and others (Budis et al., 2014; Helliwell et al., 1996) for femoral head bones. This study, however, did not observe significantly lower concentrations of Sr or Zn in fracture patients. While these results initially appear to conflict with previous research (Bruyere et al., 2007; Jamieson et al., 2006; Karaaslan et al., 2014; Riedel et al., 2017), our cross-sectional data set is not controlled for Zn or Sr supplements, although none were noted in a review of the available records. These results indicate that either: (a) Sr and Zn incorporation into bone does not significantly affect the potential for fracture beyond certain dietary deficiency thresholds; (b) Sr and Zn
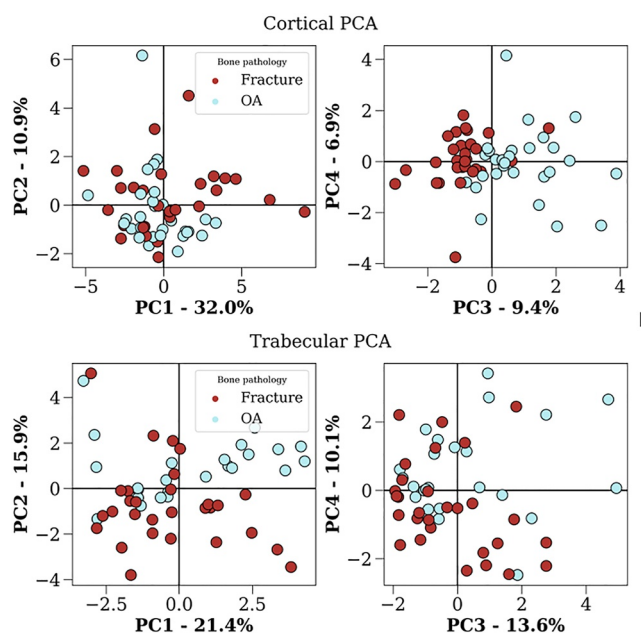
Figure 5. The relative loading scores of the first four principal components for cortical (top) and trabecular (bottom) bone.

concentrations of bones included in this study are elevated by medical/nutritional supplements that have not succeeded in preventing fracture; or (3) Sr and Zn concentrations in bone are not indicative of susceptibility to fracture.

While fractured bones do not appear to be deficient in Zn or Sr, lower concentrations of Ba and Ti were suggested by PCA analysis as being related to the tissues of fracture patients. Ba concentrations reported in this study are similar to those previously reported for healthy femoral heads on average (~3–4 ppm, Yoshinaga et al., 1995). Incorporation of Ba may have similar (or greater) effects on bone crystal structure and strength as Sr given the larger ionic radius of Ba relative to Sr. Sr is known to treat osteoporosis as a dual action bone agent (DABA), effectively retarding dissolution (resorption) of bone minerals and promoting formation of stronger bone minerals (Marie, 2007). Sr has a larger ionic radius than Ca (15% larger: Ca = 114 picometers (pm), Sr = 132 pm). Experiments have shown that small additions of Sr (50 ppm) expand the crystal lattice of bone crystal nuclei, reduces Ca-hydroxyapatite $(Ca_5(PO_4)_3)^0$ solubility (pK_s) which enhances bone mineralization, retards bone crystal dissolution, and reduces bone crystal growth, resulting in smaller and stronger bone crystals with lower susceptibility to fracture (Riedel et al., 2017; Verberckmoes et al., 2004). Here, we consider an extrapolation of the physicochemical effects to Ba incorporation, which has a larger ionic radius than both Ca and Sr (r_{ion} Ba = 149 pm; 30% larger than Ca and 12% larger than Sr). The larger ionic radius of Ba^{2+} (149 pm) may further expand hydroxyapatite crystal lattices and would thus reduce hydroxyapatite solubility (pK_s) and could create smaller and stronger bone minerals. Ba may further retard bone dissolution and perhaps more efficiently prevent bone mineral loss in osteoporosis patients. If the role of Ba can be validated in future studies, low Ba concentrations may increase susceptibility to fracture, and their potential as treatments for osteoporosis and nutritional supplements warrant further exploration.

5. Conclusions

Despite the biologically relevant abundances of trace elements in bone and their important role in bone health, there is limited research on the potential effects of many minor and trace elements in human bone (Harkness & Darrah, 2019; S. Zaichick, Zaichick, Karandashev, & Moskvina, 2011; Takata et al., 2005; Wiechuła et al., 2008). This study explored variations in trace element abundances by examining the trace element abundances in the bone minerals of surgical extracts from 29 osteoarthritis and 29 osteoporotic fracture patients. The concentrations of Pb were higher in cortical bone from patients with osteoporosis, which was expected based on several previous studies; the association between elevated Pb concentrations in cortical bone minerals reinforces the importance of lifetime exposure to Pb on health. However, our results also suggest that trace elements beyond those most commonly studied (e.g., Pb and Sr), may play a role in, or be affected by, osteoporosis. In our data, median concentrations of Sc and Cr are higher in fractured trabecular bone. While prior research into Cr has produced contradictory results, suggesting either beneficial or detrimental impacts to bone health, there is very little prior research about Sc in bones. Fractured bones from this study are also not deficient in the trace elements associated with the current suggested treatment options, Zn or Sr (even when normalized to Ca), but bone minerals from these samples do have lower concentrations of Ba and Ti in trabecular tissues. Incorporation of Ba may have similar effects on

Table 4
Loading Scores for Principal Component Analyses of Cortical Bone Data

	PCA 1	PCA 2	PCA 3	PCA 4
TM	−0.37	−0.16	−0.02	−0.12
Fe (ppm)	−0.36	−0.19	0.12	−0.06
Co (ppb)	−0.36	−0.19	0.13	−0.04
Sc (ppb)	−0.34	−0.04	0.08	0.20
Ti (ppb)	−0.34	0.13	0.16	0.07
Zn (ppm)	−0.33	−0.15	−0.05	0.08
Sr (ppm)	−0.29	−0.18	−0.16	−0.27
Cr (ppb)	−0.24	0.18	−0.22	0.32
Mn (ppb)	−0.20	0.46	0.04	0.04
Pb (ppb)	−0.15	−0.12	−0.35	−0.22
V (ppb)	−0.13	0.54	0.03	−0.10
Ba (ppm)	−0.12	0.24	0.22	−0.46
TREE	−0.10	0.02	0.07	0.44
Gd (ppb)	0.08	−0.13	0.50	−0.07
Female	−0.07	0.10	0.02	0.06
Cd (ppb)	−0.07	0.06	−0.23	−0.05
Al (ppb)	−0.06	0.28	−0.06	0.35
Mo (ppb)	−0.04	0.13	−0.08	−0.21
Age at surgery (yrs)	0.03	0.08	−0.58	−0.15
Cu (ppm)	−0.02	0.31	0.16	−0.28

Table 5
Loading Scores for Principal Component Analyses of Trabecular Bone Data

	PCA 1	PCA 2	PCA 3	PCA 4
Sc (ppb)	−0.42	−0.10	−0.12	0.03
Zn (ppm)	−0.38	0.18	−0.04	−0.03
Sr (ppm)	−0.36	0.10	−0.11	−0.06
Co (ppb)	−0.33	0.33	0.02	−0.02
Cr (ppb)	−0.29	−0.37	0.03	0.05
Cd (ppb)	−0.24	−0.23	−0.16	0.26
Pb (ppb)	−0.21	0.12	−0.05	−0.23
Mo (ppb)	−0.20	0.07	0.09	0.22
Ti (ppb)	−0.20	0.34	0.16	0.13
Mn (ppb)	−0.20	−0.34	0.22	0.24
Gd (ppb)	0.18	0.11	0.27	0.41
Age at surgery (yrs)	−0.17	−0.17	−0.16	−0.36
V (ppb)	−0.17	0.02	0.39	0.23
Female	0.13	−0.13	−0.15	0.11
Cu (ppm)	−0.12	−0.28	0.13	0.19
TREE	0.09	0.14	−0.08	0.11
Ba (ppm)	−0.07	0.40	0.13	−0.13
TM	−0.04	−0.16	0.44	−0.39
Al (ppb)	0.01	0.13	0.43	0.07
Fe (ppm)	0.01	−0.19	0.43	−0.40

bone crystal lattice structure and mineral strength as Sr, and thus the effect of Ba and Ti on the formation of bone crystal nuclei and crystal sizes warrants further consideration, potentially as a treatment for osteoporosis.

Differences in the distribution of trace element concentrations between cortical and trabecular bone were significant, with metabolically regulated elements generally being higher in cortical tissues, and median concentrations of nonmetabolically regulated elements being generally higher in trabecular tissues. The differences in trace element abundances between cortical bone and trabecular bone could lend insight into the possible mechanisms behind differing trace element concentrations in osteoporosis and osteoarthritis patients. Differences in trabecular bone may be related to symptoms of osteoporosis such as higher rates of bone resorption (loss), which may affect metabolically regulated elements more than nonmetabolically regulated elements. Whereas the longer timeframe associated with elemental accumulation in cortical bone may point more toward higher time-integrated elemental exposures throughout adolescent and adult life or preferential retention of trace elements with higher field strengths.

Future studies should be geared toward investigating the role of transition metals like Cr, Sc, and Ti in osteoporosis and the potential role of synergistic and/or antagonistic interactions between various trace elements on bone health. Presently, it remains unclear if the observed differences in trace element abundances may play a causative role (cause) or be a result of (effect) osteoporosis pathology. Ba may also be worth investigating further as a possible treatment for osteoporosis. Moreover, because various elements incorporated into bone can be released during bone resorption, trace element abundances in bone minerals deserve further investigation with respect to their potential roles in diseases of the central nervous system and peripheral neuropathies (Godwin, 2001; Sprauten et al., 2012; Travis et al., 2014), kidneys (D'Haese et al., 1999; Kasama, 2010), cardiovascular disease, tinnitus, and in influencing new bone formation (Beier et al., 2016; Carmouche et al., 2005; Dermience et al., 2015; Puzas et al., 2004; Zhu et al., 2016). For these reasons, future studies should continue to assess a broader suite of trace elements to expand the volume of available data to more fully evaluate potential relationships between these elements and bone health.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Research data for this article can be found in the OSF repository, freely available under a CC-BY and can be accessed at https://osf.io/g2tsh/, DOI 10.17605/OSF.IO/G2TSH.

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