2022

PRELIMINARY STUDY IN MORE EFFECTIVE UTILIZATION OF 87Sr/86Sr ANALYSIS IN THE FIELD OF FORENSIC ANTHROPOLOGY

Samantha Powers

Recommended Citation

PRELIMINARY STUDY IN MORE EFFECTIVE UTILIZATION OF $^{87}Sr/^{86}Sr$ ANALYSIS IN THE FIELD OF FORENSIC ANTHROPOLOGY

By

SAMANTHA ERIN POWERS

Biomedical Science B.S., Grand Valley State University, Allendale, MI, 2019
Anthropology B.S., Grand Valley State University, Allendale, MI, 2019

Thesis

presented in partial fulfillment of the requirements
for the degree of

Master of Arts
in Anthropology, Forensic Concentration

The University of Montana
Missoula, MT

May 2022

Approved by:

Scott Whittenburg, Dean of The Graduate School
Graduate School

Meradeth Snow, PhD, Chair
Anthropology

Randall Skelton, PhD
Anthropology

Mark Heirigs, PhD
Sociology

Powers, Samantha, M.A., Spring 2020 Anthropology

PRELIMINARY STUDY IN MORE EFFECTIVE UTILIZATION OF $^{87}Sr/^{86}Sr$ ANALYSIS IN THE FIELD OF FORENSIC ANTHROPOLOGY

Chairperson: Meradeth Snow, PhD

Committee Members: Randall Skelton, PhD & Mark Heirigs, PhD

Isotopic analyses are gaining attention in the field of anthropology. The current utilization of strontium isotopes primarily focuses on residence and migration patterns of past populations extracting data from tooth enamel to indicate early-life residence This study aims to identify a skeletal feature to be set as a field standard in strontium analysis of modern remains when seeking end-of-life residence, the impact of diagenesis on modern remains, and to test the accuracy of the petrous as an alternative to tooth enamel.

The results of this research were unable to suggest such a standard but gathered helpful data to be used in future efforts. The trabecular bone showed evidence of higher strontium alterations due to diagenesis. The data also suggested that the petrous bone is a suitable alternative to tooth enamel. A new technique of coring cortical bone was applied and demonstrates a less destructive extraction method when gathering material to perform isotopic and DNA analysis.

The nature of this research is preliminary, any and all data will contribute to the better understanding and capability for the application of strontium analysis of forensically modern humans. The expansion of these techniques and methods to include end-of-life data would benefit the forensic, molecular, and archaeology sub-fields. This data would provide an additional tool to aid in the positive identification of modern remains of unknown individuals.

Acknowledgements

I would like to begin by thanking my committee members; Dr. Meradeth Snow, Dr. Randall Skelton and Dr. Mark Heirigs. Their commitment to helping me complete this research was greatly appreciated.

A special thanks to Dr. Snow who encouraged me every step of the way by revising my drafts, providing advice, both academic and life, and answering emails at any hour of the day or night. Dr. Randall Skelton for taking me on as a graduate student and helping me find the right path to develop and conduct this research.

I would also like to thank my family. They never stopped believing in me, even when I took the road less traveled to achieving my dreams. I am fortunate to have too many to mention, but I will name a few. My mom who showed me what it means to be a strong woman and that finding my own way was the right way. My grandma, who cheered loudly for every accomplishment, big and small. My stepdad who probably does not even remember the conversation in the kitchen that sparked the belief that I could even go back to school after so many years. My daughter, Giana, for being the part of my soul I never knew I was missing and giving me the “reason” to never give up. For my boyfriend, Nate, for allowing me to uproot our lives and move across the country to make this all a reality. My Grandpa in heaven who helped shape me into the women I am today. He was my rock and my compass and never let me believe my life could be anything but extraordinary, if I put in the hard work.

I would like to recognize Diego P. Fernandez at the University of Utah for analyzing my samples. I would also like to thank my undergraduate professor Dr. Elizabeth Arnold for meeting with me via zoom to discuss isotopes and my results.

Lastly, but not least, my cohort, my chosen family. This could not be possible without their encouragement, coffee dates and movie nights. Thank you for being an integral part of this journey. Our futures may take us in different directions, but I have no doubt they will all be exceptional.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Anthropology encompasses a multitude of disciplines and sub-disciplines. Forensic and molecular anthropology are two of those and the focus of this research. Forensic anthropologists are often tasked with developing a biological profile that aids in establishing the identity of an individual when unknown remains are found. This profile is developed to assist other professionals, such as medical examiners and police officers, in making a positive identification. Molecular anthropologists have developed expert skills in utilizing DNA and isotopic analysis. The data from these techniques also assist in the positive identification of unknown remains.

One of the isotopes gaining popularity is strontium. The literature review that follows will cover the characteristics of this isotope and how it behaves in human bone. Due to strontium's behavior and integration into skeletal material, it may be utilized to help trace an individual's geological residence and migration patterns. In the past, tooth enamel has been heavily relied upon to provide such data of past populations. Teeth are often not recovered or contain high levels of antemortem decay, at times deeming them inadequate for isotopic analysis. However, other locales throughout the skeleton may allow for testing as effective as enamel.

This research will test Hypothesis 1: The petrous portion of the temporal bone is as effective as tooth enamel in identifying an individual's early life residence.

Test Expectations: To accept this hypothesis, the petrous and tooth enamel require a strontium signal that falls within the same range. To determine the accuracy of this measured range, the rate of diagenesis will not be significant enough to suggest alterations of the strontium signal.

There will also be a literature review of human skeletal material at a histological level and how this affects the remodeling patterns of various bones. This is crucial information when selecting appropriate skeletal features to test. Bone composition plays a role in diagenesis and must be considered when seeking scientifically accurate data. There is a lack of literature on the rate of diagenesis in forensically significant bone. In general, to be considered of forensic significance, remains are believed to be no older than 50 years. Current literature provides data on diagenetic alterations in the bone of archaeological or historical significance that have been exposed to exterior elements for extended periods.

This research will assess diagenesis by testing *Hypothesis 2:* Trabecular bone will demonstrate higher levels of diagenesis when compared to cortical bone.

Test Expectations: To accept this hypothesis, the sampled trabecular bone will show similar signals to the measured strontium signal of the Clark Fork River water and wood samples. If the measured strontium signal is more closely related to the Clark Fork River samples than other expected ranges, this would suggest diagenetic alterations resulting from close contact with the river water and embankment.

Forensic anthropologists could use isotopic analysis more effectively if end-of-life residence data collection standards were well established. This data would allow for a general geographical location of the last known residence to be identified, giving direction for a search of missing person reports and familial identification. There is currently a lack of literature on what skeletal feature would best provide the sought-after answers. This is critical in today's society as individuals tend to be relatively mobile and often reside far from their birthplace. With the identification of this feature, end-of-life strontium data may be extrapolated and coupled with early-life residence data, creating a complete picture of an individual's mobility.

This research will test the following hypothesis to identify a skeletal feature as a field standard in end-of-life residence research and determine what skeletal features are more prone to diagenesis and skewing isotopic signals.

Hypothesis 3: The diaphysis of long bones will provide the most accurate end-of-life data when performing a strontium isotopic analysis.

Test Expectations: To accept this hypothesis, the diaphysis of long bones must contain a strontium signal that falls within the expected range of end-of-life data of the individual. The measured strontium signal must fit the expected ranges better than the other skeletal features sampled.

Furthermore, this research utilizes a new extraction method, termed coring. Coring creates one small area of destruction while providing adequate material for both DNA and isotopic analysis. The testing of individuals for both DNA and isotopes equates to two areas of destructive analysis. It is often unavoidable that destructive analysis be performed to collect valid scientific and identification data. However, it is of utmost importance to anthropologists and the people close to the deceased individual that the least amount of destruction be done to their remains.

As previously mentioned, some topics and standards are not developed nor currently expressed in the literature. This research is of preliminary nature and attempts to discover a basis for further research. This is being done to bring the sub-fields of forensic and molecular anthropology one step closer to setting a standard for the application of geolocating an individual's last known residence.

This analysis will include a literature review, an examination of materials and methods, a presentation of results, and a discussion and conclusion of the findings. Limitations of this research, further research, and broader impacts of this study will also be briefly discussed.

LITERATURE REVIEW

Isotope Background in Anthropology

The utilization of isotopes in anthropological research has provided valuable methods in exploring diets and determining migration patterns of past populations. Often this research includes multiple elements and a variety of isotopic combinations. Strontium is one of many elements that provide critical pieces of valuable information in studying past and present populations.

Jonathon E. Ericson was the first anthropologist to conduct a study using strontium (Burton & Katzenber, 2018). In 1985, Ericson demonstrated the usefulness of strontium isotopes when determining the residential patterns of prehistoric societies. He was particularly interested in tracing an individual's pre-marital residence (Ericson, 1985). Erickson used Strontium (Sr) data from a cemetery sample. The Sr level of the second molar was analyzed to determine residence during early childhood, hence pre-marital residence, assuming marriage occurred after the age of twelve. Due to the remodeling bone undergoes, a sample was extracted to compare to the enamel sample, focusing on the change in level, suggesting migration. Ericson demonstrated that this technique of Sr isotopic analysis was useful in tracing the migration patterns of these individuals. By comparing the enamel sample to the geological levels of Sr he was able to determine regional intermarriage patterns (Ericson, 1985).

Strontium Background

Strontium is an alkaline earth metal that was first isolated in 1808 in the Scottish town, Strontian (Cabrera et al., 1999). It was discovered that four stable isotopes occur naturally in nature, ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr (Burton & Katzenber, 2018; Pors, 2004).

These isotopes occur in varying concentrations depending on the bedrock formation of a particular geographical location (^{84}Sr~ 0.56%, ^{86}Sr~ 9.87%,^{87}Sr~ 7.04%,^{88}Sr~ 82.53%,) (Coelho et al., 2017). ^{87}Sr is the product of the radioactive decay of Rubidium (^{87}Rb) and is present in almost equal concentrations as ^{86}Sr (Burton & Katzenberg, 2018). Due to their similar concentrations, the ratio of ^{87}Sr/^{86}Sr is often analyzed and reported as one level (Bentley, 2006). This concentration is helpful in migration research because the half-life of ^{87}Rb is an order of magnitude greater than the age of Earth, and therefore there is no measurable change of ^{87}Sr/^{86}Sr concentrations in a particular geographical location (Chesson et al., 2012; Burton & Katzenberg, 2018). The low abundance of ^{84}Sr does not make it ideal for utilization in georeferencing research. ^{88}Sr has been seen in biomedical research as a marker of calcium metabolism. Due to the differences in the natural abundance of these isotopes and not being products of rubidium radioactive decay, ^{84}Sr and ^{88}Sr are not utilized in tracing human migration patterns (Cabrera et al., 1999).

Strontium Behavior in Bone

Strontium has a multitude of other functional elemental properties that make it ideal for anthropological and forensic research; primarily the similarities to calcium (Ca). Strontium is element 38 and is found directly under calcium on the periodic table. This means Sr and Ca are both divalent (+2) and are similar in size; Sr is 200 picometre (pm) while Ca is 180pm. In addition, Sr is a bone-seeking element and has been found to replace Ca in the matrix of human and faunal bone (Burton & Katzenberg, 2018).

This process begins with eroding geological material first entering the soil, plants and local water sources then incorporate these levels (Figure 1). Animals and humans ingest the vegetation and water, further incorporating the Sr levels into their bones and other tissues via the gastrointestinal tract (Bently, 2006; Laffoon et al., 2012). Once ingested, biopurification occurs, and 10-40% of the Sr ingested is absorbed by the body (Bently, 2006). Isotopic fracturing, the partnering of the heavy and light isotopes, changing the relative concentrations, is not observed with Sr. The lack of this process allows for the Sr that is absorbed by the body and found in the bedrock to be directly correlated (Ericson, 1985).

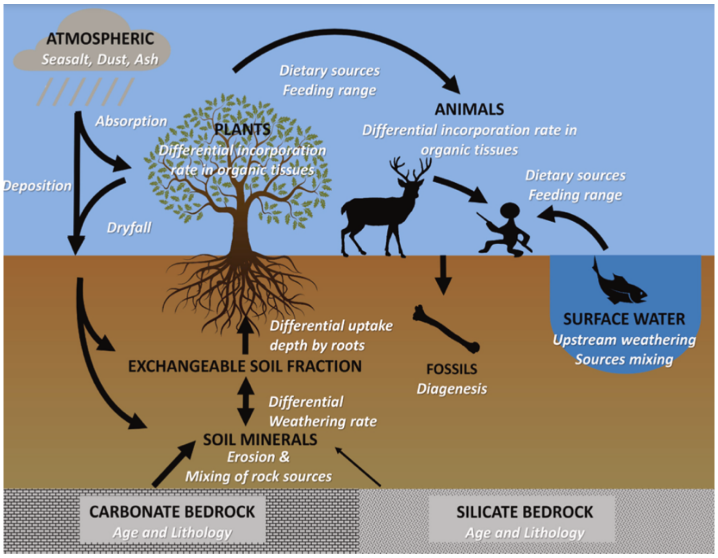

Figure 1: Schematic of Strontium Isotope Cycle from the Rocks to Ecosystem (Bataille et al., 2020)

Enamel Structure and Utilization

This has led to research that compares the level of Sr found in tooth enamel to that of a geological area of interest. In this research enamel of teeth, particularly molars, have been commonly used because the absorbed level during development has been preserved (Waterman et al. 2014). Enamel is not a living tissue, but rather mineralized calcium phosphate arranged in a crystalized hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) that concludes formation and mineralization during the early years of an individual's life. Enamel is also less prone to diagenesis from burial environments (Andres et al., 2019; Burton & Katzenberg, 2018). Though enamel can be exposed to some Sr from saliva, this is often removed during cleaning and therefore does not affect the inherent level of Sr incorporated during development from the surrounding environment (Ericson, 1985).

There is a lack of literature identifying a skeletal feature that would provide similar data to tooth enamel when enamel is unavailable. However, there is supporting data in the use of the petrous portion of the temporal bone (petrous) as an alternative to enamel in ancient DNA (aDNA) research (Pinhasi et al. 2015, Pinhasi et al. 2019). The petrous is the densest bone found in mammals and demonstrates a low level of remodeling during an individual's lifetime. The location of the petrous also allows for protection from external environmental exposure, decreasing the occurrence of diagenetic alterations (Pinhasi et al., 2015). These properties suggest the Sr levels incorporated in an individual's early life may also be preserved in the petrous, lending itself as a possible alternative to tooth enamel.

Bone Structure and Utilization

Tooth enamel is particularly useful when examining where individuals resided during their developmental years. However, this information is extraneous when attempting to determine where an individual resided during the latter part of their life (Price, 2000). This research requires the utilization of a skeletal feature, which participates in remodeling during an individual's lifetime. A standard similar to enamel has not yet been achieved in anthropological research. Current research using animal models as a proxy for humans, has explored the use of the femoral head, lumbar vertebra, rib, and iliac crest as possible sources of consistent end-of-life data (Dahl et al., 2001). The minimal research using human models is consistent in the use of these skeletal features (Barelink & Chesson, 2019).

The bone of the human skeletal system is comprised of 50-70% hydroxyapatite, 20-40% organic matrix (mostly collagen), 5-10% water and <3% lipids. There are two types of bone, 80% cortical and 20% cancellous (also referred to as trabecular) (Clarke, 2008). Both types contain osteons, the osteons of cortical bone contain haversian canals surrounding the matrix of lamellae. Within the rings of this matrix lay the lacunae, spaces containing mature bone cells known as osteocytes. In cortical bone these osteons are tightly packed producing a dense, solid structure with a porosity of 3.5% (Renders et al, 2007). In cancellous bone, osteons are arranged in a honeycomb pattern, comprised of trabeculae plates and rods. The lacuna of cancellous bone contain osteocytes and are semilunar in shape. This structure, though not tightly packed, still provides strength, and has an average porosity of 79.3% (Renders et al, 2007). These porosity differences are expected to affect the rate of diagenesis.

Another characteristic of bone that must be considered in the selection of skeletal features to test, is remodeling rates. Cortical bone has a turnover rate of 3% per year, while the cancellous bone is found to have a 26% turnover rate per year (Price, 2000). Bone must undergo remodeling as an adaptation to changing biomechanical forces, including the removal of microdamaged portions to increase integrity and strength and maintain mineral homeostasis (Clarke, 2008). The microdamage is eliminated by resorption of old bone and the formation of new bone, a process carried out by osteoclasts and osteoblasts. This process is continuous throughout an individual’s lifetime but not at a continuous rate. Perimenopausal and early postmenopausal biological females have an increased rate of remodeling that slows with aging but at a faster rate than those who are premenopausal. Biological males demonstrate a mild increase in bone remodeling with increasing age (Clark, 2008). The measured level of bioavailable Sr will be dependent upon how long the bone has been present, incorporating the Sr isotopes. Therefore, the remodeling rate is essential to consider when determining the time frame of analysis and the data being extrapolated.

Diagenesis

During an individual's lifetime the hydroxyapatite of bone participates in chemical exchange, usually by substitution with elements present in the body. This process allows bone to aid in the storage of minerals (Yadav et al. 2016). These changes in a living human are referred to as biogenesis. This process of chemical exchange persists after death with the externally available minerals found in precipitation, soil, and water that the bones are exposed to (Barelink & Chesson, 2018). These changes in a non-living individual are referred to as diagenesis. Diagenesis is problematic when performing a Sr and other isotope analysis of bone. The mineral exchange with external factors can alter the accuracy of the results when seeking to determine the isotopic concentrations that accumulated during life. These after life alterations are less of a factor in forensic research when remains have been recovered within a few days to months after death. Human skeletal tissue is not susceptible to drastic diagenetic alterations within a short time frame (Barelink & Chesson, 2018). However, not all forensically significant remains (within 50 years of death) are recovered in this short period of time. Diagenesis is a factor that must be considered and controlled for by also testing for additional trace elemental levels present in the sample. The current literature establishes an expected value for historically significant remains but has failed to provide such values for forensically significant bone samples. However, there is data that provides these values for tooth enamel of forensically significant teeth (Kamenov et al., 2018).

Forensic Case Study

Despite the need for further end-of-life strontium research and the lack of a standard skeletal feature for such analysis, isotopic research within the forensic realm of anthropology has gained traction. A combination of multiple isotopes were used in a cold case from 1971 involving a young biologically female victim referred to as "Little Miss Lake Panasoffkee" (Kamenov et al., 2014). She was discovered floating in a river by two men walking across the 1-75 bridge in Sumter County, Florida. A traditional biological profile was performed by a forensic anthropologist from the University of Florida in 1986. This profile provided valuable information but did not result in a positive identification, leaving this case to remain unsolved. In 2009 the Tampa Bay Cold Case Project began a further investigation in an attempt to identify the unidentified woman. Using Lead (Pb) and Strontium (Sr) isotopes, it was determined that this individual showed levels consistent with someone who spent their childhood in Greece (Kamenov et al., 2014). This new discovery has not led to a positive identification but did provide valuable information, such as the determination that this was not an individual born in the United States. This information also narrowed down a region of origin and a location to begin looking for missing person reports and familial ties for DNA analysis.

Similar research has been done to assist in the identification of undocumented individuals who have died crossing the United States-Mexican border. In 2008 preliminary research was performed analyzing the Sr levels found in the tooth enamel of 19 living Mexican-born individuals. This research determined that Sr could be used to narrow down origin into three regions of Mexico (Juarez, 2008). The preliminary research of this study suggests that Sr can be used to help identify where an individual may have resided prior to their entrance into the United States.

MATERIALS AND METHODS

Sample Background

The sample for this research included a single, biological male individual that is housed at The University of Montana Forensic Anthropology Lab (UMFAL). The background and residence information for this individual was provided by an autopsy report conducted by the Montana Department of Justice, Forensic Science Division, past dental records from Chester, MT and a familial account of life history, provided by a special deputy to the Missoula Country Sheriff's Office. This information indicated that the early years of life were spent in the Galata region of Montana. Later in life he moved to the area of Hammond, IN, and there is evidence of him being in Indianapolis, IN area in 2000. It is believed this individual moved to the Missoula, MT area in the early 2000's until his death in 2018. The remains were recovered in the Clark Fork River in Missoula, MT in 2018.

Skeletal Feature Selection and Extraction

Skeletal material was extracted in the dedicated drilling room of the Snow Ancient DNA Lab at the University of Montana. The conditions of this lab provide clean extraction hoods with controlled and monitored humidity. These conditions minimize the cross contamination of samples and further diagenesis. Extraction sites were selected based on bone composition (Table 1).

Table 1: Bone composition of the selected skeletal features for analysis.

Skeletal Feature	Bone Composition
Enamel of M2	Carbonated calcium-deficient hydroxyapatite
Petrous	Cortical
Rib	Trabecular
Femur Epicondyle	Trabecular
Humerus Epicondyle	Trabecular
Lumbar Vertebral Body	Trabecular
Iliac Crest	Trabecular
Femur Diaphysis	Cortical
Humerus Diaphysis	Cortical
Parietal	Trabecular
Mandible	Cortical
Clavicle	Cortical

Prior to extraction no cleaning procedures of the skeletal remains were performed. This sample was previously used for DNA research. Those researchers reported cleaning the remains by soaking them in a 3.75% solution of sodium hypochlorite (bleach) for 15 minutes. The remains were then rinsed 3 times with distilled water and were left overnight in the drilling room of the Snow Ancient DNA Lab to dry.

Bone samples were extracted using two methods. The first method of creating bone powder is the technique that is commonly found in the literature (Jørkov et al. 2007) A second method of creating a bone core is less common but was found to be effective when extracting samples from cortical bone. This method creates bone powder that can be utilized in DNA analysis as well as a core sample that can be utilized for isotopic analysis. This method is less destructive due to the creation of a small single site of destruction that provides adequate material for multiple analysis.

Extraction of skeletal material creating powder was performed using a handheld Dremel with a 1mm dental burr drilling bit (Microscopy Dental, HP8) at speed 3. Powder was collected using a small weigh boat. Drill bits and weight boats were sterilized by a Spectronics Corporation SPECTROLINKER^{TM} XL-1500 UV Crosslinker. A new weigh boat was used for each sample to prevent cross contamination. The powder was transferred to a 2 mL collection tube that was sterilized in the above mentioned crosslinker. Weight in grams was measured using a US Solid scale and recorded on an excel spreadsheet. A minimum of 20-30 mg is required for isotopic analysis. Tubes were labeled with a black sharpie on both the lid and side of the tube and secured with tape. The labeled collection tubes were placed in a plastic bag that contained the same label, written with black sharpie. The labels used for the test tubes and plastic bags contained the first two or three letters of the skeletal feature and the number 1, indicating this was the first extraction.

Extraction of the core samples were created using the above procedure with the exception of the Dremel attachment. For the coring of the sample, a 3/16-inch diamond core bit commonly used for glass and tile was used (Figure 2). Excess powder was also collected and utilized for the purpose of DNA analysis in a separate project.

Figure 2: Set of diamond core bits. Arrow indicating the selected size used in the coring method.

Collection of Control Samples

The background information provided suggested this individual spent their early years of life residing in the rural community of Galata, MT (48°28’32”N 111°21’08”W). To develop a control sample for the enamel and petrous portion of the temporal bone, a sample of wood was collected in the town of Galata by removing a branch from a tree. A sample of 7.62 cm-10.16 cm with a 1.27 cm diameter is required to perform an isotopic analysis. A larger sample, 19.05 cm with a 12.70 cm diameter was collected to allow for areas of exposure and possible diagenesis to be removed.

Table 2: Sample Selection: The location and amount of material prepared for analysis.

LABEL	MATERIAL	Amount*	Bone	Side	Location
H_20 A	Water	42.5			Clark Fork River
H_20 B	Water	50.0			Clark Fork River
M1 W1	Wood	19.69 x 20.32			Missoula
GA W1	Wood	19.05 x 12.7			Galata
En 1	Powder	0.092	M2	Right	Enamel
Pet 1	Powder	0.049	Petrous	Left	
Rib 1	Powder	0.012	Rib	Left	Thoracic/Shaft
Lum 1	Powder	0.152	Lumbar Vertebra		Body
IC 1	Powder	0.15	Iliac Crest	Left	Anterior/Superior Iliac Spine
PA 1	Core	0.033	Parietal	Left	Middle
MA 1	Core	0.098	Mandible	Left	Inferior to oblique line of body
CL 1	Core	0.031	Clavicle	Left	Near Subclavian Grove
FE (E) 1	Powder	0.100	Femur	Left	Proximal End
HU (E) 1	Powder	0.137	Humerus	Left	Proximal End
FE (D) 1	Core	0.101	Femur	Left	Mid Shaft
HU (D) 1	Core	0.083	Humerus	Left	Mid Shaft

* Volume was measured in mL, length and width was measured in cm, and weight was measured in g.

The remains of this individual were found in the Clark Fork River in Missoula, MT (46°52’57”N 114°2’21”W). A wood and water sample was collected from this area to provide a control to demonstrate possible diagenesis, or contamination of the remains. A sample of wood was collected from a tree on the river’s edge. This sample measured 19.69 cm with a diameter of 20.32 cm. A water sample was collected in two 50 mL tubes. Due to large levels of ice and snow build-up at the river’s edge, this sample was collected by adhering the tubes to a long branch with duct tape and string to allow for the tube to reach the area of running river water. This setup is best described as resembling a fishing pole and motions similar to casting were used to reach the desired water for sampling. Tube A contained 42.5 mL of water and Tube B contained 50 mL of water. All wood from Galata, Missoula, and the Clark Fork River water samples followed the same procedures as the skeletal samples by being labeled, secured, and placed in a separate plastic bags. All sample materials are provided in Table 2.

Isotopic Analysis

All collected samples were sent to the ICP-MS Metals and Strontium Isotopes Lab in the Department of Geology and Geophysics at the University of Utah. A Thermo-Fisher Scientific Neptune MC Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine the metallic composition of both liquid and solid samples. ICP-MS provides one of the most sensitive analytical readings. Due to the high plasma temperature, all chemical bonds are broken, creating cations and providing a reading of total elemental concentration. All samples were analyzed for the following trace elements, $^{87}Sr/^{86}Sr$ ratio, Magnesium (Mg), Aluminum (Al), Calcium (Ca), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Strontium (Sr), Cerium (Ce), and Lead (^{208}Pb). The levels of these trace elements were reported in milligram/kilogram (mg/kg) (equivalent to parts per million, ppm).

Acid leaching of the samples was carried out by the lab as a cleaning measure. This was done with a 10% HCl solution contained in a large plastic capped container. These containers were filled leaving ~10% volume headspace. Samples were then placed in a Ziplock bag. The bags were contained on a Pyrex tray and placed in the oven at 65^{o} C for one day. The bags were then turned over and left in the oven for a second day. The containers cooled down to room temperature, and the 10% HCL solution was removed.

Elemental solid samples (bone, enamel, and wood) were then prepared using laser ablation, altering the material to an aerosol form needed for analysis by the ICP-MS. Solid samples were prepared on Petrographic slides (25x50 mm) and 25 mm diameter round mounts that were loaded in the laser ablation cell. The standards used for this process and ICP-MS was USGS MACS-3 (calcium carbonate).

The water samples were standardized using the NIST 163f standard as well as the NIST 987 (strontium carbonate) and USGS EN-1 (carbonate rock) for the analysis of the $^{87}Sr/^{86}Sr$ ratio of aqueous solutions.

These procedural steps were extracted from the website for the Department of Geology & Geophysics Strontium Lab at the University of Utah (https://earth.utah.edu/research_facilities/earth-core-facility/icp-ms.php).

RESULTS

The results from the isotopic analysis from the University of Utah were returned to the researcher via email, in spreadsheet form. The data has been divided into the following two tables (Table 3 & 5). All analyses were performed using expected ranges for the geographical locations provided by known residential and migration patterns of the individual.

Table 3: Measured Bioavailable $^{87}Sr/^{86}SR$

Sample	$^{87}Sr/^{86}Sr$	SE(1s)
Water	0.71293	0.00001
Water	0.71293	0.00001
Wood	0.71544	0.00001
Wood	0.70870	0.00002
M2	0.70940	0.00001
Petrous	0.70954	0.00001
Rib	0.71042	0.00001
Lumbar Vertebral body	0.71110	0.00001
Iliac Crest	0.71119	0.00001
Parietal	0.70975	0.00001
Mandible	0.71035	0.00001
Clavicle	0.71019	0.00001
Femoral epiphysis	0.71053	0.00001
Humeral epiphysis	0.71061	0.00001
Femoral diaphysis	0.70972	0.00001
Humeral diaphysis	0.70964	0.00001

Analysis Using Expected Ranges from a Bedrock/Water Isoscape

The data was compared to the expected $^{87}Sr/^{86}Sr$ ranges found in an Isoscape map of the United States of America (Figure 3). This Isoscape map was created by previously measured $^{87}Sr/^{86}Sr$ bedrock and water levels (Bataille & Bowen, 2012; Chesson et al., 2012). The measured levels of this research sample were deemed consistent if the measured value fell in the expected ranges provided by the Isoscape Map (Figure 3). The geographical locations chosen for comparison were Galata, MT., Missoula, MT., Hammond, IN., and Indianapolis, IN.

It was found that the Galata, MT wood sample, enamel of M2, and the petrous were consistent with the expected range of Galata, MT. The rib sample was not consistent with any expected ranges of the selected geographical locations. The measured values of the lumbar vertebra, iliac crest, parietal, mastoid, clavicle, femoral epiphysis, humeral epiphysis, femoral diaphysis, and humeral diaphysis samples were all consistent with the expected ranges of Indianapolis, IN and Hammond, IN.

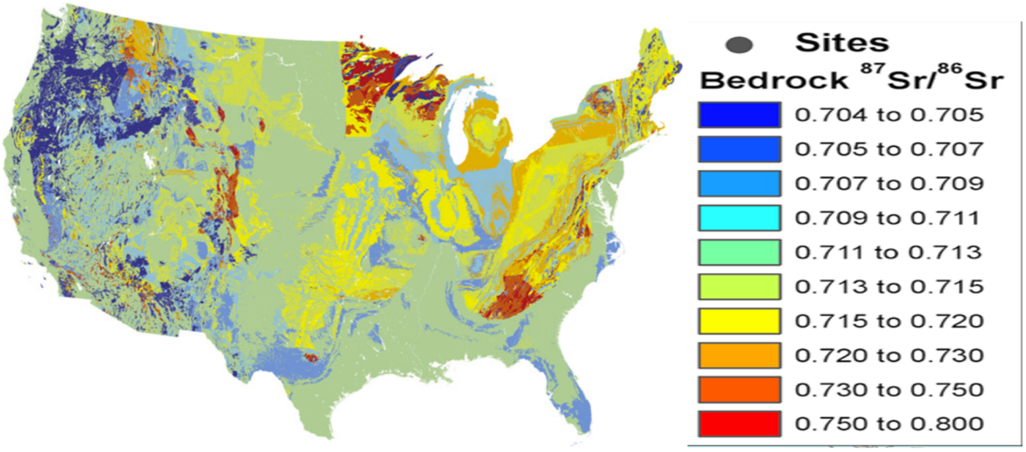

Figure 3: An Isoscape map of the United States of America: based on measured bedrock and water ratios of ^{87}Sr/^{86}Sr (Bataille & Bowen, 2012).

Analysis Using Expected Ranges from an Isoscape with Multiple Variables

The measured bioavailable ^{87}Sr/^{86}Sr was analyzed using expected ranges from an Isoscape that included the previously used bedrock and water levels with the addition of measured tap water levels and the bedrock models. All models were combined to provide a range of expected values (Table 4). The geographical locations of this study varied slightly from those previously used due to the data presented (Chesson et al., 2012). Cut

Bank, MT was selected in place of Galata, MT based on the close proximity. Dillon, MT was selected in place of Missoula, MT due to the close proximity, as well as the proximity to the Bitterroot Mountain Range. Bloomington, IN was selected in place of Indianapolis, IN based on the close proximity and similar latitude. Chicago, IL was selected in place of Hammond, IN due to the close proximity. It was found that the Galata, MT wood sample, enamel of M2, and petrous were consistent with the expected ranges of Cut Bank, MT The rib, parietal, mastoid, mandible, clavicle, femoral diaphysis, and humeral diaphysis were all consistent with the expected ranges of Dillion, MT, Bloomington, IN, and Chicago, IL. The lumbar vertebra, femur epiphysis, and the humeral epiphysis were consistent with the expected ranges of Dillion, MT and Bloomington, IN. The iliac crest was consistent with the expected range for Bloomington, IN.

Table 4: Expected ranges developed from four methods of $^{87}Sr/^{86}Sr$ measurements: measured tap water, bedrock model, major bedrock model, and local water model (Chesson et al., 2012).

Geographical Location	**Expected Range of $^{87}Sr/^{86}Sr$ (ppm)**
Cut Bank, MT	**0.7059 - 0.7129**
Dillon, MT	**0.7049 - 0.7113**
Bloomington, IN	**0.7089 - 0.7136**
Chicago, IL	**0.7091 - 0.7104**

Analysis Using Control Samples as Expected Values

It was found that the enamel of M2, petrous, rib, and parietal are consistent with the wood sample collected from Galata, MT. The lumbar and iliac crest are consistent with the wood and water samples of the Missoula, MT sample. No other skeletal feature analyzed was found to be consistent with either geographical location.

Analysis of Possible Diagenesis

The isotopic analysis performed at the University of Utah tested for a lead (^{208}Pb) isotope and trace elements (Table 5). The additional analysis was performed to indicate possible diagenesis. Previous literature provides normal ranges of these metals in the enamel of a modern population (Kamenov et al., 2018). The tested levels in this sample were compared to the published expected ranges. The sample for this research was determined to be consistent, if the measured value fell into the expected range. A measured value outside of the expected range could suggest diagenesis and that the elevated trace elemental signal is from external contamination (Table 6). It was found that the measured values of this sample were consistent with the expected ranges for Ca, Fe, Cu, Zn, Sr, and ^{208}Pb. The measured values did not fall into the expected range for Mg, Mn, and Ce.

Table 5: Measured Trace Elements of Sample

Label	Material	Weight	Side	Area	Mg	Al	Ca	Mn	Fe	Cu	Zn	Sr	Ce	208 Pb
H2O A	Water	42.5mL			11.7	0.009	40	0.0033	0.016	0.004	<0.02	0.2	0.000029	0.000139
H2O B	Water	50mL			9.2	0.023	39	0.0033	0.016	0.002	<0.02	0.2	0.000026	0.000112
M1 W1	Wood	7.75x.8(in)			134.1	0.1	1722	2.4530	1.7	0.2	14.7	4.9	0.000489971	0.01
GA W1	Wood	7.5x.5(in)			42.4	1.0	96	0.1095	0.7	0.4	<0.3	1.5	0.001051197	0.01
En 1	Powder	0.092	right	M2	4328.9	8.7	440527	6.5	16.4	1.1	251.0	114.2	0.052	4.64
Pet 1	Powder	0.049	left		5217.4	54.4	347704	7.7	92.0	1.3	204.3	83.2	0.289	6.39
Rib 1	Powder	0.012	left	Thoracic	2973.17	<9	258944	<0.7	92.7	0.7	113.1	57.6	0.407	0.49
Lum 1	Powder	0.152	right	Body	11381.80328	17.3	331641	<0.7	215.9	0.3	171.3	71.0	0.999	0.65
IC 1	Powder	0.15	left	Anterior/Superior Iliac Spine	8658.4	28.5	386851	1.8	178.6	0.7	180.8	83.3	1.109	0.68
PA 1	Core	0.033	left	Middle	5340.7	<8	384058	3.3	62.2	1.0	135.2	80.6	0.701	1.86
MA 1	Core	0.098	left	inferior to oblique line of body	7513.1	<13	494541	2.2	18.3	0.5	190.1	116.0	0.364	1.37
CL 1	Core	0.031	left	near subclavian groove	4561.0	<9	356454	<0.8	83.0	0.7	126.0	74.2	0.820	0.86
Fe (e) 1	Powder	0.1	left	proximal end	4578.8	21.7	304751	<0.8	34.7	0.5	141.1	60.9	0.581	0.61
Hu (e) 1	Powder	0.137	left	proximal end	5346.6	48.5	327827	<0.8	326.9	1.0	168.4	63.1	1.076	0.95
Fe (d) 1	Core	0.101	left	mid shaft	8459.1	<9	586695	<0.8	10.9	0.6	211.9	116.1	0.723	1.60
Hu (d) 1	Core	0.083	left	mid shaft	6241.1	<9	461673	<0.8	7.6	0.3	147.6	89.2	0.476	1.44

Table 6: Metal Averages of Modern Tooth Enamel Compared to Measured Sample Levels (Kamenov et al., 2018).

Sample	Mg	Ca	Mn	Fe	Cu	Zn	Sr	Ce	^{208}Pb
Expected Range	**3075 +/- 623**	**391417 +/- 18230**	**1.95 +/- 1.72**	**15 +/- 19**	**3.6 +/-7.4**	**215 +/-77**	**168 +/- 178**	**0.010 +/- 0.015**	**6.55 +/- 8.6**
Measured Value	**4328.9**	**440527**	**6.5**	**16.4**	**1.1**	**251.0**	**114.2**	**0.052**	**4.64**
Consistent with Expected Range		*		*	*	*	*		*

* indicates measured sample levels falling within the normal range of modern tooth enamel

DISCUSSION

The results of this research suggest that the petrous portion of the temporal bone would provide similar $^{87}Sr/^{86}Sr$ isotopic signals as tooth enamel of the second molar. The measured $^{87}Sr/^{86}Sr$ concentration of this sample fell within a Sr range that would indicate the same early life geographical residence. The results of the petrous and enamel fell in the expected range generated from the bedrock Isoscape, the expected ranges of the Isoscape including tap water, and the wood sample collected from Galata, MT. This data would support the acceptance of the hypothesis (H1) that the petrous portion of the temporal bone is as effective as tooth enamel in identifying an individual's early life residence.

The hypothesis (H2), the diaphysis of long bones will provide the most accurate end-of-life data when performing a strontium isotopic analysis, was not supported by this research. The diaphysis of long bones was hypothesized to be the most accurate due to the slower rate of cortical bone remodeling and the ability to resist diagenesis. The result of this research concluded that the measured $^{87}Sr/^{86}Sr$ ratio of the femoral and humeral diaphysis did not provide a distinct signal from all other skeletal features. Without a distinct signal and significant level of superior accuracy, this hypothesis is not accepted. Further research could indicate the usability of the diaphysis of long bones to provide end-of-life residence. However, at this time there is no indication that these features are more useful than others.

The third hypothesis of this research was developed to explore skeletal features that undergo increased rates of diagenesis. It was hypothesized that cancellous bone will demonstrate higher levels of diagenesis when compared to cortical bone. There is supporting evidence found in this research. The lumbar vertebra and iliac crest both had the most similar measured $^{87}Sr/^{86}Sr$ ratios to both the Clark Fork River sample and the Missoula, MT wood sample. Based on the recovery report of these remains, it is believed that these skeletal features underwent a longer exposure to the Clark Fork River. The combination of similar measured $^{87}Sr/^{86}Sr$ ratio and the water exposer, it is believed that these cancellous skeletal features demonstrate the highest level of diagenesis of this sample. Hypothesis 3 is accepted.

The coring method utilized in this research in the extraction of cortical bone was deemed successful and accurate. This method was able to produce sufficient sample material for both an isotopic and DNA analysis, while producing one small site of destruction. These factors suggest the coring method should be further validated and implemented in the extraction of cortical bone.

The results of this research must be evaluated within the context of a preliminary study. All findings require further analysis, testing these hypotheses with a larger sample size and such variables as age and biological sex. The ideal sample will also contain a detailed and accurate account of the individual residence patterns.

CONCLUSION

The anthropological literature demonstrates a growing interest and utilization of isotopic analysis. Currently, there is ample research in the sub-field of archeology using tooth enamel and the role diagenesis plays in ancient remains. The same standard skeletal feature for end-of-life residence has not been accepted for forensic casework. Such a standard would allow isotopic analysis in forensically modern remains produce useful and accurate results. In order to develop this standard, a better understanding of the rate of diagenesis is needed. Little is known about short term rates of diagenesis in environments where remains are in direct contact with soil or natural water features. This research also suggests that the petrous is an adequate alternative to tooth enamel for early-life residence. This data would benefit multiple sub-fields of anthropology. Though hypotheses 1 and 3 were supported in this research, further investigation must be performed to fully support these findings. The same holds true for the rejection of hypothesis 2.

LIMITATIONS

The limitations of this research include the small sample size of a single individual. To fully accept these findings in developing an anthropological standard skeletal feature for end-of-life isotopic analysis, these results must be consistent when analyzing a much larger sample. The same holds true for the use of the petrous in place of tooth enamel when analyzing early life residence. Due to this small sample size, this research is considered preliminary work in the field of anthropology.

The limitation found in the isotopic analysis was the need to send samples to a lab outside of the University of Montana. This limitation diminished the hands-on experience and control of sample analysis. Though collaboration is encouraged and acceptable, the actual analysis being performed by the researcher would be beneficial to the overall project.

Though life history information was provided, it was general in nature. A specific timeline of an individual's residence and migration pattern would help to provide a more accurate comparison of isotopic levels.

FUTURE RESEARCH

As previously mentioned, this is preliminary research in determining a skeletal feature to be used for end-of-life isotopic analysis as well as determining the accurate utilization of the petrous in place of tooth enamel. Future research should be done using a larger sample size and include individuals of different sexes and ages. In addition, individuals included in the larger sample should contain specific information pertaining to their life history.

Extracting bone using the core method in place of powder or chunking should be repeated with a larger sample to confirm the benefits of replacing these commonly used methods. This method has evidence of being superior, especially in situations where isotopic analysis is coupled with DNA.

BROADER IMPACTS

According to namus.gov, as of January 2022, there are currently 21,397 unidentified individuals in the United States of America. Isotopic analysis is a tool that can be utilized when biological profiles and DNA have not proven successful. The georeferencing of the unidentified individuals can provide a geological location of birth and end-of-life residence. This information can direct those tasked with identification to a location to begin looking through missing person reports and locating familial samples for DNA analysis.

The use of human georeferencing has shown especially useful in individuals that reside in the countries of South and Central America (Juarez, 2008). Strontium isotopic analysis could be applied to the overwhelming number of unidentified individuals who have perished crossing the United States Southern border.

The methods are also beneficial to bioarcheologists looking to research or revisit previous research on migration patterns when tooth enamel was not available. This also allows for research questions in this field to be asked that may have previously been unable to be answered. This greatly broadens our understanding of past populations.

The field of anthropology seeks to conduct research with the least amount of destruction possible. The method of coring bone samples provides a less destructive form of extraction. Coring provides a method to perform multiple analyses with a single extraction. This increases the likelihood of permission for destructive analysis to be granted.

WORK CITED

Anders, Dominic, Amira Osmanovic, and Marina Vohberger. 2019. “Intra- and Inter-Individual Variability of Stable Strontium Isotope Ratios in Hard and Soft Body Tissues of Pigs.” *Rapid Communications in Mass Spectrometry* 33 (3): 281–90. https://doi.org/10.1002/rcm.8350.

Bataille, Clément P., and Gabriel J. Bowen. 2012. “Mapping 87Sr/86Sr Variations in Bedrock and Water for Large Scale Provenance Studies.” *Chemical Geology* 304–305 (April): 39–52. https://doi.org/10.1016/j.chemgeo.2012.01.028.

Bataille, Clement P., Brooke E. Crowley, Matthew J. Wooller, and Gabriel J. Bowen. 2020. “Advances in Global Bioavailable Strontium Isoscapes.” *Palaeogeography, Palaeoclimatology, Palaeoecology* 555 (October): 109849. https://doi.org/10.1016/j.palaeo.2020.109849.

Bartelink, Eric J., and Lesley A. Chesson. 2019. “Recent Applications of Isotope Analysis to Forensic Anthropology.” *Forensic Sciences Research* 4 (1): 29–44. https://doi.org/10.1080/20961790.2018.1549527.

Bentley, R. Alexander. 2006. “Strontium Isotopes from the Earth to the Archaeological Skeleton: A Review.” *Journal of Archaeological Method and Theory* 13 (3): 135–87. https://www.jstor.org/stable/20177538.

Burton, James, and M. Anne Katzenberg. 2018. “Strontium Isotopes and the Chemistry of Bones and Teeth.” In *Biological Anthropology of the Human Skeleton*, 505–14. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119151647.ch15.

Cabrera, Walter E., Iris Schrooten, Marc E. De Broe, and Patrick C. D’Haese. 1999. “Strontium and Bone.” *Journal of Bone and Mineral Research* 14 (5): 661–68. https://doi.org/10.1359/jbmr.1999.14.5.661.

Chesson, Lesley A., Brett J. Tipple, Glen N. Mackey, Scott A. Hynek, Diego P. Fernandez, and James R. Ehleringer. 2012. “Strontium Isotopes in Tap Water from the Coterminous USA.” *Ecosphere* 3 (7): art67. https://doi.org/10.1890/ES12-00122.1.

Clarke, Bart. 2008. “Normal Bone Anatomy and Physiology.” *Clinical Journal of the American Society of Nephrology : CJASN* 3 (Suppl 3): S131–39. https://doi.org/10.2215/CJN.04151206.

Coelho, Inês, Isabel Castanheira, João Moura Bordado, Olivier Donard, and José Armando L. Silva. 2017. “Recent Developments and Trends in the Application of Strontium and Its Isotopes in Biological Related Fields.” *TrAC Trends in Analytical Chemistry* 90 (May): 45–61. https://doi.org/10.1016/j.trac.2017.02.005.

Dahl, S. G, P Allain, P. J Marie, Y Mauras, G Boivin, P Ammann, Y Tsouderos, P. D Delmas, and C Christiansen. 2001. "Incorporation and Distribution of Strontium in Bone." *Bone* 28 (4): 446–53. https://doi.org/10.1016/S8756-3282(01)00419-7.

Ericson, Jonathon E. 1985. "Strontium Isotope Characterization in the Study of Prehistoric Human Ecology." *Journal of Human Evolution* 14 (5): 503–14. https://doi.org/10.1016/S0047-2484(85)80029-4.

Jørkov, Marie Louise S., Jan Heinemeier, and Niels Lynnerup. 2007. "Evaluating Bone Collagen Extraction Methods for Stable Isotope Analysis in Dietary Studies." *Journal of Archaeological Science* 34 (11): 1824–29. https://doi.org/10.1016/j.jas.2006.12.020.

Juarez, Chelsey A. 2008. "Strontium and Geolocation, the Pathway to Identification for Deceased Undocumented Mexican Border-Crossers: A Preliminary Report*." *Journal of Forensic Sciences* 53 (1): 46–49. https://doi.org/10.1111/j.1556-4029.2007.00610.x.

Kamenov, George D., Erin H. Kimmerle, Jason H. Curtis, and Darren Norris. 2014. "Georeferencing a Cold Case Victim with Lead, Strontium, Carbon, and Oxygen Isotopes." *Annals of Anthropological Practice* 38 (1): 137–54. https://doi.org/10.1111/napa.12048.

Kamenov, George D., Ellen M. Lofaro, Gennifer Goad, and John Krigbaum. 2018. "Trace Elements in Modern and Archaeological Human Teeth: Implications for Human Metal Exposure and Enamel Diagenetic Changes." *Journal of Archaeological Science* 99 (November): 27–34. https://doi.org/10.1016/j.jas.2018.09.002.

Laffoon, Jason E., Gareth R. Davies, Menno L. P. Hoogland, and Corinne L. Hofman. 2012. "Spatial Variation of Biologically Available Strontium Isotopes (87Sr/86Sr) in an Archipelagic Setting: A Case Study from the Caribbean." *Journal of Archaeological Science* 39 (7): 2371–84. https://doi.org/10.1016/j.jas.2012.02.002.

Namus.2022."Reports&Statistics". https://namus.nij.ojp.gov/library/reports-and-statistics

Pinhasi, Ron, Daniel Fernandes, Kendra Sirak, Mario Novak, Sarah Connell, Songül Alpaslan-Roodenberg, Fokke Gerritsen, et al. 2015. "Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone." *PloS One* 10 (6): e0129102. https://doi.org/10.1371/journal.pone.0129102.

Pinhasi, Ron, Daniel M. Fernandes, Kendra Sirak, and Olivia Cheronet. 2019. "Isolating the Human Cochlea to Generate Bone Powder for Ancient DNA Analysis." *Nature Protocols* 14 (4): 1194–1205. https://doi.org/10.1038/s41596-019-0137-7.

Pors Nielsen, S. 2004. "The Biological Role of Strontium." *Bone* 35 (3): 583–88. https://doi.org/10.1016/j.bone.2004.04.026.

Price, T. Douglas. n.d. “Immigration and the Ancient City of Teotihuacan in Mexico: A Study Using Strontium Isotope Ratios in Human Bone and Teeth | Elsevier Enhanced Reader.” Accessed January 19, 2022. https://doi.org/10.1006/jasc.1999.0504.

Renders, G A P, L Mulder, L J van Ruijven, and T M G J van Eijden. 2007. “Porosity of Human Mandibular Condylar Bone.” *Journal of Anatomy* 210 (3): 239–48. https://doi.org/10.1111/j.1469-7580.2007.00693.x.

University of Utah, 2021. “ICP-MS Metals and Strontium Isotope Facility” https://earth.utah.edu/research_facilities/earth-core-facility/icp-ms.php

Waterman, Anna J., David W. Peate, Ana Maria Silva, and Jonathan T. Thomas. 2014. “In Search of Homelands: Using Strontium Isotopes to Identify Biological Markers of Mobility in Late Prehistoric Portugal.” *Journal of Archaeological Science* 42 (February): 119–27. https://doi.org/10.1016/j.jas.2013.11.004.

Yadav, Manisha C, Massimo Bottini, Esther Cory, Kunal Bhattacharya, Pia Kuss, Sonoko Narisawa, Robert L Sah, et al. 2016. “Skeletal Mineralization Deficits and Impaired Biogenesis and Function of Chondrocyte-Derived Matrix Vesicles in Phospho1–/– and Phospho1/Pit1 Double-Knockout Mice.” *Journal of Bone and Mineral Research* 31 (6): 1275–86. https://doi.org/10.1002/jbmr.2790.