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Testing the Iron Deficiency Anemia Hypothesis using p-XRF

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McNair Scholars Program

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Acknowledgements: With great appreciation, I would like to thank the 2018 and 2019 McNair
Scholars cohorts for their countless support

Summary:

Problem / Gap: Within the discipline of bioarchaeology, research has both supported the hypothesis that iron deficiency anemia (IDA) causes porotic hyperostosis (PH) and cribra orbitalia (CO) (Faccini et al., 2004; Macadam 1985, 1987, 1989; Oxenham & Cavil 2010), as well as rebutted it (Rothschild 2012; Wapler et al. 2003; Walker et al., 2009; Zaringa et al., 2016). As a whole, this hypothesis of causation between IDA, CO, and PH is what we will refer to as the iron-deficiency anemia hypothesis. CO and PH are two pathological conditions that are identified by porosities along different areas of the human skull, CO on the upper eye orbit, and PH on the skull vault. Both conditions are exclusively found to be active in younger individuals, while porosities which show healing are typically found in adults (Walker et al, 2009; Steyn et al, 2016). Results of various studies on the iron deficiency anemia hypothesis have often been contradictory. For example, as discussed later on, Oxenham & Cavil 2010 directly refute Walker et al., 2009's conclusion that IDA cannot possibly cause PH and CO. The answer to what causes these two conditions is not clear, and in large part, the research done looking into the etiology of the lesions associated with CO and PH is done by bioarchaeologist, their predecessors, and people in related fields, rather than by medical professionals. Both Oxenham & Cavil 2010 as well as Walker et al., 2009 cite medical literature when making their cases, but future research done by medical researchers is necessary to shed light on this question, especially considering the technologically equipped nature of the field. Past research trying to address the question of the relationship between IDA and CO/PH has used destructive and time-consuming methods like spectrometry and isotope analysis to determine element concentration differences. For example, Wapler et al. (2003) had to take a sample from each skull, which was refilled with plaster. Even when remains are available to be destructively analyzed, if they are able to be used in a study

will depend on several factors including size, so only those that are still intact to a certain degree can be analyzed by current methods (Facchini, 2004; Nagaoka, 2017).

Purpose: This research will assess the IDA hypothesis as an explanation for PH and CO. Our primary concern is determining whether differences in iron concentration exists between skulls with and without CO / PH. If assumed that the IDA hypothesis is correct, we expect to see lower iron concentrations in skulls showing signs of CO and PH and higher concentrations in those without. This research will apply portable X-ray fluorescence (p-XRF) to a question within bioarchaeology previously untested by this specific technology. Regardless of the results, we will supply data on whether or not p-XRF can be useful when assessing the IDA hypothesis in an attempt to close the gap of knowledge. Because p-XRF has not been used in the past to test the IDA hypothesis, we will be testing the applicability of this method for the first time. Given its positive characteristics (nondestructive, rapid data collection, portable, relatively inexpensive), this research hypothesizes that recommendations will be made on behalf of the future use of p-XRF in addressing the IDA hypothesis. The results generated through this research will exemplify the broad spectrum of results capable of being produced by p-XRF that is unseen in previous research trying to address the IDA hypothesis.

Significance and Justification: The debate on whether IDA can directly cause CO and PH is still active and controversial within the field of bioarchaeology. Determining whether there is a direct link between the two is a matter of importance because of the implications these results can have on the credibility of research done citing IDA as a cause for CO / PH, and those conditions as an identifier of the life health challenges an individual would have faced as hypothesized by the IDA hypothesis, and as discussed shortly. The results of this research will impact our capability to analyze the health of skeletal remains. Analyzing PH and CO as they

relate to the IDA hypothesis can provide us a fantastic insight into the life history of past populations, provided that p-XRF is an effective methodological approach. By analyzing the health status of individuals, we can infer information about their social class, social stressors, environmental stressors, diet, health during childhood, and many more factors that can give us an insight into the lives of people in archaeological contexts. Therefore, methods used to understand life history need to be trusted within the field of anthropology, as well as in this specific bioarchaeological context. Also, understanding the development of disease by analyzing the past helps us learn about factors that can contribute to our own health today. As discussed later, IDA is a prevalent condition that still impacts much of the world's population.

P-XRF could prove to be an immensely powerful tool in a bioarchaeological context. I expect a greater openness to research conducted with p-XRF, given its positive characteristics (nondestructive, rapid data collection, portable, relatively inexpensive), when compared to destructive methods currently used. P-XRF's positive characteristics should make institutions holding skeletal collections that could be useful for testing bioarchaeological hypotheses to be more amenable to providing access to their collections, subsequently allowing for an expansion of data sets by incorporating collections previously untouched due to their sensitivity. Aside from testing the validity of this new method, our main interest lies in determining whether the p-XRF shows support for the iron-deficiency anemia hypothesis. Given the conflicting evidence related to the IDA hypothesis, our research may help settle the debate.

Background: From Red Blood Cell to Lesion

Even in our modern age of medicine, iron deficiency anemia is still prevalent, affecting over a quarter of the world's human population and is the top cause of anemia worldwide

(Camaschella, 2015). The development of bony lesions such as cribra orbitalia and porotic hyperostosis is not something that happens overnight. These bony lesions develop as a result of being born with a genetic condition like thalassemia major or sickle cell anemia; or lesions can also develop as the result of environmental factors (Cafey, 1937). Environmental factors that can lead to CO and PH include nutrient deficiency anemias, parasites, and eating foods that inhibit iron absorption in the intestine. When the absorption rate or ample amount of iron in the body is lacking, the body responds by releasing erythropoietin, a hormone. This hormone accelerates red blood cell production to combat hypoxia, or lack of oxygen, a characteristic of anemia (Fandrey, 2004). If this method fails, the body attempts to produce more red blood cells by expanding the bone marrow. In childhood, one of the regions that promote the most growth for red blood cells is the red bone marrow above the orbital socket, while in adulthood this area switches to the vertebrae, sternal, and coastal regions. (Hoffbrand and Lewis, 1981). Here depending on age, a process known as marrow hypertrophy will occur (Moseley et al., 1966). This process involves the expansion of the diploe, a spongy layer of bone that surround the bone marrow which produces red blood cells.

Once the diploe begins to expand, it will naturally reabsorb the areas surrounding the expansion, leading to what we see as CO of the orbital roof and PH of the skull vault. Anemia could be causing CO/PH in children, given that the cranial vault produces more red blood cells during this time in life (Hoffbrand and Lewis, 1981; Halvorsen and Bechensteen, 2002). The observation supports the shift in many cases showing lesions in children as active, with no healing typically found, while adults typically show CO/PH related healing (Walker, 1985). Many authors in the past (Britton et al., 1960; Burko et al., 1961; Eng 1958; Sheldon, 1936) have tied porotic hyperostosis and cribra orbitalia to iron deficiency anemia. However, more modern

analyses have suggested that iron deficiency anemia cannot sustain the number of red blood cells needed to cause marrow hypertrophy and consequently absorb the outer layers leading to the condition (Walker et al., 2009). These authors suggest that other nutrient deficiencies, such as deficiencies in vitamin B12 and folate, may be responsible for the marrow hypertrophy seen with CO and PH. Thus, the link between CO and PH and IDA is still unclear.

Literature Review:

The iron deficiency anemia hypothesis gained most significant traction in the mid to late twentieth century (Angel, 1966; El- Najjar et al., 1975; Macadam, 1985,1987, 1989), but the relationship between IDA and CO/PH has been questioned in recent years (Rothschild et al., 2012; Walker et al., 2009; Wapler et al., 2003). The first professional within the field of anthropology to be cited for their work linking iron deficiency to CO and PH was Angel (1966). Angel (1966) introduced the concept that genetically acquired anemias could not be the cause of PO and CO in the New World. His article was the first to hint at the probability of iron deficiency anemia as the cause. Macadam (1987) built on this hypothesis with research done using contemporary populations and medical technology. Macadam (1987) used radiographic technology to show the “hair on end” effect associated with CO and PH found in X-rays of living humans. The individuals used to conduct this research had iron deficiency anemia at the time. The claims made by Angel (1966) and Macadam (1987) were challenged by Walker and colleagues (2009), Rothschild (2012) and others, as discussed previously.

The most comprehensive argument against the hypothesis was made by Walker et al. (2009), which says that iron deficiency anemia prohibits the erythropoiesis necessary to expand the diploe enough to absorb the surrounding area. Walker et al. (2009) suggested that iron deficiency anemia cannot sustain the massive erythropoiesis needed to cause CO and PH. Given

this reasoning, Walker et al. (2009) argue that iron deficiency anemia cannot logically be linked to CO and PH. They go on to promote the hypothesis that megaloblastic anemias is one of the true probable culprits. The authors conclude that “iron deficiency anemia cannot sustain the massive red blood cell production that causes the marrow expansion responsible for these lesions” (Walker et al., 2009). Their main piece of evidence is anatomical studies showing that iron deficiency is minimal while B12 and folate deficiencies are more likely the culprits leading to CO and PH. The work of authors today challenging the idea that IDA causes CO and PH is what Rothschild (2012) considers combating a negatively implied “collective consciousness” within anthropology. I believe Rothschild (2012)’s quote goes to show just how seriously the rift is between opposing schools of thought.

Although Walker and colleague’s critique of the IDA hypothesis has gained acceptance in the field of bioarchaeology, there is still no definitive answer. Oxenham and Cavill (2010) argue that Walker et al. (2009) is citing literature incorrectly and that iron deficiency anemia is still a probable cause for PH and CO. Their reasoning behind this rebuttal is yes, in all cases leading up to PH and CO, such as in megaloblastic anemia, defective erythropoiesis exist, however, this would simultaneously lead us to conclude that all anemias producing defective erythrocytes (megaloblastic anemia included) could not be the cause of PH and CO. Thus, refuting the possibility that megaloblastic anemia, which is proposed by Walker and colleagues’ (2009) to be causing CO and PH, also could not be the cause.

Regardless of what eventual research will show us to be the etiology of cribra orbitalia and porotic hyperostosis, the iron-deficiency anemia hypothesis cannot be ruled out because of problems with hidden heterogeneity as proposed by McIlvaine (2015): individuals who in life were impacted by several anemias such as megaloblastic and iron deficiency anemia, since they

typically co-occur, would show no signs of CO and PH since IDA would inhibit lesion production regardless of megaloblastic anemia being present and attempting to expand the diploe as proposed by Walker et al. (2009). Therefore, the IDA hypothesis deserves further testing before being dismissed, given the complications that arise from its dismissal. We see that the articles discussed in this section go back and forth, relying on different interpretations of medical literature. By using medical literature to substantiate their claims in a manner that ultimately proved contradictory, the authors have opened up the question of the true nature of CO/PH for future researchers in the field of medicine.

As reviewed, research contradicts each other on the matter of whether IDA causes CO and PH. Further research will be needed given the importance of relying on this link to tell us about health in past populations. A direct link between anemia that is not genetically acquired, such as iron deficiency anemia and megaloblastic anemia, and CO / PH has not been established. Therefore, we seek to answer the question: do elemental concentrations measured via XRF technology support the iron-deficiency anemia hypothesis? By addressing this question, we will be adding data to the greater breadth of knowledge available to be used to solve the controversy surrounding the iron-deficiency anemia hypothesis.

Materials and Methods:

Portable X-ray fluorescence (p-XRF) is a handheld technology that uses an X-ray source to emit a photon. The photon emitted by the XRF knocks an electron out of its orbital shell when it encounters atoms in the sample. Each element in the periodic table releases a specific amount of electron volts of energy in the form of a second photon when this electron displacement happens. The p-XRF unit measures these returned photons, and from these spectral data elemental concentrations can be estimated. The photon counts from each electron volt energy

level are measured and translated into element parts per million concentrations through internally validated calibrations. The p-XRF requires a reference data set point to calibrate the measurements from the sample and quantify ppm element concentrations. In this case, the internal calibration “Mudrock Air” (Bruker Nano) was used. The “Mudrock Air” calibration was built using samples with physical consistency similar to bone, and that is why it makes a good reference point our dataset. Our primary independent variable is iron concentrations and other element concentrations found within our skull sample set.

Our sample data set includes 13 skulls housed within a collection at the University of Northern Colorado's Anthropology Department. Before taking element concentration measurements, I conducted a visual analysis to build the biological profiles of our individuals, as found in previous literature (Acsadi and Nemeskeri, 1970) (see fig. 2), as well as to determine the extent in which each skull showed evidence of CO and PH using methods presented by Macadam (1982). Biological profile building is used to learn information about individuals via their remains, including sex and age. Features looked at to determine the sex of each individual in the collection include the nuchal crest, mastoid process, orbital margin, glabella, and the mental eminence (in individuals that had a mandible associated with it) (see fig.1). All the features listed are rated on scales of one through five, with one characterizing the features most commonly found on remains that are biologically female. In the same regard, individuals falling with the range closer to five are more likely to be biologically male. All individuals had their sex estimated except for a child individual in the collection. Humans do not develop the markers used to determine sex until later in life.

Fig 1)Sex estimation reference material

Fig 2.) Age estimation based on dental eruption

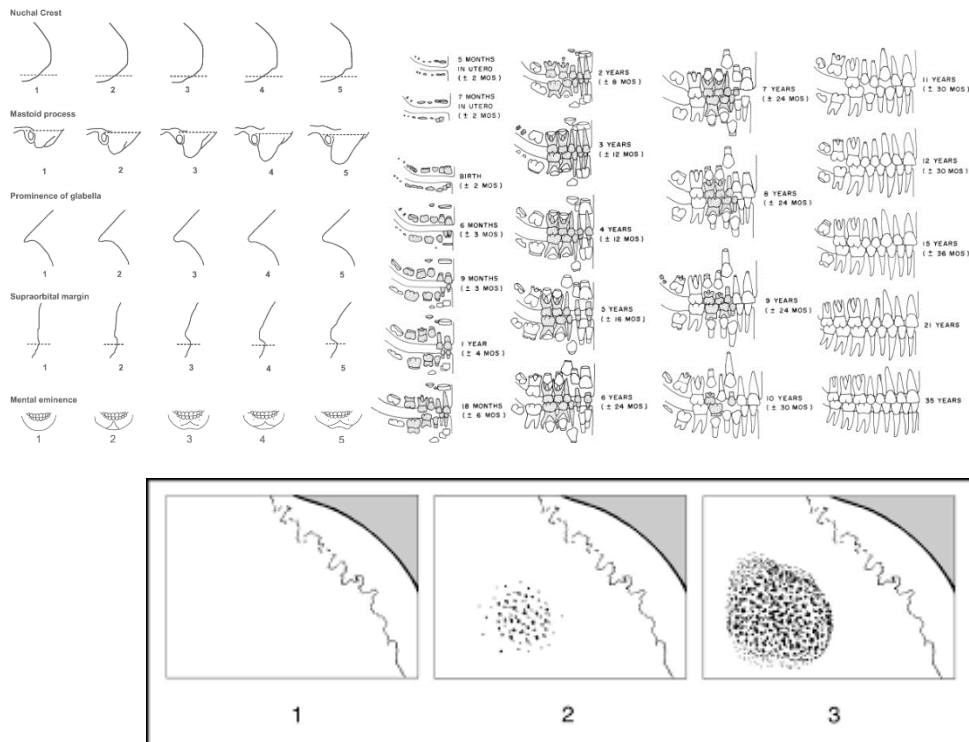


Fig 3.) Rating porotic hyperostosis severity reference

To determine the age of each individual, methods which include observing tooth development or eruption and cranial suture closures (Ubelaker, 1989 ; Mendel and Lovejoy, 1985) were used. Dental eruption in our individual's where compared to the standards put forward by Ubelaker, 1989, based on the idea that teeth erupt at various ages during development (see fig.2). This method is only able to confidently determine age up to 20 years of age because after that time the teeth of all adults have matured and erupted to their potential. In five individuals within our collection, we were unable to determine the age using tooth development because the mandible was missing. Thus, we relied exclusively on cranial suture closure.

To assess age using suture closures along the crania, Mendel and Lovejoy (1989) use 10 different sutures and give each a rating between 0 and 3, with zero indicating no suture closure and three signifying that the sutures have entirely fused. After these observations are generated for each suture, two separate calculations are used to come up with a composite score which is

then associated with ages estimated by Mendel and Lovejoy (1989). After these calculations are done, we are given an age range estimate, with an associated standard deviation.

Once age and sex were determined, I examined each skull for presence of CO and PH. PH and CO are both rated on a scale of 1-3, with 3 being a significant case, and 1 showing no signs (see fig. 3). PH is observed along the parietal bone and surrounding area, while CO is observed in the upper eye orbit. The observer is looking for signs of porosities. It is important to ensure that damage and contamination are not mistaken for porosities associated with these two conditions. For example, if an area along the parietal bone of an individual showed what appeared to be porosities, they should be analyzed to make sure that the color of wear matches the entire skull consistently so as not to confuse CO or PH with taphonomic damage. One could expect recent damage to the bone to show a different coloration than the bone around it. In addition to looking at PH and CO, I also looked at linear enamel hypoplasia, another pathological condition to get an overall idea of the health of the collection. This condition causes lines to be physically present horizontally along the teeth of individuals suffering from this condition.

Once the data associated with the biological profiles of each individual was complete, iron and other element concentration measurements were collected with the p-XRF. An area in the Archaeology Laboratory at UNC was used as a stable surface for p-XRF data collection. P-XRF requires the user to be at least an arm's length away from the device and to be standing to the rear of the handheld unit, away from the nose of the instrument which emits the X-ray (see fig. 4). Although the p-XRF can be used portably and handheld, in this case it was used stationary due to the large number of scans being taken. Additionally, p-XRF requires the lens through which it emits its x-rays to be flat with the surface of the sample. Any crevice in

between the sample and the lens causes x-rays to be emitted outwards, therefore measuring the outside air and not the sample.

Fig 4.) p-XRF data collection taking place inside UNCO Archaeology Laboratory



Several sections along the crania of each individual were scanned with the p-XRF. These areas included the left parietal, right parietal, occipital, and any area along the skulls, which seemed to have the least contamination present on the surface. These scans gave us parts per million concentration along with its error rate of light and heavy elements. This type of scan takes 90 seconds to generate, but p-XRF is also capable of producing a spectral graph, which can be interactively engaged by the user. This spectral analysis is described as being semi qualitative because it shows raw photon counts but does not calibrate the data to estimate parts per million concentrations of each element. It shows the energy peaks found in each element, therefore allowing the user to see the proportion of each element to one another.

To determine if there were significant differences between groups with and without CO/PH, I used several two-tailed T-tests. This statistical method shows us if there is a statistically significant difference ($p < 0.05$) in element concentrations between the two groups, those with CO and/or PH and those without. Once we determined what our data shows us, we were able to come to evaluate the IDA hypothesis and assess if XRF iron concentration shows a

connection between iron deficiency, CO, and PH. Data derived from these tests helped me evaluate the credibility of XRF as a method for addressing the IDA hypothesis. For example, if I had noticed a high fluctuation in all our element concentrations and was unable to come to any conclusion, then we might be able to assume the XRF is not a viable method for testing the IDA hypothesis or we might say that XRF data do not support the IDA hypothesis. If we do conclude that XRF supports the iron-deficiency anemia hypothesis, then we could further focus on analyzing iron element concentration differences for our research, while taking the possibility of XRF being a viable method much more seriously.

I assured quality control by making sure that each scan used in the t-test came from the same location on each skull. The t-tests included data from the scans taken from the area along each cranium, which had the least amount of visible contamination. Also, I assured the quality of my research by following standard methods used to rate PH and CO as well as those used to build the biological profiles of individuals.

In our case, like all others, the matter of diagenesis must be determined on an individual basis. This has to do with the fact that different environmental factors will determine diagenetic alterations and their severity (Nelson, 1986). Common contaminants such as silicon and potassium were looked at to determine if contamination differed between individuals were present. It is worth noting that Izci et al. (2013) pointed out that iron might be one of the elements most susceptible to diagenesis.

Results:

An overall analysis of the data collected in regards to the sex and age of each individual led me to estimate that the collection used for this research was composed of five females, six males, one adult who's sex I found to be ambiguous, and the child who's sex could not be

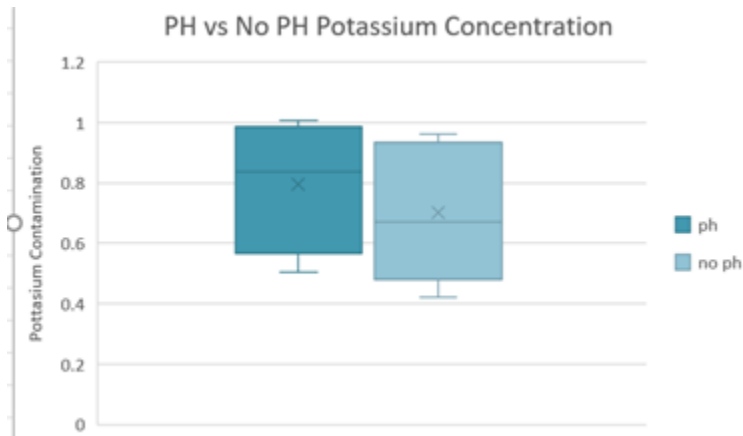
determined due to reasons previously discussed (see fig 5)... In each of the six adult individuals in which tooth development could be assessed, the age for all individuals is estimated to be at over 35 years of age. Additionally, methods using suture closure led to the estimation that all individuals in this collection excluding the child, were between their early forties and mid 50's. The child in the collection is believed to be 6 years of age +/- 2 years.

Fig 5.) Sex estimation Final Results

sex estimation	nuchal	mastoid p	orbital m	glabella	f eminence	sex
8	4	2	1	2.5	0 female	
1	3	2	4	2	2 female	
3	4	5	2	4	3 male	
16	3	4	2	5	0 ambiguous	
9	1	2	1	1	2 female	
11	5	4	1	5	0 male	
10	1	2	1	1	0 female	
5	5	5	1	5	3 male	
13	5	5	5	5	2 male	
4	5	5	5	5	5 male	
6	3	3	1	2	2 female	
15	4	5	1	1	4 male	

Fig.6) Pathology Final Results.

Fig. 7) Graph showing potassium concentrations in sample groups with Porotic hyperostosis and those without



	<i>ph potassium concentration</i>	<i>no ph potassium concentration</i>
Mean	0.7373	0.702
Variance	0.05453473	0.047269577
Observations	5	8
P(T<=t) two-tailed	0.792347728	

Fig. 8) t-test of potassium concentrations in sample groups with Porotic hyperostosis and those without

Nine of the thirteen individuals had CO (see fig. 6). The CO found in each of these individuals would be described as mild in severity. No individual in this collection showed a severe case of CO. PH was found in four of the thirteen individuals. In two of these cases, individuals showed evidence of severe PH, with the rest being described as mild. An overview of linear enamel hypoplasia showed that four of the individuals showed present and severe cases. While linear enamel hypoplasia was not examined directly in regard to element concentrations, it is an indicator of overall life health.

The first two-tail t-test conducted included adult individuals with and without PH (N1 = 4, N2= 8, p= 0.27). An additional t-test comparing the adult individuals with CO to those without, also failed in showing support for an alternative hypothesis indicating a difference in iron concentrations between the groups (N1=9, N2=3, p= 0.76). A final one-tail t-test done comparing the child individual with PH to the adult individuals with PH showed a statistically significant difference in iron concentrations between the groups (N1=1, N2=8, p= <0.01).

To account for contamination, common contaminants found in soil, such as silica, and potassium were compared to see if any differences existed. Student's t-test showed that there was no difference between potassium and silicon concentrations amongst sample groups (with PH and without, including the child) (Silica, N1= 5, N2=8, p= 0.50) (Potassium, N1 = 5, N2 = 8, p= 0.79). (see fig's. 7 & 8).

Discussion:

An analysis of the pathology found in the collection led to the determination that several of the individuals in this collection faced health related complications in life. The amount of

PH/CO found in this collection indicate that anemic conditions could have been widespread throughout the population from which these individuals came from. The LEH found in four individuals also could indicate environmental stressors, such as malnutrition, which caused the teeth of these individuals to stop maturing for some time.

The first two t t-test done comparing the individuals with PH/CO to those without did not show any difference in iron concentrations between the individuals. Only adults were used in this first t-test because as mentioned before, PH/CO is only found active in young individuals. Therefore, if the IDA hypothesis is supported, we would expect iron concentrations measurements to be different in a younger individual showing signs of CO/PH. We can say with more certainty that a child would have been actively experiencing the environmental challenges which lead to IDA and the osteological reconstruction that follows closer to their time of death. PH/CO porosities show reconstruction and inactivity during adulthood, so PH/CO would be a more certain indicator of their health in the past than it would be of their current health standing. However, because the adults experienced the environmental conditions which lead up to PH/CO during childhood, it can also be deduced that they would have faced the same conditions in adulthood.

The next t-test done comparing the child individual (had signs of PH) to adults which showed signs of PH succeeded in showing the expected difference between the iron concentration of these two groups as previously discussed. However, the results are not what is expected via the IDA hypothesis. The iron concentration in the child sample alone was significantly higher than that of the adults who had PH ($p < 0.01$). If the IDA hypothesis were true, we would expect the opposite of this to be present in our data.

In addition, although the t-test comparing adult individuals with PH to those without found no statistical significant difference, a visual analysis of the data graphically indicates the iron concentration trend being higher in individuals with PH, both with and without the child individual added (see fig. 9 &10). Again, this would be on the contrary of what is expected if the IDA hypothesis is true.

Fig.9) Box plot showing iron concentrations of individuals who showed signs of PH and those who did not.

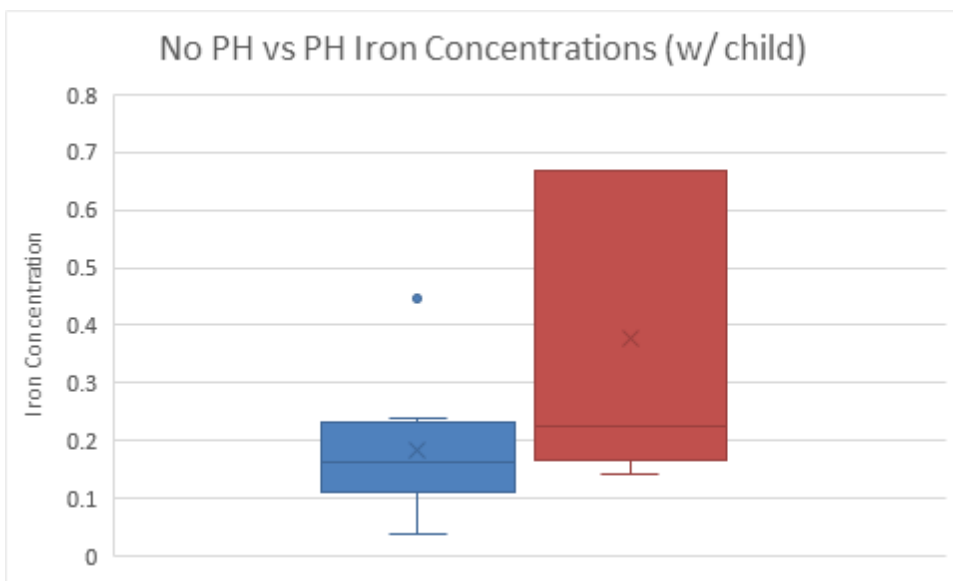
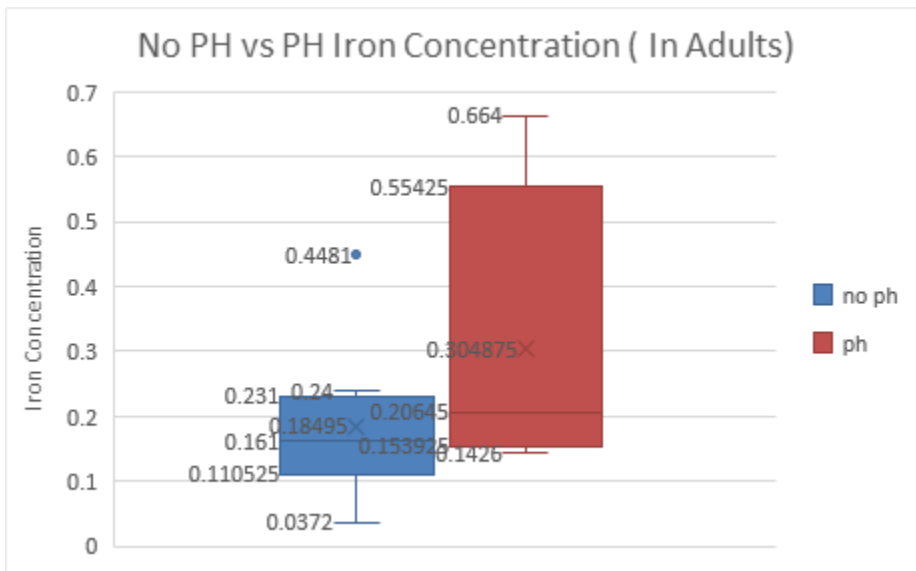


Fig. 10) Box plot showing iron concentrations in adults who showed signs of PH and the child who also showed signs of PH.

Additional t-test done on potassium and silica in individuals with PH/CO showed no difference in contamination. This means that there is a strong likelihood that the iron concentration readings we generated with the p-XRF were in fact accurate, and that outside contamination did not skew results in a noted manner. Taking this into account adds more complexity to results we gathered which show iron concentrations being higher in the child when compared solely to the adults with PH.

Conclusion:

The purpose of this research was to test whether p-XRF could be used in future research testing the IDA hypothesis. However, the independent results generated by the p-XRF through this research do not support the IDA hypothesis alone. On the contrary, p-XRF showed that iron concentrations were higher in our individuals that had PH. This is the opposite of what we would expect with the IDA. As noted earlier, common contaminants were taken into account, and these did not show a difference between sample groups. Therefore, contamination is not an explanation for the differences we see. P-XRF generates a tremendous amount of data and further time spent analyzing individual element concentrations for each individual could lead to greater insight about the relationship between CO/PH and element concentration. Another possible explanation could be because red blood cell production happens largely in younger adults in the cranial vault (Hoffbrand and Lewis, 1981), active cranial bone marrow in the child at the time of death could have retained more iron containing blood at the time of death than the adults, leading to higher concentration readings being generated by the p-XRF.

P-XRF proved to be a useful tool for research involving human remains. Its ability to be both stationary and portable make it useable in the field. P-XRF's many other positive features still improve the possibility of conducting research in a manner that is entirely nondestructive and carries virtually no health complications when done appropriately. However, future research is needed to understand the uses and limitations of using p-XRF to examine element concentration in skeletal remains and to better test the IDA hypothesis.

Not being able to analyze the soil in which the individuals used for this study where originally interred proved to be the most significant complication because the reality of altered element concentrations, poses a series of challenges. In a situation where the provenience of a sample collection is not in question, then I believe that p-XRF could truly further research into the IDA hypothesis. Unfortunately, we only have basic information on the original site from which our collection was disinterred from. Therefore, any future test attempting to determine the possible contamination found in each of the individuals through an analysis of the original burial material or contextualization of the site and its history, is impossible. In future research the soil composition and other burial artifacts collected at a site could be accounted for indefinitely. A true understanding of an archaeological site and the provenance of the remains collected would allow a researcher to examine the iron concentrations of each sample while taking into account all known possible contaminants.

Based on my results, using p-XRF in the future to test the IDA is a genuine possibility. Technology like this inherently has an appeal in archaeological work due to its nondestructive nature. Additionally, p-XRF is a cost-effective method in comparison to standard spectrometers. All p-XRF's positive characteristics make it into a tool that has extensive possibilities for use in archaeological and historical contexts alike.

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