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Exploring the Relationship Between Nutrition and Cribra Orbitalia: The Comparison of Dietary Stable Isotope Comparisons of Juveniles from Kuelap, Peru

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EXPLORING THE RELATIONSHIP BETWEEN NUTRITION AND CRIBRA ORBITALIA: THE COMPARISON OF DIETARY STABLE ISOTOPE COMPOSITIONS OF JUVENILES FROM KUELAP, PERU

by
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ABSTRACT

A juvenile's dependency on their caregiver is significant to the overall development of nutritionally related pathological lesions. However, not all skeletal pathology is caused by nutritional stress; despite anemia being the usual inferred cause, the origin of Cribra Orbitalia (CO) – lesions on the orbital roofs of the cranium– is undetermined. The purpose of this research is to compare the reconstructed diets of juveniles with and without CO and explore connections to dietary patterns (inferred from stable isotope ratios of carbon and nitrogen). Rib bone samples of 79 juveniles with and without CO were sampled from the Kuelap archaeological site in Chachapoyas, Peru (AD 800–1532) – known for its archaeological diversity. Stable isotope analysis was conducted (δ^{13}C and δ^{15}N values) to statistically analyze each group's values. Samples were further subdivided into age cohorts of infants (0–3 years), juveniles (4–11 years), and adolescents (12–18 years). The diets of juveniles with and without CO were determined to have no statistically significant difference between each other. However, a significant statistical difference did exist between the diets of the different juvenile age cohorts regardless of CO status, indicating that weaning and early dietary transitions through childhood affected the juvenile's nutritional regime in the region. The research presented is the first study of the relationship between nutrition and CO from Kuelap; significantly, it further explores the lifestyle of past individuals in Chachapoyas through the understanding of childhood diets.

DEDICATION

I want to dedicate this to my parents for always believing in me and

giving me the opportunity for an amazing education to find passion in scientific research.

ACKNOWLEDGEMENTS

I want to thank Dr. Toyne for giving me the opportunity to work in her lab and gain a profound research interest in stable isotope analysis of skeletal remains in Kuelap, Peru. Dr. Toyne and Dr. Wheeler have supported me throughout the journey, despite all the challenges, and believed in me. Thanks to the both of them, I enjoy research and am proud of how far they have helped me come as an aspiring researcher.

I also am very grateful for all the support and love my parents have expressed to me during my years of undergraduate studies. They have fought hard to provide me the best life with endless doors of opportunities, and I cannot thank them enough.

Statement of the use and destruction of human remains as done following accepted ethical protocols in the lab. Statement that samples were exported legally under Peruvian Ministry of Culture export permit RVM No 095-2015 VMPCIC/MC by Dr. J. Marla Toyne.

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Chapter 1. INTRODUCTION

Purpose of Research

The purpose of this research is to reconstruct the diets of juveniles from Kuelap, Peru using the stable isotope analysis of carbon and nitrogen to explore the relationship between pathology and nutrition. Regarding pathological conditions, the relationship of Cribra Orbitalia (CO) – a bony lesion identified on the orbital roofs of juveniles – and nutrition will be explored using stable isotope analysis of bone samples from 79 juveniles, ranging in age from 0 to 18 years of age. The bone samples are divided into two groups: those with and without CO. The group samples consist of 14 juveniles with CO and 66 juveniles without CO. These skeletal remains were excavated from the pre-Hispanic archaeological site of Kuelap in the Chachapoyas region of Peru. The diets and lifestyle of juveniles are of interest to further understanding the lives and death of past individuals.

This thesis addresses two main questions:

1. Is there a relationship between CO and nutrition? It could help determine the differences in the dietary patterns of the juveniles of the two groups and provide supplemental information on the impact and identification of disease in the past within the region.
2. Is there a relationship between diet and age of individuals?

Addressing these questions using isotopic analyses may help determine whether nutrition is a factor of developing CO between the two groups of bone samples.

The Bioarchaeological Approach

According to Larsen (2015), skeletal remains are significant in archaeological research. These ancient skeletons create the foundation for understanding the past life of individuals for further analysis and comparison to life in that region today (Larsen, 2015; Sutton, 2020). They help in exploring questions about past lives: what were their lifestyles and nutritional regimes like and were they affected in any way by environmental or pathological factors? Bioarchaeological approaches are important in understanding these questions, especially in understanding three key components: diet, health, and lifestyle.

In relation to this research, the analysis of human skeletal remains is crucial to paleopathology (Ortner, 2011; Sutton, 2020). The soft tissues and bones can be analyzed for evidence of active or healed lesions indicative of metabolic or nutritional stress (Brickley, 2018; Nagaoka et al., 2017; Zarifa et al., 2016). The juvenile rib bone samples that were collected at Kuelap, Peru were previously analyzed for the presence of porotic lesions on the orbital roofs and other signs of trauma or disease prior to exporting the bone samples to the United States. Juveniles with CO were identified due to the presence of the active or healed cranial lesions, separating them from the individuals without it. Signs of pathological bone conditions are a depiction of the nutritional and social lifestyles of these individuals. They help bioarchaeologists understand patterns in disease conditions and how disease stress may have impacted past life and society (Ortner, 2011).

Juvenile skeletal remains also play a significant role in understanding paleopathology due to the presence of active lesions (Lewis, 2006; Bogin, 1997; Halcrow and Ward, 2018). As a result, juveniles are significant to addressing anthropological and bioarchaeological research

questions. In order to understand the change and pattern over the entire subadult period, infant, juvenile, and adolescent bone samples were analyzed as well.

In furthering the understanding of paleopathology and its connection to nutrition, which plays a factor, stable isotope analysis of skeletal remains can be used. It continues to answer those anthropological questions: what was their range in dietary variation? What does their diet say about nutrition and paleopathology? This thesis focuses on stable isotope analysis in further exploring the concepts in the research to compile a detailed analysis of life in the Chachapoyas region of Peru.

Stable Isotope Analysis

To reconstruct the diet of past juveniles from Kuelap, stable isotope analysis was performed on archaeological human bone samples. Stable isotopes are the non-radioactive forms of an element with the same number of protons and different number of neutrons (Reitsema et al., 2018). The most common stable isotopes in bioarchaeology are carbon-13 (δ^{13}C) and nitrogen-15 (δ^{15}N) these forms will be the foci of the stable isotope analysis in this research (Reitsema et al., 2018). Stable isotopes are part of everything we drink and consume; these atoms will reside in the bones, making them useful to determine the dietary compositions of one's diet through stable isotopic variations (Katzenberg, 2008).

Carbon originates from the macromolecules within the food that was consumed, while nitrogen originates from protein (Katzenberg and Lovell, 1999; Kusaka et al., 2010). Nitrogen determines one's trophic level within the food chain, depending on the amount of consumed protein; additionally, its isotopic values can depict nutritional stress (Reitsema, 2013). The isotopic values of carbon and nitrogen are determined through collagen extraction from the

bones of human skeletal remains. Collagen extraction is important in the process of determining the stable isotope ratios; rib collagen can reflect the dietary patterns of the last three to five years of an individual before death (Gil et al., 2009). This protocol is significant in the study, as we can analyze the diets of many juveniles who died shortly after birth; this can help better understand the social developments of a child and their transitions through weaning. Stable isotope analysis is the main and crucial part of diet reconstruction of juveniles.

Significance of Kuelap

Despite the increase in bioarchaeological studies in the Chachapoyas region of Peru, there have been few studies on juvenile skeletal remains; however, juvenile skeletal remains have generally popularized over the years in studies due to the identification of its importance to paleopathology and understanding past behaviors (Andrushko et al., 2011; Beauchesne and Agarwal, 2018). In terms of pathology, multiple studies have been conducted within the region, especially in Kuelap, on different types of paleopathological conditions and its relation to nutrition and lifestyle (Tran, 2016; Toyne, 2015; Toyne and Narváez, 2017; Toyne et al., 2020) . According to Van Valkenburgh and colleagues (2020), Kuelap is the most studied archaeological site in the region; its studies have varied from isotopic variation to architecture. Although, CO has only been minimally studied as the focus in studies done in the archaeological site, especially its connection to nutrition.

The lack of studies on this topic highlight the importance of new research being focused on juvenile skeletal remains and CO at the site. Previous research reconstructed the diet of adults and juveniles at Kuelap during the Inca period (Denierio, 2018); Toyne et al., 2020). From Kuelap, 79 rib bone samples from juveniles were used for stable isotope analysis. Of the total

sample, 14 rib bones were from juveniles identified to have CO present. Even though there is not an equal number of samples between the two groups, the CO samples will be used to address the research questions and test the statistical significance with the sample of juveniles with no orbital lesions. The research at Kuelap will provide information on aspects of paleopathology that were not previously studied at the site but also further the understanding of life in the Chachapoyas region of Peru.

Hypotheses

For the research study, two research questions are being explored and the following hypotheses are tested:

1. Is there a relationship between nutrition (as inferred from stable isotopes) and CO? Hypothesis tested: Are there differences in the stable isotope compositions of carbon and nitrogen of juveniles with and without orbital roof lesions? Since the juvenile skeletal remains were excavated from the archaeological site of Kuelap, there is a possibility of similarities in lifestyle, including diets, regardless of evidence of skeletal pathology.
2. Is there a relationship between age and dietary patterns? Hypothesis tested: Are there differences in the stable isotope compositions of carbon and nitrogen in infants, juveniles, and adolescents? Due to the dependence of infants to their caregivers compared to juveniles and adolescents, there is a possibility of dietary variations.

These hypotheses are being tested through statistical analysis of the two groups and the age ranges within the groups. In hypothesizing the presence or absence of statistical significance between the stable isotope ratios of the groups, a non-parametric Mann-Whitney

U Test will be used. The statistical test is significant in comparing independent samples that are not normally distributed; it determines whether the two groups could have derived from a single original population (Wayne, 2017).

If statistical significance is observed among the two groups, then the juveniles with and without CO would have similar dietary compositions. Therefore, a connection between nutrition and CO would not be present. In contrast, if no statistical significance is observed among the juveniles with and without CO, then the juveniles would have differing dietary compositions. This difference in diet could indicate that the presence of CO could be nutritionally-related. Nutritional deficiencies could play a factor in the development of the pathological lesion. However, other factors could be possible. In order to give the best interpretation of the results, a literature review follows to fully understand the significance of nutrition on paleopathology and stable isotope analysis, as well as the significance of juveniles in archaeological research.

CHAPTER 2. BACKGROUND

This chapter focuses on a literature review on the bioarchaeological approach to paleopathology and dietary patterns and the studies conducted on cribra orbitalia from human skeletal remains from archaeological contexts. It will help in further understanding the foci of the research leading up to the research questions addressed in this thesis.

Bioarchaeology of Juveniles

Challenges arose with studying juvenile skeletal remains in the past due to the misinterpretation of what being a child characterized archaeologically. According to Perry (2008), age is based on skeletal development. Archaeologists have different definitions on the subcategories of age, but based on Lewis (2006), infants could be up to 5 years of age and juveniles are between 1 and 15 years of age; adolescences are between 15 and 17 years of age. The ages within these categories may vary with archaeologists but are important in the studies of juvenile skeletal remains. However, childhood is not solely based on age but is also based on social and cultural experiences (Lewis, 2006).

According to Bogin (1997), juvenile skeletal remains are sensitive indicators to environmental change. As bioarchaeological studies have emerged, juveniles have become important due to the paleopathological information that could be interpreted from their remains. Skeletal lesions on juveniles are more apparent compared to adults as a result of a faster bone turnover rate (Lewis, 2006). With adults, bone remodels in a healing lesion, resulting in a change in the original surface morphology. Therefore, these active lesions could help archaeologists gain a further understanding on the biological and social context of the childhood past and associate

these behaviors with possible cause of dietary variation, disease, or stress (Beauchesne and Agarwal, 2018; Bogin, 1997; Lewis, 2006; Halcrow and Ward, 2018).

Childhood is a social construct due to a child's dependency on their caregiver. During infancy, the infant depends on their mother for nutrition through lactation. The nutrients in breast milk initially nourish the infant, but as the child develops, those nutrients are not sufficient for complete nourishment (Beauchesne and Agarwal, 2018). More energy is required for the child which solid foods could provide. As the infant stage ends and the juvenile stage begins, weaning – the shift to solid food – takes place. However, a child's transition to the weaning stage depends on the resources available and their economic situation. Infants, who end the infancy stage at 3 years of age, in low economically developed countries (LECs) must depend on lactation, potentially resulting in chronic nutritional deficiencies (Bogin, 1997). Overall, juveniles have some dependency on their caregivers as to the foods they eat; the foods eaten are the ones provided and accessible to them. The diet created for them or by them could have a pathological impact on their health or nutritional intake.

Diet and Pathology of Stable Isotope Analysis

A child's dependency on their caregiver gives them limited decision on their nutritional regime. Dependent on the food resources available per location or family based on socioeconomic standing, diets may vary per juvenile. This might result in diets with incomplete nourishment for a growing child. With the lack of nutrients consumed among juveniles or adults, metabolic and nutritional stress weigh on the body due to nutritional deficiencies. These deficiencies can produce chronic pathological conditions, such as scurvy (vitamin C deficiency) or anemia (iron deficiency), which can result in osteological markers (Snoddy et al., 2018).

Various studies have been published on the association of diet and pathology (Koci, 2021; Curto, 2019; Turner and Armelagos, 2012). Pathological lesions have become evident as possible markers for malnutrition and further understanding the nutrients and foods lacking in the diet. However, most osteological markers are non-specific and do not provide information to the general reconstruction of diets of past individuals. Instead, stable isotopic analysis of bone tissue could help provide information on the isotopic compositions, which can indicate a general class of foods included in the dietary regime.

Stable isotopes reflect on the idea that "you are what you eat," as the foods consumed by an individual have different isotopic compositions for comparison of dietary variation (Curto, 2019; Katzenberg and Waters-Rist, 2019). There are two types of carbon stable isotopes: δ^{12}C and δ^{13}C. These values represent the whole diet – carbohydrates, proteins, and lipids. However, the carbon isotopes differ in the photosynthetic plant pathways. CAM plants are usually succulents and cacti; depending on the environmental conditions of the past society, the δ^{13}C values will fall between the values for C_3 and C_4 plants. C_4 plants (i.e. maize) belong in a hotter and drier climate; the range of δ^{13}C values is –14‰ to –9‰ (Katzenberg, 2008). In comparison, C_3 plants (i.e. shrubs) belong in temperate environments; the range of δ^{13}C values are – 35‰ to –20‰ (Katzenberg, 2011). These values could help determine the type of plants consumed in past societies. Due to fractionation, these values shift about 5‰. This causes an enrichment in bone collagen of 5.1‰, which reflects the dietary protein within the diet (Ambrose and Norr, 1993)

There are two types of stable isotopes for nitrogen: δ^{14}N and δ^{15}N. These isotopes reflect protein consumption, as nitrogen determines the trophic level of an individual within the food chain (Larsen, 2015). The higher an individual's position within the food chain, the more protein the individual consumed. This is reflected in high δ^{15}N values, which can also be an indicator of

nutritional stress, based on a study of starving birds (Hobson et al., 1993). The information stable isotope analysis provides on the diet of past individuals could help determine the nutritional lifestyle in the past and its connection to pathologies.

Cribra Orbitalia

In 1888, Welcker published a study on a porotic lesion discovered on the orbital roofs of the skull, known as cribra orbitalia (CO), through the analysis of skulls internationally (Welcker, 1888). The connection between race and CO were analyzed by Welcker, but the notion was wrongly implied. However, the discovery of CO led to significant research on its connection to pathological and nutritional etiology.

CO is present on the superior wall of the eye orbit (*pars orbitalis* or orbital plate) near the lacrimal gland. With layers of bone forming, the blood vessels within the lacrimal gland appear with each layer of bone (Cole and Waldron, 2019). This causes marrow hyperplasia, which results in the expansion of the diploe – spongy trabecular bone in between the inner and outer cortical bone of the skull – and results in perforations of the cortical bone, and a porotic appearance of the orbital roofs (Steyn et al., 2016). Lesions that are active are more common in juveniles, as the remodeling of the cortical bone in adults results in healed and inactive lesions (Walker et al., 2009). Therefore, the skeletal remains of juveniles are significant within bioarchaeological research in providing information on past life. Figure 1 below shows a juvenile skull observed by Dr. Toyne at Kuelap archaeological site with evidence of CO.

Figure 1. A juvenile skull from Kuelap with evidence of CO (Courtesy of Dr. Toyne).

Some conditions have orbital lesions similar to CO, resulting in the misidentification of the type of condition, as well as misleading CO causes. These conditions are rickets, scurvy, leprosy, as well as trauma (Cole and Waldron, 2019; Zdilla et al., 2021; Steyn et al., 2016). Scurvy (vitamin C deficiency) and trauma can both induce subperiosteal bleeding, causing similar porotic lesions to CO; however, with trauma, the expansion of the diploe is not present (Ortner, 2003). Leprosy is a bacterial infection, which can affect the eyes and sometimes cause porosity within the orbital roofs. Rickets (vitamin D deficiency) produces similar lesions, but the porosity is thinner compared to CO (Steyn et al., 2016). All these nutritional pathologies have been associated with CO, but the cause of CO is unknown, although anemia is the theoretical cause.

No specific cause of CO has been identified, although several hypothetical causes have been determined in various studies. Anemia has become the common theoretical cause due from iron deficiency in juveniles; some studies have supported the cause, while other studies have discarded it and connected CO to other nutritional deficiencies or parasitic infections (Facchini et al., 2004; Walker et al., 2009; Oxenham and Cavill, 2010). Zarifa et al. (2016) conducted a study

on the stable isotope analysis of juveniles in Latvia and identified a connection between CO and low iron levels, which was correlated to anemia. Unrelated to anemia, vitamin B-12 or vitamin C deficiency was a causal factor for CO for ancestral Puebloans due to unsanitary living conditions (Walker et al., 2009). These unsanitary conditions in past societies have been correlated with parasitic infections, as well as malaria, with evidence of lesions in juvenile remains (Cole and Waldron, 2019; Walker et al., 2009). Blom et al. (2005) concluded that in pre-contact South America, anemia was a main factor of CO, but it was caused by parasitic infections and malnutrition. This highlights that there could be many underlying factors to the main cause, which could be anemia or other nutritional or non-nutritional causes (Scaffidi, 2020).

However, in a study regarding skeletal remains analyzed from Machu Picchu, Peru, researchers did not find a nutritional association to CO based on stable isotope analysis (Turner and Armelagos, 2012). Due to the differences in analyses and conclusions related to the nutritional causes of CO from multiple studies, geographic origin or lifestyle could be possible major factors contributing to CO.

Archaeology of Chachapoyas

The modern capital of the Amazonian region of Peru, Chachapoyas, lies 2,000 to 3,000 meters above sea level within an array of high-altitude forests: tropical dry forests, deciduous forests, and the "lush cloud forest." This region is known for its archaeological culture, "Chachapoya," which existed (AD 900-1470) before the Inca conquest of the region in AD 1470. Among the cultural features are circular houses made of limestone between 5 to 8 cm in diameter located on mountaintops within a walled communal village (Guengerich, 2014). Within the Chachapoyas region, its culturalism and architectural style stood out due to the intricate designs

of the geometric and stone mosaic friezes that covered the circular houses (Von Hagen, 2002). Stone molding was applied to cornices around the houses to prevent flooding, while clay and stones were used to cover the floor to minimize humidity within the buildings (Von Hagen, 2002).

Based on the Chachapoya culture, the Chachapoya people depended on cultivation of high and low elevation plants. Quinoa, potatoes, yuca, and maize were some of the plants consumed by past societies in the region (Michell, 2018; Toyne et al., 2016), although, maize was a major plant source. During the drier climates, cacti and succulents were plants that were grown. In relation to protein courses, hunting was a large part of the culture. Large domestic animals (camelids and deer), guinea pigs, rodents, and fish were of the many protein sources the people depended on (Michell, 2018; Toyne et al., 2020).

One of the largest and most significant archaeological sites in the central region of Chachapoyas is Kuelap. Its attention comes from the architectural beauty of a large wall surrounding the site and settlements, making up about 450 hectares of land. The wall is made of cut limestone and has a height range of 10 to 20 meters (Church and Von Hagen, 2008). It has more than 400 circular houses and occupies 6 hectares of land, along with communal tombs. These houses are divided among high and lower sectors, which is indicative of social division amongst the Chachapoya people, according to Narváez (1988).

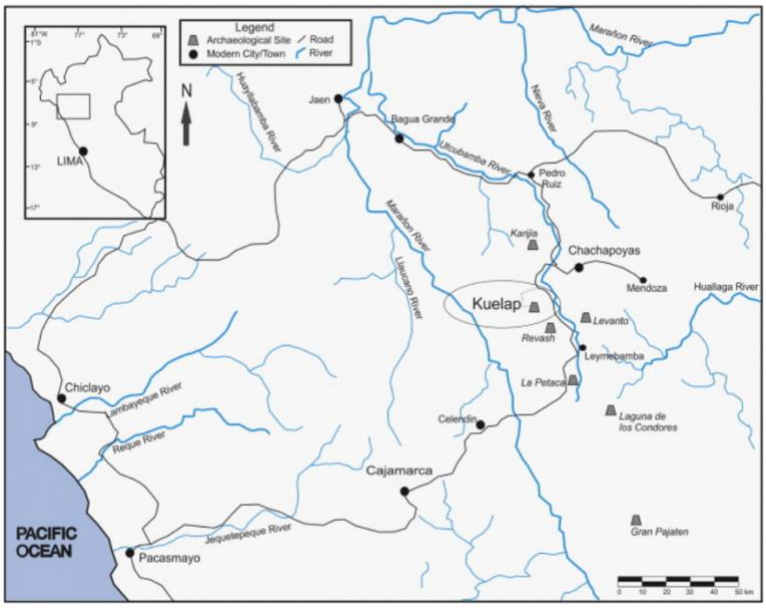

Figure 2. A map of the Chachapoyas region of Peru, including Kuelap, the study site. Adapted from *Tibial Surgery in Ancient Peru* (p. 30), by J. M. Toyne, 2015, International Journal of Paleopathology. Copyright 2014 by Elsevier Inc.

Kuelap is significant to this research, as juveniles played a major role in the Andes as social constructs. When they reached the weaning stage, dietary variations were possible, as their caregiver often changed to a grandparent or sibling (Baitzel, 2018). In addition, these children were raised to become less dependent at this stage, as they were responsible for carrying water or herding and helping in textile or ceramic production (Bolin, 2006).

Due to the role juveniles play in the Andes region, studies on the dietary patterns of individuals in relation to health and lifestyle have been important. This present research focuses on exploring the relationship of diet and pathology and determining whether CO may be nutritionally related. The next chapter focuses on the methodology used to obtain the necessary results to identify the diet-pathology relationship.

CHAPTER 3. MATERIALS AND METHODS

This chapter presents the skeletal samples and methods used in this research. Relative to the research, the sample composition of the two groups of juveniles – with and without CO – will be defined, along with the different age groups. The scientific methodology of collagen extraction for stable isotope analysis of C and N values, as well as the statistical analysis performed are described.

Skeletal Samples

At the Kuelap site, the skeletal remains of 613 individuals of all ages were analyzed by Dr. J. Marla Toyne. Out of the skeletal sample, 220 of them were juveniles under 18 years of age; statistically, 35.9% of the skeletal remains belonged to juveniles. For the purpose of this study, 79 juvenile skeletal remains were chosen for analysis and age groups are defined as infants (0-2 years), juveniles (3-10 years), and adolescents (11-18 years). The distribution of the total sample from Kuelap is presented in Table 1, while the study sample is presented in Table 2.

Table 1. Distribution of the total samples from Kuelap.

	Sample Distribution	**Statistical Distribution (%)**
Juveniles (< 18 years)	220	35.9
Adults (18+)	393	64.1
Individuals with CO	33	5.38
Juveniles with CO (< 18 years)	26	4.24
Total Sample	613	-

Table 2. Age group categorization of the total juvenile skeletal collection from Kuelap.

Age Group	Total
Infants (0-2 years)	45
Juveniles (3-10 years)	119
Adolescents (11-18 years)	56
Total	220

Two groups of samples of juvenile skeletal remains were analyzed: those with and without CO. Out of the total of 613 individuals collected, 33 (5.38%) individuals had CO and 26 (4.24%) of those individuals were juveniles under 18 years of age. Bone samples of 79 individuals were chosen for this study (Table 3). Fourteen individuals with CO were used as samples for the stable isotope research. The sample with CO consisted of juveniles ranging from 6 months to 13 years old; no adolescents had CO. Sixty-five individuals without CO were used as comparative samples for this research. The sample without CO consisted of juveniles ranging from 1 month to 18 years. Table 3 describes the number of juveniles in the different age categories for each group used in this research.

Table 3. Age group categorization of the juvenile samples with and without CO.

Age Group	Sample Size		Total
	Without CO	With CO	
Infants (0-2 years)	8	5	13
Juveniles (3-10 years)	36	9	45
Adolescents (11-18 years)	21	0	21
Total	65	14	79

Juvenile samples were the focus due to paleopathological and stress indicators being more apparent on juvenile skeletal bones rather than on adults. Juveniles have a faster turnover rate, making them useful in studies of understanding life in the past (Lewis, 2006). Also,

comparing the different age groups will further the understanding of the comparison of study patterns between the different age groups.

For most of these samples, rib bones samples were used as part of the stable isotope analyses; however in some cases, rib bones were not available. In these cases, the fibula and cranial bones were used; the types of bones used and the ages of the juveniles are identified in Appendix A and B for each group.

Methods

Cribra Orbitalia Indication. In the Chachapoyas region of Peru, Dr. J. Marla Toyne conducted skeletal analyses of the human remains collected from the Kuelap archaeological site from 2004 to 2015. Osteological studies were performed, in which the skeletal remains for individuals were observed for any skeletal paleopathological or stress indicators. In indicating whether juveniles presented evidence for cribra orbitalia, lesions on the orbital roof of the cranium were identified. Observations for each individual indicated the location of the porosity (right, left, or both orbits), the categorical degree of affliction (minor, moderate, pronounced), area of orbital plate affected (small <1cm, moderate 1-2 cm, large >2cm), and activity (active, remodeling, or well-remodeled). For this study only presence or absence is used for comparison. Along with identifying any pathological indicators, the human remains were analyzed to estimate sex and age at death. After all the observations and notes were recorded, samples of juveniles with and without CO were obtained for collagen extraction and stable isotope analysis. Skeletal samples of the individuals selected for stable isotope analyses were packaged properly and exported to the U.S. for further analysis under the permit RVM No 095-2015 VMPCIC/MC from the Peruvian Ministry of Culture. Figure 2 shows the comparison between a juvenile with CO and without CO.

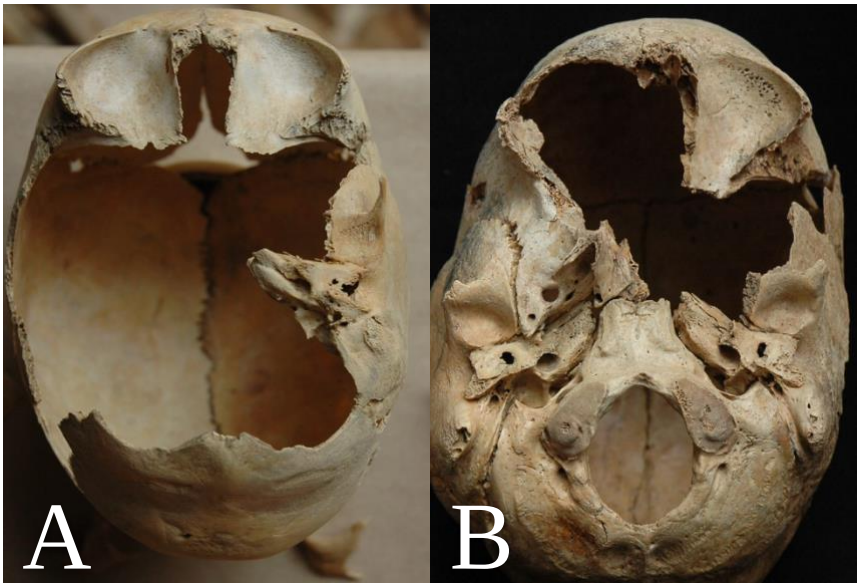

Figure 3. Juvenile skulls collected from Kuelap without (A) and with (B) signs of CO (courtesy of Dr. Toyne).

Collagen Yield. Collagen yield determines how well-preserved the sample is and whether it should remain within the data set for the research. It could be expressed as a weight percentage or weight ratio (Klinken, 1999). Relative to weight percentage, a sample with a collagen yield < 1% would be poorly preserved and discarded as a sample due to a high possibility of contamination from other external sources (Ambrose, 1990). A sample with a collagen yield of > 10% is well-preserved, but samples with a collagen yield of 1- 8% could still remain in the sample data set as sufficiently preserved (Ambrose, 1990). The equations used for the calculation of collagen yield is expressed:

$$\text{Collagen Yield (\%)} = \frac{\text{collagen weight (before chemical preparation)}}{\text{dry collagen weight (after heated)}} \times 100$$

In addition to using this calculation, the C/N atomic ratios can evaluate the status of sample preservation. According to Ambrose (1990), if the atomic ratio is within the range of 2.9-3.6, the sample is well-preserved. Another method of determining preservation is weight (wt) % C and wt % N. For carbon, the collagen is good if it has an approximate 35 wt %; the higher the percentage, the more organic carbon is present, while the lower the percentage, the more inorganic carbon is present (Klinken, 1999). For nitrogen, a range of 11-16 wt % will be indicative of good collagen bone (Klinken, 1999).

Collagen Extraction. In analyzing the stable isotope ratios of the juvenile skeletal samples, collagen extraction took place using the modified Longin (1971) method. The process began by weighing an empty glass centrifuge tube. Then, the bone sample was cleaned by removing the trabecular bone from the collagen bone. Once cleaned, the bone sample was cut in approximately equal sized pieces and placed inside a centrifuge tube, which was weighed on an analytical balance. The goal weight of collagen bone sample was between 1 gram. After the sample was successfully prepared, distilled water was added into the sample-containing tube, which was placed in the ultrasonicator for 10 minutes to remove any dirt. The bone fragments were then soaked in about 4 mL of 0.25 M hydrochloric acid (HCl) for days up to weeks until the sample was fully demineralized. After the HCl solution was removed, 2 mL of 0.1 M sodium hydroxide (NaOH) were added into the demineralized sample to remove any humic and fulvic acids. To ensure the collagen sample had a neutral pH of 7, the sample was rinsed with distilled water a few times. About 2 mL of 0.25 M HCl were added again to the sample, which was placed in an oven at 90°C to dissolve. The HCl solution was removed, and the sample was placed back into the oven to dry to complete the collagen extraction. The collagen samples were taken out of the oven and weighed for collagen yield.

Stable Isotope Analysis. The extracted collagen samples were sent to the University of Florida Light Stable Isotope Mass Spec Laboratory in Gainesville, Florida for stable isotope analysis. The stable isotope analysis was achieved using an isotope-ratio mass spectrometer coupled with a carbon and nitrogen elemental analyzer (EA-IRMS). Within the IRMS process, the samples are sent into the elemental analyzer where after combustion, nitrogen (N_2) and carbon dioxide (CO_2) gases are separated; the N_2 and CO_2 ions are sent into the ion source of the IRMS where the mass to charge ratio (m/z) of the carbon and nitrogen components in the samples are measured (Ogawa et al., 2010). The isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ expressed as parts per mil (‰) were determined using a standardized equation (Ogawa et al., 2010; Kusaka et al., 2010):

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

According to Muccio and Jackson (2008), IRMS is used to measure the variations in isotopic ratios of light elements. In relation to the research, variations in $\delta^{13}C$ and $\delta^{15}N$ will be taken into account between the two groups of juveniles. The isotopic ratios are measured in comparison to a standard to reduce systematic error; the standard for carbon is Vienna Peedee Belemnite (VPDB), while the standard for nitrogen is typically air (Muccio and Jackson, 2009).

Statistical Methods

Descriptive Statistics. Once the isotopic ratios of well-preserved collagen bone samples were measured, statistical calculations were conducted – average, range, and standard deviation – for each group of juveniles and their age groups. These calculations were used to indicate any direct observations between the isotopic variations of the two groups, as well as the age categories within each group.

Mann Whitney U Test. For an accurate analysis of comparative variation, a non-parametric Mann Whitney U Test was used since the sample size was small and the data set did not represent a normal distribution (MacFarland and Yates, 2016). Within this test, the averages of the isotopic ratios between both groups were compared using a p-value of 0.05 to determine the status of statistical significance; a similar procedure occurred with the age groups to determine whether the diets between different age groups varied and how it has affected the group as a whole. In applying the test, the SPSS data editor (IBM SPSS Version 26) was used for all necessary calculations.

Statistical significance (p-value > 0.05) implies that the isotopic ratios between the juveniles with and without CO had distinct variation. In the case of this research, the diets of both groups of juveniles varied in nutritional content, so the possibility of diet being a factor of CO could be present. Different diets could indicate different lifestyles between the juveniles and further the understanding of this division. No statistical significance (p-value < 0.05), however, implies that the isotopic ratios are similar and variation is small. Relative to the research, the diets of juveniles with and without CO would be similar, so diet would not play a factor in CO.

Overall, the stable isotope methodology and use of descriptive statistics will obtain the necessary statistical findings to further understand the dietary variation present between juveniles with and without CO, as well as the different age cohorts. The following chapter will present these results.

CHAPTER 4. RESULTS

This chapter presents the statistical results between the $\delta^{13}C$ and $\delta^{15}N$ values of the isotopic diets of juveniles. Statistics are performed between juveniles with and without CO to determine whether there is a significant difference between their isotopic diets. To further understand CO and dietary composition, the isotopic values among age categories (infants, juveniles, and adolescents) are compared statistically. The original $\delta^{13}C$ and $\delta^{15}N$ stable isotope compositions obtained are found in Appendix C and D for juveniles separated by those with and without CO.

Descriptive Statistics

Descriptive statistics were calculated to have a comparison between the average, range, and standard deviation of the isotopic values for the two groups of juveniles: with and without CO (Table 4). The standard deviations between the two juvenile groups are similar, even though the sample size between the two groups differ. Statistical comparisons were also conducted between age groups (infants, juveniles, and adolescents). A summary table for the age groups with CO are below in Table 5, while Table 6 is a summary table for those without CO.

Table 4. Standard results (average, range, and standard deviation) of juveniles with and without CO.

Juvenile Group	Isotopic Variable	Average (‰)	Range	Standard Deviation
Without CO (n= 65)	$\delta^{13}C$	–12.85	7.15	1.33
	$\delta^{15}N$	+7.90	6.71	1.39
With CO (n= 14)	$\delta^{13}C$	–13.11	4.34	1.35
	$\delta^{15}N$	+8.68	5.13	1.70

Table 5. Statistical results (average, range, and standard deviation) of age categories with CO.

Age Categories (with CO)	Isotopic Variable	Average (‰)	Range	Standard Deviation
Infants (n= 5)	$\delta^{13}C$	–13.26	3.54	1.46
	$\delta^{15}N$	+10.43	0.39	0.94
Juveniles (n= 9)	$\delta^{13}C$	–13.03	1.36	1.26
	$\delta^{15}N$	+7.71	1.28	3.84
Adolescents	$\delta^{13}C$	-	-	-
	$\delta^{15}N$	-	-	-

Table 6. Statistical results (average, range, and standard deviation) of age categories without CO.

Age Categories (without CO)	Isotopic Variable	Average (‰)	Range	Standard Deviation
Infants (n= 8)	$\delta^{13}C$	–12.04	4.68	1.73
	$\delta^{15}N$	+9.38	2.72	0.93
Juveniles (n= 36)	$\delta^{13}C$	–12.92	4.73	1.35
	$\delta^{15}N$	+8.07	6.30	1.42
Adolescents (n= 21)	$\delta^{13}C$	–13.05	4.26	1.04
	$\delta^{15}N$	+7.04	3.33	0.84

Box and Whisker Plots

For a visual understanding of statistical significance for the $\delta^{13}C$ and $\delta^{15}N$ values among the two groups of juveniles and the age cohorts, box and whisker plots were constructed. For an overall comparison of dietary composition, the isotopic values were compared between the general two groups of juveniles with and without CO with a box and whisker plot, as shown in Figure 4. Visually, the average nitrogen values are lower compared to the average carbon values; however, even though there is not an equivalent number of samples per group, the carbon values fell within the same range between the juveniles with and without CO, while the nitrogen samples for the juveniles with CO were lower on average than the juveniles without CO.

Box and whisker plots were constructed between different age groups from the juvenile groups with and without CO. Major differences were seen in some comparisons of the isotopic

values of the age cohorts, especially with the infants likely due to breastmilk consumption. Figure 4 to 10 display the plots for each of the comparisons (infants vs. infants, juveniles vs. juveniles, infants vs. juveniles, infants vs. adolescents, and juveniles vs. adolescents). No comparisons between adolescents with and without CO were made due to the absence of adolescents within the sample set of juveniles with CO.

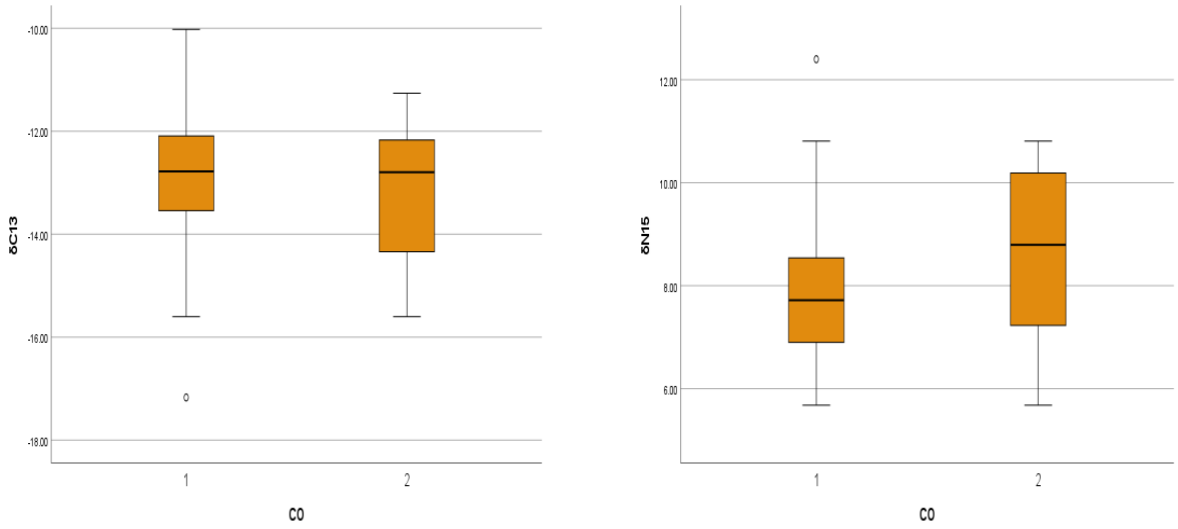

Figure 4. Box and whisker plots for **A.** δ^{13}C and **B.** δ^{15}N for all age groups without (1) and with CO (2).

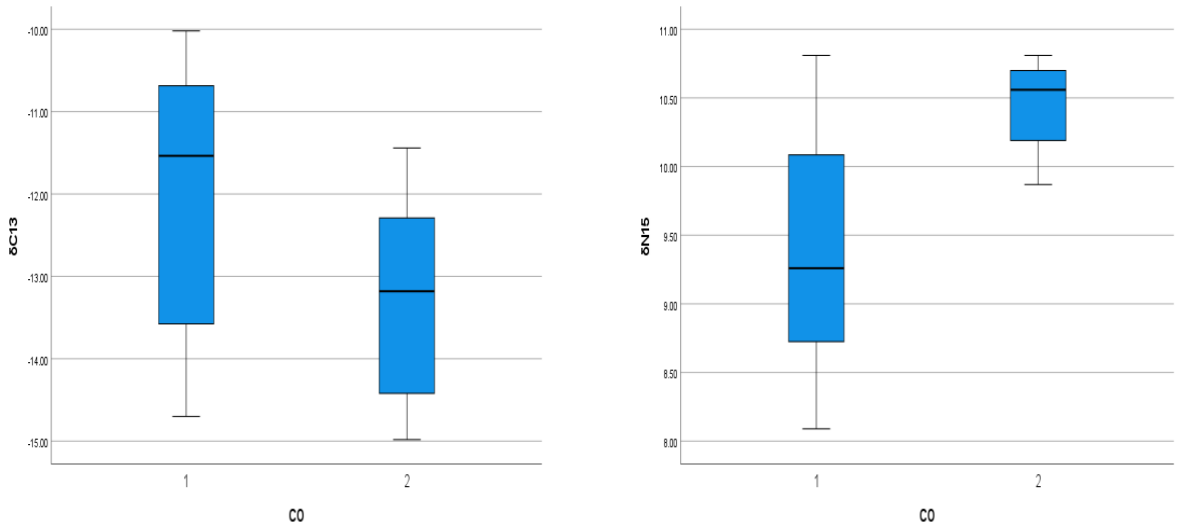

Figure 5. Box and whisker plots for the **A.** δ^{13}C and **B.** δ^{15}N values for infants without (1) and with CO (2).

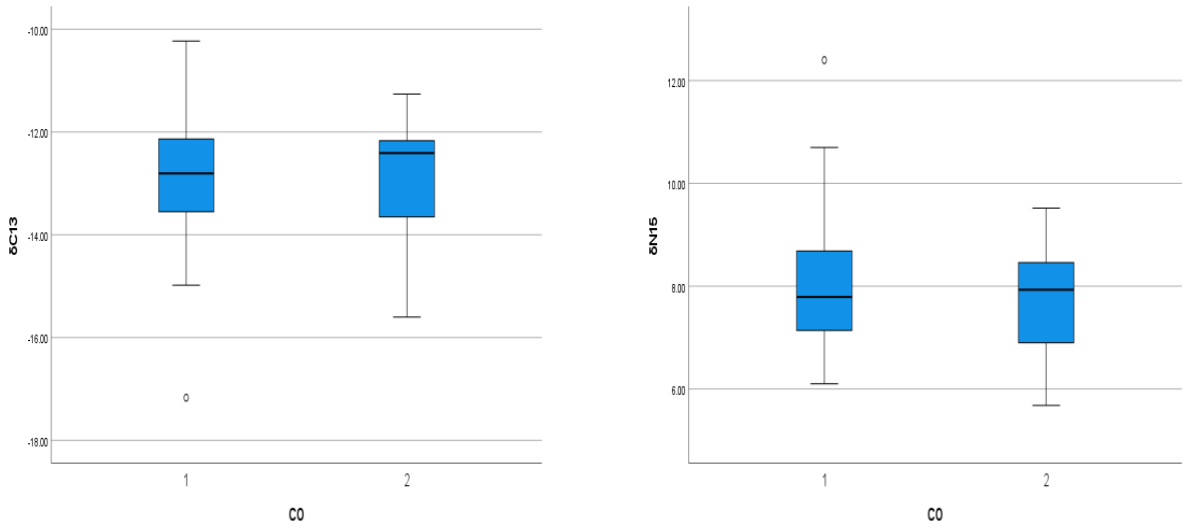

Figure 6. Box and whisker plots for the **A.** δ^{13}C and **B.** δ^{15}N values for juveniles without (1) and with CO (2).

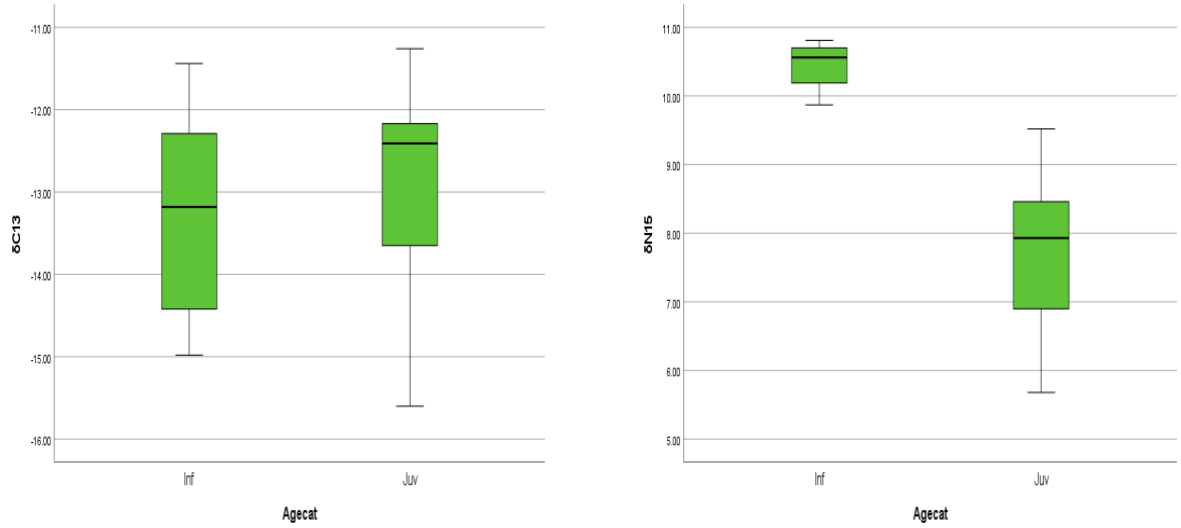

Figure 7. Box and whisker plots for the **A.** δ^{13}C and **B.** δ^{15}N values for infants (Inf) and juveniles (Juv) with CO.

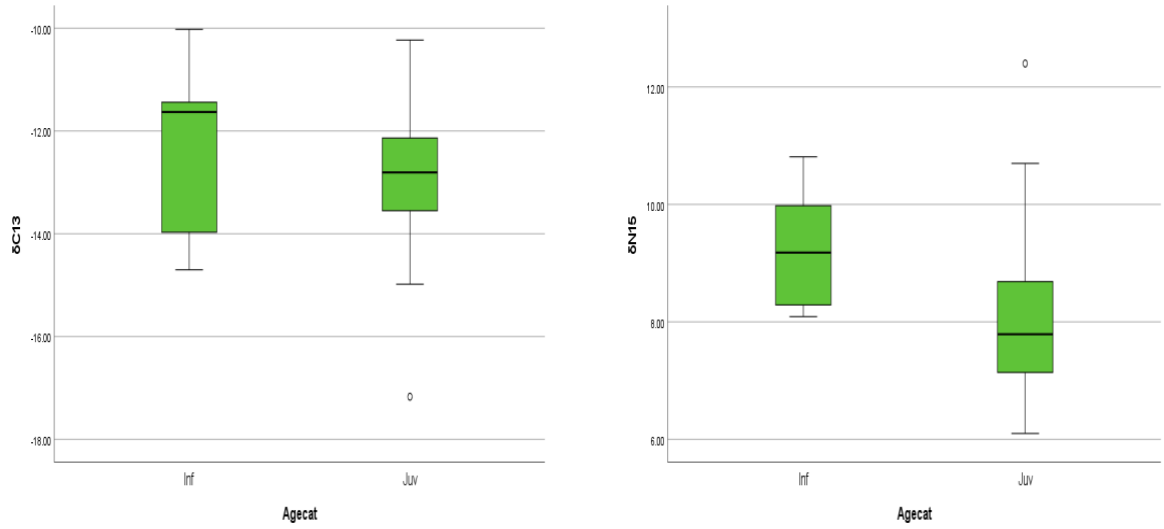

Figure 8. Box and whisker plots for the **A.** δ^{13}C and **B.** δ^{15}N values for infants (Inf) and juveniles (Juv) without CO.

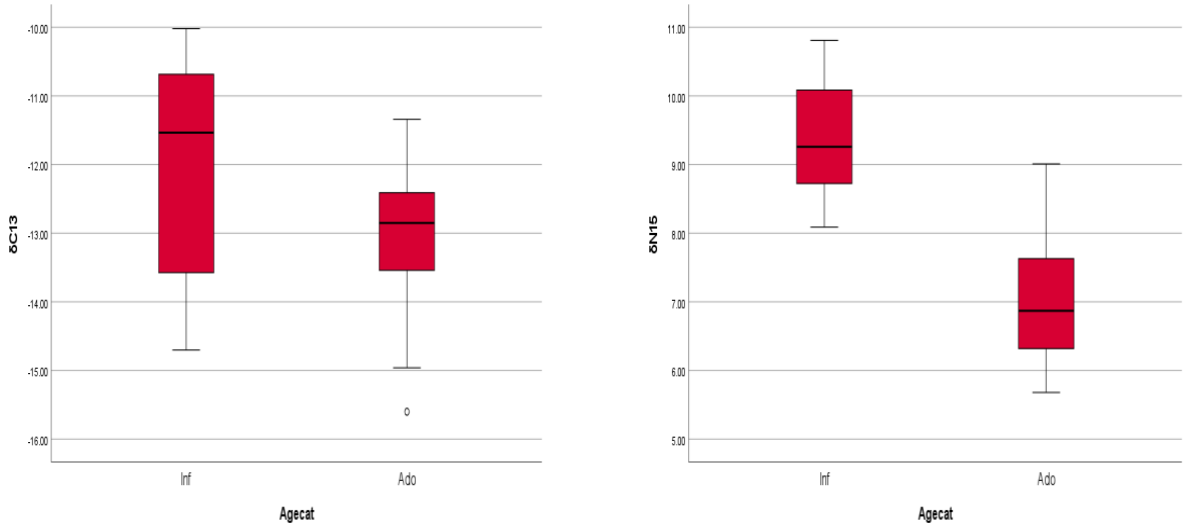

Figure 9. Box and whisker plots for the **A.** δ^{13}C and **B.** δ^{15}N for infants (Inf) and adolescents (Ado) without CO.

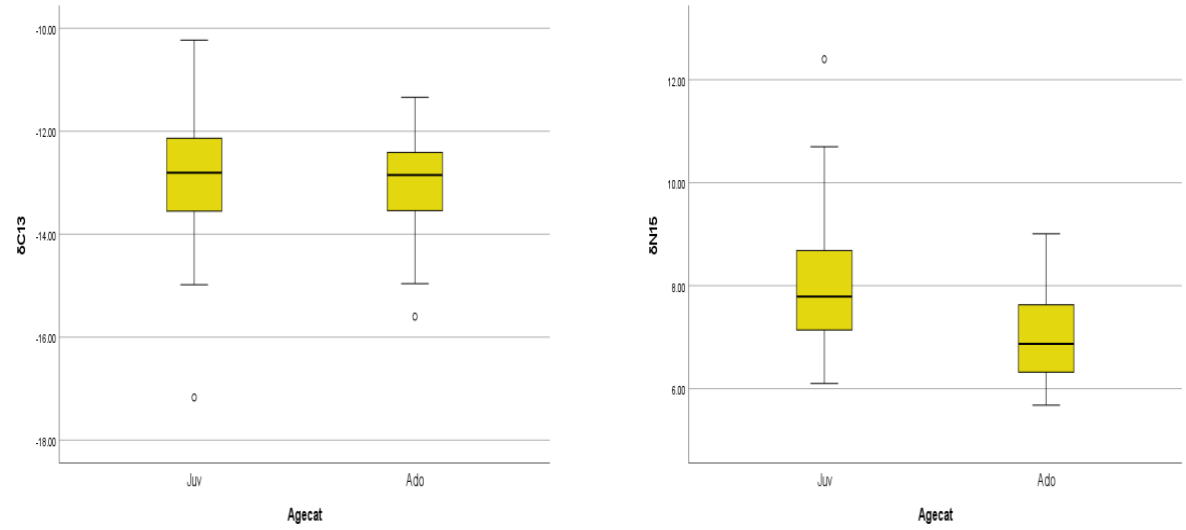

Figure 10. Box and whisker plots for the **A.** δ^{13}C and **B.** δ^{15}N for juveniles (Juv) and adolescents (Ado) without CO.

Bi-plots

To directly visualize the pattern between the isotopic values of juveniles with and without CO, a bi-plot in Figure 11 was constructed. Due to limited sample size of juveniles with CO,

there were less samples to compare with the ones without CO; however, the bi-plot can physically show similarities within the values, including any individuals who may be outliers to the general pattern. Figure 12 and 13 are additional bi-plots comparing the isotopic values of the age categories between the juveniles with and without CO to observe any patterns.

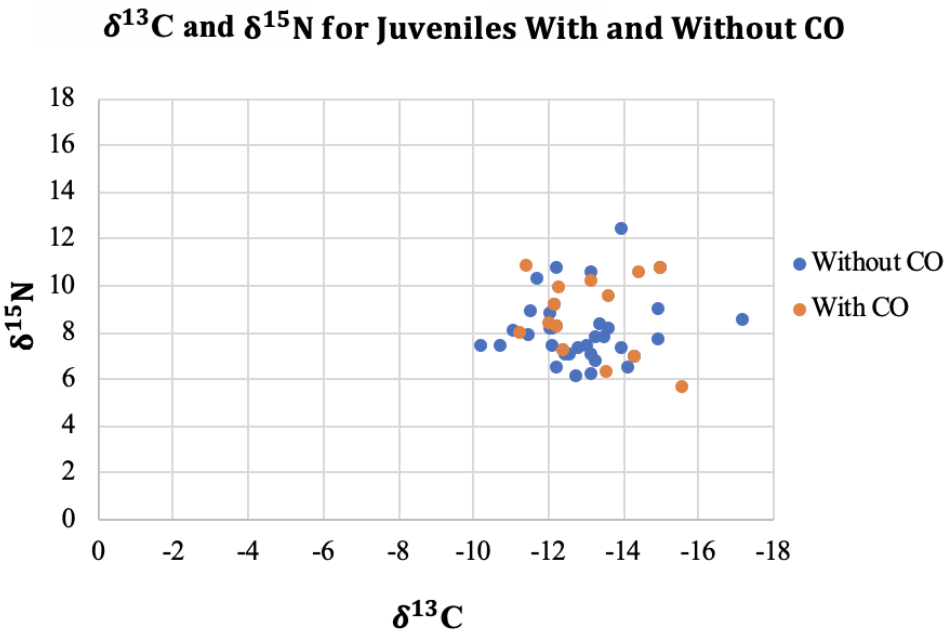

Figure 11. Bi-plot between δ^{13}C and δ^{15}N values of all juveniles with and without CO.

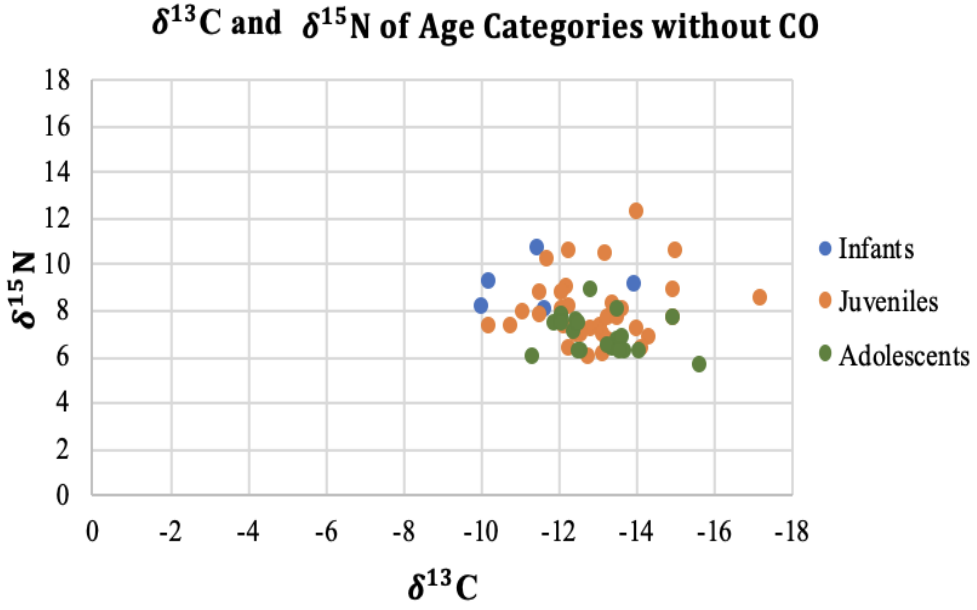

Figure 12. Bi-plot between δ^{13}C and δ^{15}N values of age categories without CO.

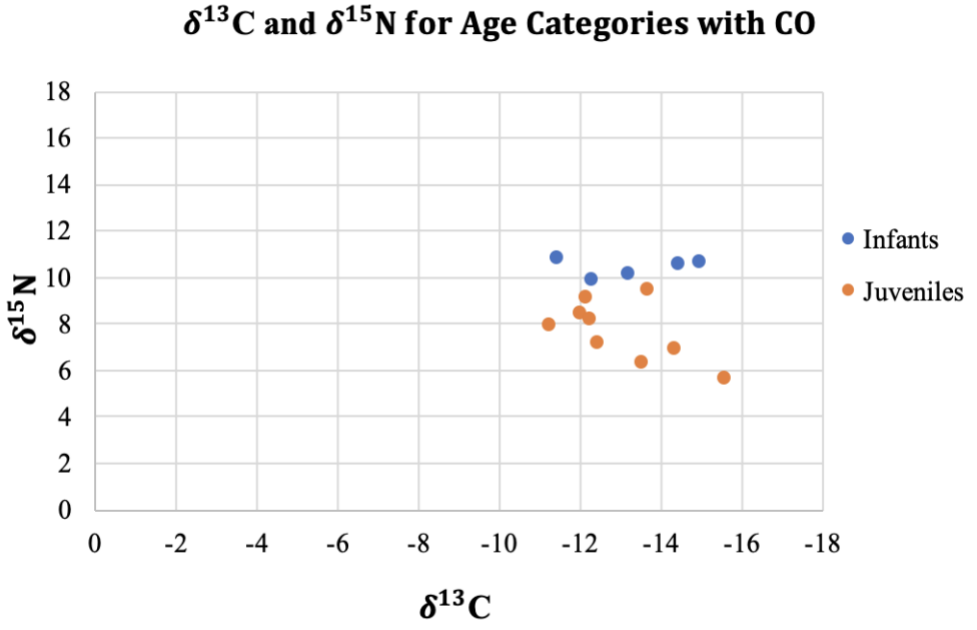

Figure 13. Bi-plot between δ^{13}C and δ^{15}N values of infants and juveniles with CO.

Statistical Analysis

In further determining whether diet is a factor of CO, a non-parametric statistical test was performed to compare the isotopic values of the juveniles. The Mann-Whitney U Test produced statistical values of significance for $\delta^{13}C$ and $\delta^{15}N$ isotopic values for juveniles with and without CO, which are summarized within Table 7. The $\delta^{13}C$ of the juvenile's diet is the same between the groups with and without CO, as the p-value was at a value of 0.572, greater than 0.05. However, the $\delta^{15}N$ isotopic values were significantly different between both groups as the p-value was less than 0.05. To develop a better understanding of the dietary variations of the juveniles, a Mann-Whitney U Test was performed to compare significance among the age groups (infants vs. infants, juveniles vs. juveniles, infants vs. juveniles, juveniles vs. adolescents, and infants vs. adolescents). Summary tables for all age groups are below in Tables 8 to 13.

Table 7. Summary table of $\delta^{13}C$ and $\delta^{15}N$ distribution between all age groups (infants, juveniles, and adolescents) with and without CO.

Distribution between with CO vs. Without CO	Mann-Whitney U Test Value of Significance	P-Value
$\delta^{13}C$	0.319	0.572
$\delta^{15}N$	3.027	0.082

For the infants with and without CO (Table 8), the p-value for the $\delta^{13}C$ of both groups was 0.222, which indicates the $\delta^{13}C$ of both diets are not statistically significant. The $\delta^{15}N$ of both diets are not statistically significant based on the p-value of 0.065. No statistical significance was also observed for the $\delta^{13}C$ and $\delta^{15}N$ of the diets of juveniles with and without CO; according to Table 9, the p-values of $\delta^{13}C$ and $\delta^{15}N$ between both groups were 0.856 and 0.727. The same age groups (infants vs. infants and juveniles vs. juveniles) with and without CO had no statistical significance between their diets when compared.

Table 8. Summary table of $\delta^{13}C$ and $\delta^{15}N$ distribution among infants with and without CO.

Distribution between Infants with and without CO	Mann-Whitney U Test Value of Significance	P-Value
$\delta^{13}C$	11.000	0.222
$\delta^{15}N$	33.00	0.065

Table 9. Summary table of $\delta^{13}C$ and $\delta^{15}N$ distribution among juveniles with and without CO.

Distribution between Juveniles with and without CO	Mann-Whitney U Test Value of Significance	P-Value
$\delta^{13}C$	155.500	0.856
$\delta^{15}N$	149.00	0.727

There was statistical significance observed in the $\delta^{15}N$ of the diets when the different age groups were compared among each other. Table 10 had p-values of 0.699 and <0.001 for the $\delta^{13}C$ and $\delta^{15}N$ of the diets of infants and juveniles with CO. Since the p-value of $\delta^{15}N$ was less than 0.005, that portion of the diet for both groups were significantly different. Infants and juveniles without CO (Table 11) had a similar p-value of 0.699 for the $\delta^{13}C$ values of the diet but a p-value of 0.004 for $\delta^{15}N$, which indicated statistical significance. For Table 12, no statistical significance was observed among the $\delta^{13}C$ of the diets of infants and adolescents without CO, as the p-value was 0.103. A p-value of <0.001 for the $\delta^{15}N$ of the diet of both groups indicate statistical significance. Lastly, with the diets of juveniles and adolescents without CO being compared in Table 13, the p-values of $\delta^{13}C$ were 0.568, indicating no statistical significance. The p-value of $\delta^{15}N$ was 0.005, which is equivalent to the p-value threshold for statistical significance.

Table 10. Summary table of $\delta^{13}C$ and $\delta^{15}N$ distribution among infants and juveniles with CO.

Distribution between Infants and Juveniles with CO	Mann-Whitney U Test Value of Significance	P-Value
$\delta^{13}C$	26.000	0.699
$\delta^{15}N$	0.000	***<0.001***

Table 11. Summary table of $\delta^{13}C$ and $\delta^{15}N$ distribution among infants and juveniles without CO.

Distribution between Infants and Juveniles without CO	Mann-Whitney U Test Value of Significance	P-Value
$\delta^{13}C$	26.000	0.699
$\delta^{15}N$	52.000	0.004

Table 12. Summary table of $\delta^{13}C$ and $\delta^{15}N$ distribution among infants and adolescents without CO.

Distribution between Infants and Adolescents without CO	Mann-Whitney U Test Value of Significance	P-Value
$\delta^{13}C$	50.000	0.103
$\delta^{15}N$	3.000	***<0.001***

Table 13. Summary table of $\delta^{13}C$ and $\delta^{15}N$ distribution among juveniles and adolescents without CO.

Distribution between Juveniles and Adolescents without CO	Mann-Whitney U Test Value of Significance	P-Value
$\delta^{13}C$	343.500	0.568
$\delta^{15}N$	206.500	0.005

To explore the multiple anthropological questions on the origin or patterns of CO, critical analyses of the results need to take place. Therefore, the following chapter will discuss the significant interpretation of the results and what the overall interpretation signifies towards the relationship between CO and nutrition.

CHAPTER 5. DISCUSSION

This chapter discusses and interprets the results obtained through stable isotope analysis and statistical tests in relation to diet and health. In comparing the stable isotopic ratios for juveniles with and without CO, the relationship of nutrition to CO could be determined and examined further. In addition, the determination of dietary variation based on stable isotope values amongst the age cohorts (infants, juveniles, and adolescents) could develop a greater understanding between the stable isotopic compositions in each age group and its relation to pathology.

1) Stable isotope Variation of All Juveniles With and Without Cribra Orbitalia

One possible cause of CO is anemia, an iron deficiency; however, its true origin is unknown. What are possible factors resulting in these pathological lesions? Since various studies have linked CO to nutritional deviancies, nutrition could be a possible factor. In identifying any nutritional relation to CO, stable isotope analyses were conducted between juveniles samples with and without CO.

These stable isotopic values were initially analyzed through descriptive statistics (average, standard deviation, and range). Table 4 compares the statistical findings between juveniles with and without CO. In general, the bone samples of juveniles without CO obtained an average δ^{13}C value of –12.85‰ and average δ^{15}N value of +7.90‰. For juveniles with CO, the δ^{13}C value was –13.11‰ and average δ^{15}N value was +8.86‰. The average of the carbon isotopic values for both groups were similar, which can be seen in the box and whisker plot in Figure 4. The range in variation was larger for the juveniles with CO compared to the range of variation for juveniles without CO within the box and whisker plot.

An outlier is observed within the bone samples of juveniles with CO; the outlier was K-442 with a $\delta^{13}C$ value of –15.60‰, which was beyond the range of variation. With juveniles without CO, the average carbon values reflected a diet composed of a mix of C_3 and C_4 plants; however, the juvenile (K-442) had a carbon value reflecting a diet composed of a higher proportion of C_3 plants, which have $\delta^{13}C$ values between –15‰ and –18‰ (Szpak et al., 2013). Visual differences can be observed in Figure 4 between both groups, as the average of $\delta^{15}N$ values for juveniles with CO was lower than the average for juveniles without CO. Unlike the ranges for the carbon isotopic values, the $\delta^{15}N$ values for both groups fell within the same range of +6‰ and +11‰.

The bi-plot in Figure 11 visualizes the dietary patterns among the two groups. A few data points of juveniles with CO had $\delta^{13}C$ values within a range of –11‰ and –12‰, but the rest of the data points scattered towards more negative carbon isotopic values. For juveniles without CO, the data points were evenly distributed within a range of –10‰ and –15‰. Overall, the carbon isotopic values represent those of C_4 plants, which fall within a range of –8‰ and –15‰ for plants in the Andes (Toyne et al., 2020). This diet could include some portion of kiwicha and maize, which are the main domesticated C_4 plant sources in the Chachapoyas region of Peru (Michell, 2018; Toyne et al., 2020; Toyne et al., 2016).

For the groups with and without CO, the $\delta^{15}N$ values were evenly distributed and overlap. Nitrogen isotopic values reflect protein consumption within a diet, and the nitrogen isotopic values could indicate a slightly higher protein consumption for the juveniles with CO. Nutritional, physiological, and biological stress are reflective of enriched nitrogen values. Based on a study conducted by Deschner et al. (2011), $\delta^{15}N$ values of nutritionally stressed bonobos within a controlled feeding environment were higher. Even though there was no major difference

among the δ^{15}N values between all the juveniles, juveniles with CO had slightly higher δ^{15}N values, which can be a result of nutritional or biological stress (Toyne and Turner, 2020).

In determining whether there is any statistical difference between the diets of the isotopic compositions, a Kruskal-Wallis Test was performed. The p-value for significance for δ^{13}C values was 0.572, while the value for δ^{15}N values was 0.082, as shown in Table 4. Thus, no statistical difference was observed among the diets of juveniles with and without CO, indicating there is no nutritional relation to CO. However, the sample size for juveniles with CO is smaller compared to those without CO, which might be a factor affecting statistical results. Therefore, dietary variation was compared within the age groups to observe any major differences that could have affected the general isotopic values.

Relationship of Nutrition to Cribra Orbitalia

After interpretation of the statistical analyses obtained, nutrition does not play a factor in relation to the presence or absence of CO. Theoretically, the cause of CO has commonly been thought to be anemia, but the true cause of CO is unknown. Some studies have correlated CO to anemia, but others have linked it to other nutritional deficiencies such as vitamin B or C deficiency (Zarifa et al., 2016; Klaus, 2020;Walker et al., 2009). However, there are studies linking CO to a non-nutritional factor, such as geography (Scaffidi, 2020).

CO is a pathological bone condition, in which children with it might be ill and not feeling as well as a healthy child. With sickness, the diet changes to relieve any symptoms and make one feel better, but no statistical dietary variation existed in the research. This relates to the Osteological Paradox, which indicates that an ill individual may not survive for long enough for a pathological lesion to form, whereas individuals who actually survive and have skeletal lesions

were healthier (Wood et al., 1992). Many of the bone samples with CO were of juveniles that died at a different stage of their life; one juvenile (K-442) died between 10 to 13 years of age. They underwent adaptive plasticity, according to McFadden and Oxenham (2020); these ill individuals were able to adapt to the stressors surrounding their illness to survive. Relative to the research and the results, even though some of the juveniles had CO, through diet and stable isotope analysis, it was challenging to spot major dietary variations between juveniles without CO.

As previously mentioned in the literature review, Turner and Armelagos (2012) conducted a study on the association of CO and SI within a population of skeletal remains in Macchu Picchu, Peru. Unlike the current research presented with the use of $\delta^{13}C$ and $\delta^{15}N$ isotopic values, their study focused on a multi-isotope analysis of Strontium ($\delta^{87}Sr/ \delta^{86}Sr$), Oxygen ($\delta^{18}O$), and lead ($\delta^{20n}Pb/ \delta^{204}Pb$) to identify nonspecific factors of porotic hyperostosis (PH) and CO. Not only did their study determine whether there was a relationship between CO and nutrition, but it also identified any association with residential etiologies. Using the Kruskall Wallis Test and other statistical parameters, the researchers concluded that there was a relationship between the residential origin of an individual rather than diet. The research presented in this paper had a similar conclusion between the association of CO and diet; however, other isotopic variables were not used to determine residential association, which could be part of a future direction.

Stable Isotope Variation Among the Age Groups

In comparing the dietary compositions between the different age cohorts, the transitions of diets through each age group could statistically and visually be observed from breastfeeding to

weaning and childhood dietary regimes. It was established with the first research question that there is no association between CO and SI. Since CO is not a factor in diet, could age be one? To determine the relation between age and dietary patterns, a comparison between the stable isotopic composition of the three age groups – infants, juveniles, and adolescents – of children without CO will be conducted. Now, what happens when age and CO together are compared? Since there was not a relationship between CO and nutrition, could there be one between CO and age? In finding these answers, the three age groups of children with CO will be compared and compared as well to the children without to determine if CO is perhaps a major factor.

2) Age Groups and Dietary Patterns

All Age Groups without CO

The $\delta^{13}C$ values for all age groups without CO were similar and statistically not significant. The average $\delta^{13}C$ value for infants without CO was –11.5‰. Even though infants were not consuming solid foods at that stage, their mother's nutrition contributes to the nutrition they were receiving. The carbon isotopic values fall within the source of C_4 plants between –15‰ to –8‰, which typically included maize, potatoes, yuca, kiwicha, quechua, and other types of tropical grasses (Michell, 2018; Toyne et al., 2016; Szpak et al., 2013). Some outliers existed for the carbon and nitrogen values of juveniles without CO. Juvenile sample K-299 had a $\delta^{13}C$ value of –17.17‰, while the average carbon value for the entire sample of juveniles without CO was –12.92‰, as shown in Table 6. The carbon value reflects that of higher contributions of C_3 plants (succulents and cacti), which have a range between –-18‰ to –15‰ (Toyne et al., 2020); marine sources have carbon values near that of C_4 plants (Larsen, 2015). However, the $\delta^{15}N$ values differed between the age groups without CO.

During the infancy period, infants consume all their nutrients from that of their mother. As breastfeeding continues, the infants are higher on the food chain at a trophic level above their mothers, resulting in higher δ^{15}N values (Fuller et al., 2006). These nitrogen isotopic values reflect protein consumption, which infants were directly getting from their diet from consuming their mother's tissues when being breasted, therefore, elevating their protein intake (Fuller et al., 2006). When infants move into the weaning stage as juveniles, the δ^{15}N values begin to decline due to the introduction of solid foods. Within this stage, the isotopic values can be seen in Figure 8 to shift among juveniles without CO, as their diets are dependent on resources available, as well as nutritional choices made by their caregivers.

A dietary pattern of lower δ^{15}N values was observed among the age groups without CO (infants, juveniles, and adolescents), which may be relative to the decreased dependency of a child to their caregiver, as well as the food choices made available to the Chachapoya people. Chachapoyas focused on subsistence practice relative to hunting and tending domesticated animals (guinea pigs and camelids) for protein source, as well as cultivation of grains and C_3 and C_4 plants (Church and Von Hagen, 2008). As children transition into the adolescent stage, more dietary freedom on supplementary foods may be available. According to Turner and colleagues (2017), the introduction of solid foods and the end of breastfeeding causes a decrease in δ^{15}N values as the diet becomes that similar to adults and late juveniles and adolescents. This is indicated in Figure 12, as the majority of juveniles and adolescents have δ^{15}N values that fall within the range of +6‰ and +8‰, while infants are observed having δ^{15}N values averaging above +8‰.

A commonality between juveniles and adolescents is that solid foods are assumed to be part of their diets. A slight difference, however, is that adolescents may have had more freedom

in regards to their diet compared to juveniles. Juveniles are still young, so they have some dependence on their caregivers and the food the caregivers provide for them. The δ^{15}N values based on the bi-plot in Figure 9 are quite similar, inferring that the juveniles and adolescents had similar plant and animal resources available to them, especially with cultivated techniques and hunting in Chachapoyas (Michell, 2018).

The δ^{15}N values of adolescents were lower than those of juveniles. The diet of juveniles evolve as new foods and beverages are introduced as they become older. With these new foods, microbes are introduced to the body, causing biological stress on the body, including potentially elevating δ^{15}N values (Toyne and Turner, 2020). Based on the Mann-Whitney U Test, a statistical value of 0.005 was obtained for the δ^{15}N values, indicating a statistical difference for the nitrogen isotopic values of juveniles and adolescents without CO. The protein portion of the diet might possibly have differed between juveniles and adolescents, but there is also a possibility that the diets were similar and the environmental stresses encountered were differed. Biological stress contributes to the introduction of microbes within new supplementary foods within the diets of juveniles and children, causing an enrichment in δ^{15}N values, which is represented in Figure 10 with juvenile δ^{15}N values being higher than that of adolescents (Toyne and Turner, 2020).

Similar to that of juveniles, adolescents have more dietary variation compared to infants. With this, the diets of infants and adolescents without CO were different statistically with a statistical value of <0.001, specifically in their δ^{15}N values. The average δ^{15}N value of adolescents was lower than that of infants; this can be explained from the higher trophic level of infants from breastfeeding and the possible reduction in protein intake of adolescents due their growth and development (Williams et al., 2007; Fuller et al., 2006). Another outlier was juvenile

sample K-282 with a δ^{15}N value of +12.40‰; the average δ^{15}N value was +8.07‰. The possibility of the high nitrogen values could be due to nutritional or pathophysiological stress; even though, the juvenile did not have CO, the juvenile could have possibly been ill and suffer from a different pathological condition. Also, environmental stress is another possible factor of enriched nitrogen values; coastal regions have higher values. Therefore, K-282 could have relocated to Kuelap from living at a coastal region just before death (Scaffidi, 2020).

3) Age Groups and CO Status

Infants and Juveniles with CO

Due to the dietary and social transitions from infancy to the juvenile stage, the δ^{13}C and δ^{15}N values between infants and juvenile with CO are significantly different. The δ^{13}C values for the juveniles with CO were higher than for the infants with CO, which is reflective of the introduction to solid foods. More plant and nutritional sources are part of the nutritional regimes for juveniles compared to that of infants. Juveniles with CO may have had a diet slightly different from a healthy child, as more nutritional and medicinal food (i.e., soup) could have been offered in the diet for an ill child. After comparative analysis through Mann-Whitney U test, the δ^{13}C values between infants and juveniles with CO were not distinct, despite some visual differences in the values with the box and whisker plots in Figure 6.

In comparing the δ^{15}N values, the Mann-Whitney U Test produced a test value of 0.001, which is indicative of a statistical difference between the infants and juveniles with CO. The infants with CO had higher δ^{15}N values compared to the juveniles with CO. Lactation is the main contributing factor to these values, but nutritional or other types of stress, as mentioned before, are also possible. With juveniles consuming solid food, many choices could be made relative to

the food's nutritional value to the body. In addition, rapid growth of a child from infancy to the juvenile stage could result in lower δ^{15}N values, as consumed protein will be used in tissue building (Williams et al., 2007). Therefore, the significant difference in N conforms to expectations.

All Age Groups Regardless of CO

If stable isotopes are assumed to reflect only dietary resources, then the age groups of all samples show that infants have a different diet compared to juveniles and adolescents likely due to breastfeeding from their mothers. Broadly, age did differentiate SI. Very specifically, infants with CO were distinct isotopically from all other groups. This is dietary but also perhaps linked to elevated stress associated with the unknown casual factor for CO and infant physiology. The same was not seen when comparing with all juveniles with and without CO. So a clear dietary variation existed when comparing the age groups to infants, but juveniles and adolescents, regardless of CO, had similar dietary compositions. Therefore, when age and CO are combined relative to diet, the infants with CO have elevated δ^{13}C and δ^{15}N values compared to infants and the other age groups without it.

CHAPTER 6. CONCLUSION

The aim of this research was to explore the relationship between CO and nutrition, as well as age and dietary patterns through the stable isotope analysis of bone samples collected from Kuelap, Peru of juveniles with and without CO. The relationship was explored through the comparison of isotopic compositions of the different age cohorts (infants, juveniles, and adolescents). The anthropological questions were explored based on the results obtained from the research. Overall, this thesis was organized to answer the question that many anthropologists have attempted to find through their studies: does CO impact nutritional variation? However, it was determined that nutrition does not influence dietary stable isotope values, relative to the Osteological Paradox.

Since CO lesions are active in juveniles, bone samples of juveniles with and without CO were used for this research. Stable isotope analysis of the bone samples was conducted to reconstruct their diets and explore if there was a nutritionally related relationship. In statistically and visually comparing the two groups using the Mann-Whitney U Test and bi-plots, the $\delta^{13}C$ and $\delta^{15}N$ were similar, demonstrating there is no dietary variation between all juvenile samples with and without CO. This comparison alone indicates dietary sources does not play a strong role, but a dietary comparison between the different age cohorts indicates nutrition changes during early life. Based on the nitrogen isotopic values of infants, dietary variation existed in comparison to juveniles and adolescents due to breastfeeding being a major part of an infant's nutritional regime. Higher protein intake was a result of breastfeeding; however, with the introduction of supplementary foods after breastfeeding is complete, the protein intake decreases, resulting in lower nitrogen values for juveniles and adolescents. Additional reasons for higher isotopic values for infants could have been resulted from stress (nutritional, metabolic,

physiological, or environmental), especially with infants with CO. Carbon values among the age groups were not statistically significant, aside from outliers reflective of a coastal diet. Overall, nutrition is an underlying factor in isotopic variation as expected, but other factors are possible and could be determined.

Limitations

This research was successful. Improvements within the research could have been made for a better conclusive determination of the research questions and hypotheses tested.

Sample Size

Within the research study, a total of 79 juvenile bone samples were used, which is a sufficient sample; however, there was an inequivalent sample between juveniles with and without CO. A total of 65 bone samples of juveniles without CO were used to compare its isotopic variations with 14 bone samples of juveniles with CO. A larger sample size of juveniles with CO would possibly greatly define the statistical significance between the isotopic compositions of carbon and nitrogen of juveniles without CO. It would provide a better representation of juveniles with CO and their dietary variations compared to those without. Since CO lesions are rare at Kuelap, the bone sample of juveniles could also be reduced for a more equivalent comparison and representation. In addition, adolescents with CO were not present within the isotope sample set, so no comparison of dietary variation was able to be made with adolescents without CO for a better understanding of the role diet played in children with CO. Due to selective sampling, Dr. Toyne did not collect bone samples for all individuals with CO. It

is possible that there were adolescents with CO who could have been included within the isotope data set to further understand the relationship between dietary variation and age.

Type of Bone

In analyzing the stable isotope composition of juveniles with CO, rib bone samples were mostly used. However, CO is identified on the orbital roofs of the crania. Using a piece of the orbital roof in which the porotic lesion is visually present could possibly give a better representation of dietary reconstruction of juveniles with CO. In determining whether a difference in the stable isotope composition is obtained, a rib bone and orbital roof bone sample could be used from the same juvenile and the results could be compared (Fahy et al. 2017). Rib bones are the most common type of bone used in archaeological studies due to the higher frequency of them present in the human body.

CO Lesions

Porotic lesions on the orbital roofs of skeletal samples is a primary indication of CO; however, there are two possible reasons that could cause misclassification of skeletal samples as having CO. Since orbital roof bones are not always found during excavation for certain juvenile samples, the archaeologists will not be able to classify them as to having CO. Additionally, the lesions could have been healed or active at the time of death. These possibilities will prevent there from being a more accurate representation of statistical frequency of CO in Kuelap for juveniles and adults.

Future Directions

This research focused on identifying if there is a relationship between nutrition and CO through stable isotope analysis of juvenile bone samples from Kuelap, Peru. Even though nutrition did play a role in CO based on the dietary variation of infants between juveniles and adolescents, no statistical significance was observed among juveniles and adolescents, as well as the groups of juveniles with and without CO. In this case, there are other plausible factors in the production of CO lesions in children that work independently or with nutritional deficiencies.

According to Scaffidi (2020), CO is more prevalent in coastal regions and is linked to climatic and geographic variables. After determining CO's relationship with nutrition, more research could be conducted in determining the relationship of CO with geography within Kuelap, Peru. In doing so, stable isotope analysis could be conducted with other isotopic variables (strontium and oxygen) that might help determine if CO has any residential/environmental etiologies rather than nutritional. To determine the stable isotope compositions of the skeletal samples, tooth and hair samples could be used. These samples have geographical trace, which can further validate the residential etiologies of juveniles with CO by comparing them with juveniles without.

Rib bones were used in determining the anthropological questions of the study, but if bones with lesions were used, there might have been different results. Even though CO lesions are present on the orbital roofs of the crania, rib bone samples of juveniles with CO were used within the research due to a faster analysis and preparation time. However, it is a possibility that the use of the orbital roof bone where the CO lesion appeared could result in slightly different results of isotopic composition. In future research, the orbital roofs bones could be used in undergoing stable isotope analysis, and the stable isotopic compositions and statistical

significances could be compared with the present research. This could determine if any differing results will occur and help archaeologists develop a better understanding of the types of bone samples used and its effect on testing the research questions and hypotheses. It will further the idea of the relationship of CO with nutrition and with other possible factors.

Overall, this research was conducted to explore the relationship between nutrition and pathology. Despite CO being hypothetically caused by nutrition, SI analysis was used on skeletal samples of juveniles to determine the correlation between diet and CO. This research helped to either validate or refute the hypothetical cause and raise questions as to other possible factors. CO was determined to not be differentiated by SI, but age was tested as well to identify its relationship to diet. After the interpretation of the results of both research questions, it can be concluded that nutrition is not related to CO and that the diet of infants differ from juveniles and adolescents due to infant physiology and their nutritional regime. More specifically, SI and CO patterns are complicated, especially when combined; however, testing skeletal bone samples begins to clarify any archaeological questions researchers have.

APPENDICES

Appendix A: Sample List of Juveniles without CO including Age Estimate and Type of Bone

Sample ID	Age Categories	Estimated Age	Bone
K-07	INF	6 mos	rib
K-11	JUV	8-10 yrs	cranium
K-13	JUV	4-6 yrs	rib
K-15	ADO	12-15 yrs	cranium
K-28	JUV	6-8 yrs	tibia fragment
K-29	JUV	6-8 yrs	rib
K-31	JUV	4-5 yrs	cranium
K-32	INF	9 +-3mos	rib
K-39	JUV	9 +-3 yrs	rib
K-54	JUV	3 +/-1 yrs	rib
K-59	ADO	15 +/-3 yrs	cranium
K-65	JUV	2 yrs +/-8mos	rib
K-79	JUV	5 +/-1.5yrs	rad
K-80	JUV	8 +/-2 yrs	rib
K-101	ADO	10-15 yrs	rib
K-105	INF	18 +/-6mos	rib
K-109	JUV	4 +/- 1yr	rib
K-115	ADO	14-16 yrs	rib
K-122	INF	9 +-3 mos	rib
K-124	JUV	9 +- 3 yrs	rib
K-128a	JUV	8-10 yrs	rib
Kue-75	JUV	8-10 yrs	fibula
K-130	JUV	2 +/-8mos	rib
K-133	ADO	14-16 yrs	rib
K-135	INF	1 +/-4 mos	rib
K-145	JUV	-	rib
K-148	ADO	13-16 yrs	rib
K-228	INF	NB-6mos	humerus
K-253	JUV	7-9 yrs	radial fragment
K-255	JUV	5-7 yrs	rib
K-257	ADO	10-12 yrs	rib
K-259	JUV	2-4 yrs	rib
K-261	ADO	8-10 yrs	rib
K-263	ADO	10-12 yrs	rib
K-265	ADO	11-13 yrs	rib
K-267	ADO	16-18 yrs	rib
K-269	JUV	6-9 yrs	rib
K-271	JUV	2-4 yrs	rib
K-282	JUV	Juvenile	rib

K-289	INF	Infant	maxilla fragment
K-290	JUV	Juvenile	rib
K-296	JUV	Juvenile	maxilla fragment
K-299	JUV	Juvenile	mandible fragment
K-308	JUV	Juvenile	rib
K-309a	INF	Infant	rib
K-366	JUV	2-4 yrs	rib
K-368	JUV	2-3 yrs	rib
K-376	JUV	6-8 yrs	rib
K-378	JUV	5-7 yrs	rib
K-384	ADO	11-13 yrs	rib
K-388	JUV	7-9 yrs	rib
K-390	ADO	10-12 yrs	rib
K-402	ADO	13-15yrs	rib
K-406	JUV	8-10 yrs	rib
K-410	ADO	11-13 yrs	rib
K-416	ADO	14-16 yrs	rib
K-421	ADO	14-17 yrs	rib
K-423	ADO	12-15 yrs	rib
K-425	JUV	7-10 yrs	rib
K-428	ADO	8-12 yrs	rib
K-430	JUV	8-11 yrs	rib
K-433	JUV	7-10 yrs	rib
K-434	JUV	6-8 yrs	rib
K-440	ADO	8-11 yrs	rib
K-442	ADO	10-13 yrs	rib

Appendix B: Sample List of Juveniles with CO including Age Estimate and Type of Bone

Sample ID	Age Categories	Age	Bone
K-425	JUV	7-10 yrs	rib
K-440	JUV	8-11 yrs	rib
K-442	JUV	10-13 yrs	rib
K-280	INF	3-9 mos	rib
K-23	JUV	6-8 yrs	rib
K-25	JUV	2-4 yrs	cranium
K-47	JUV	3-5 yrs	rib
K-79	JUV	5 +/-1.5yrs	radius fragment
K-80	JUV	8 +/-2 yrs	rib
K-105	INF	18 +/-6mos	rib
K-135	INF	1 +/-4 mos	rib
K-130	INF	2 +/-8mos	rib
K-145	JUV	2-4 yrs	rib
K-146	INF	6 mos	rib

Appendix C: Stable Isotope δ^{13}C and δ^{15}N values for Juveniles without CO

Sample ID	δ^{13}Ccoll (‰, VPBD)	δ^{15}N (‰, AIR)
K-07	–11.19	+9.16
K-11	–12.12	+8.14
K-13	–11.10	+8.02
K-15	–11.34	+6.11
K-28	–11.52	+8.89
K-29	–12.08	+8.14
K-31	–14.95	+8.96
K-32	–10.18	+9.34
K-39	–12.78	+6.10
K-54	–11.71	+10.28
K-59	–13.49	+8.15
K-65	–12.26	+10.70
K-79	–12.17	+9.13
K-80	–12.26	+8.19
K-101	–12.10	+7.75
K-105	–13.18	+10.19
K-109	–12.05	+8.83
K-115	–12.55	+6.27
K-122	–14.70	+9.98
K-124	–12.83	+7.31
K-128a	–10.75	+7.36
Kue-75	–11.49	+7.91
K-130	–14.98	+10.70
K-133	–12.85	+9.01
K-135	–11.44	+10.81
K-145	–14.34	+6.90
K-148	–11.86	+7.47
K-228	–10.02	+8.29
K-253	–13.06	+7.39
K-255	–13.26	+7.79
K-257	–12.41	+7.15
K-259	–14.96	+7.72
K-261	–14.96	+7.72
K-263	–12.09	+7.86
K-265	–12.54	+6.31
K-267	–13.50	+6.77
K-269	–14.16	+6.43
K-271	–13.62	+8.14
K-282	–13.99	+12.40
K-289	–11.63	+8.09
K-290	–13.18	+10.57
K-296	–13.48	+7.79
K-299	–17.17	+8.54
K-308	–13.41	+8.30
K-309a	–13.97	+9.18
K-366	–13.13	+6.21
K-368	–12.15	+7.43
K-376	–13.15	+6.99
K-378	–12.25	+6.44
K-384	–13.41	+6.42

K-388	–12.57	+7.05
K-390	–14.08	+6.35
K-402	–13.61	+6.87
K-406	–10.23	+7.40
K-410	–12.46	+7.63
K-416	–12.08	+7.56
K-421	–13.72	+6.25
K-423	–12.52	+7.56
K-425	–12.41	+7.23
K-428	–13.25	+6.55
K-430	–13.98	+7.29
K-433	–13.28	+6.76
K-434	–12.46	+7.00
K-440	–13.54	+6.32
K-442	–15.60	+5.68

Appendix D: δ^{13}C and δ^{15}N values for juveniles with CO

Sample ID	δ^{13}Ccoll (‰, VPBD)	δ^{15}N (‰, AIR)
K-425	–12.41	+7.23
K-440	–13.54	+6.32
K-442	–15.60	+5.68
K-280	–14.42	+10.56
K-23	–12.01	+8.46
K-25	–11.26	+7.93
K-47	–13.65	+9.52
K-79	–12.17	+9.13
K-80	–12.26	+8.19
K-105	–13.18	+10.19
K-135	–11.44	+10.81
K-130	–14.98	+10.70
K-145	–14.34	+6.90
K-146	–12.29	+9.87

REFERENCES

Ambrose, S. H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science*, *17*(4), 431-451. https://doi.org/https://doi.org/10.1016/0305-4403(90)90007-R

Ambrose, S. H., & Norr, L. (1993). Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In *Prehistoric human bone: Archaeology at the molecular level* (pp. 1–37). Springer, Berlin, Heidelberg. Retrieved from https://doi.org/10.1007/978-3-662-02894-0_1.

Andrushko, V. A., & Messer, D. (2011). The pathological consequences of cranial vault modification: an analysis of human skeletal remains from Cuzco, Peru. *American Journal of Physical Anthropology*, *144*.

Baitzel, S. (2018). Parental grief and mourning in the ancient Andes. *Journal of Archaeological Method and Theory*, *25*(2), 1-24. https://doi.org/10.1007/s10816-017-9333-3

Beauchesne, P., Agarwal, S. C., & Larsen, C. S. (2018). *Children and childhood in Bioarchaeology*. *Oxford Academic*. University Press of Florida. Retrieved from https://doi.org/10.5744/florida/9780813056807.001.0001.

Blom, D. E., Buikstra, J. E., Keng, L., Tomczak, P. D., Shoreman, E., & Stevens-Tuttle, D. (2005). Anemia and childhood mortality: Latitudinal patterning along the coast of pre-Columbian peru. *American Journal of Physical Anthropology*, *127*(2), 152–169. https://doi.org/10.1002/ajpa.10431

Bolin, I. (2006). *Growing up in a culture of respect: Child rearing in Highland Peru*. *JSTOR*. University of Texas Press. Retrieved from https://doi.org/10.7560/709829.

Bogin, B. (1997). Evolutionary hypotheses for human childhood. *American Journal of Physical Anthropology*, *104*(25), 63-89. https://doi.org/https://doi.org/10.1002/(SICI)1096-8644(1997)25+<63::AID-AJPA3>3.0.CO;2-8

Brickley, M. B. (2018). Cribra orbitalia and porotic hyperostosis: A biological approach to diagnosis. *American Journal of Physical Anthropology*, *167*(4), 896-902. https://doi.org/https://doi.org/10.1002/ajpa.23701

Church, W. B., & Hagen, A. V. (2008). Chachapoyas: cultural development at an Andean cloud forest crossroads. In *Handbook of South American Archaeology* (pp. 903–926). Springer New York.

Cole, G., & Waldron, T. (2019). Cribra orbitalia: dissecting an ill-defined phenomenon. *International Journal of Osteoarchaeology*, *29*(4), 613–621. https://doi.org/10.1002/oa.2757

Curto, A. (2019). Diet and disease in Tomar, Portugal. In *Annual meeting of the European Association of Archaeologists*.

Denierio, M. (2018). *Feeding the Children: A Paleodietary reconstruction of Juveniles from Kuelap, Peru* (thesis). STARS.

Deschner, T., Fuller, B. T., Oelze, V. M., Boesch, C., Hublin, J.-J., Mundry, R., Richards, M. P., Ortmann, S., & Hohmann, G. (2012). Identification of energy consumption and nutritional stress by isotopic and elemental analysis of urine in bonobos (*pan paniscus*). *Rapid Communications in Mass Spectrometry*, *26*(1), 69–77. https://doi.org/10.1002/rcm.5312

Facchini, F., Rastelli, E., & Brasili, P. (2004). Cribra Orbitalia and Cribra cranii in roman skeletal remains from the Ravenna area and rimini(i–IV century AD). *International Journal of Osteoarchaeology*, *14*(2), 126–136. https://doi.org/10.1002/oa.717

Fuller, B. T., Fuller, J. L., Harris, D. A., & Hedges, R. E. M. (2006). Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotope ratios. *American Journal of Physical Anthropology*, *129*(2), 279–293. https://doi.org/10.1002/ajpa.20249

Gil, A. F., Neme, G. A., Tykot, R. H., Novellino, P., Cortegoso, V., & Durán, V. (2009). Stable isotopes and maize consumption in central western Argentina. *International Journal of Osteoarchaeology*, *19*(2), 215–236. https://doi.org/10.1002/oa.1041

Guengerich, A. (2014). The architect's signature: the social production of a residential landscape at Monte Viudo, Chachapoyas, Peru. *Journal of Anthropological Archaeology*, *34*, 1-16. https://doi.org/https://doi.org/10.1016/j.jaa.2013.12.005

Halcrow, S. E., & Ward, S. M. (2018). Children in Bioarchaeology and Forensic Anthropology. In *Encyclopedia of Global Archaeology* (pp. 1-6).

Hobson, K. A., Alisauskas, R. T., & Clark, R. G. (1993). Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. *The Condor*, *95*(2), 388–394. https://doi.org/10.2307/1369361

Katzenberg, M. A. (2008). Stable isotope analysis: a tool for studying past diet, demography, and life history. In *Biological Anthropology of the Human Skeleton* (2 ed., pp. 411-441). John Willey & Sons, Inc.

Katzenberg, M. A., & Lovell, N. C. (1999). Stable isotope variation in Pathological Bone. *International Journal of Osteoarchaeology*, *9*(5), 316–324. https://doi.org/10.1002/(sici)1099-1212(199909/10)9:5<316::aid-oa500>3.0.co;2-d

Katzenberg, M. A., & Waters-Rist, A. L. (2019). *Biological anthropology of the human skeleton* (3rd ed.). John Wiley & Sons Inc.

Klaus, H. D. (2020). Metabolic diseases in Andean paleopathology: Retrospect and prospect. *International Journal of Paleopathology*, *29*, 54-64. https://doi.org/https://doi.org/10.1016/j.ijpp.2019.06.008

Klinken, G. J. V. (1999). Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *Journal of Archaeological Science*, *26*(6), 687–695. https://doi.org/10.1006/jasc.1998.0385

Koci, M. (2021). *Understanding the Dietary Behavior of a 7th - 8th Century Avar Community from Jagodnjak, Croatia Using Stable Isotope Analysis of Bone Collagen* (thesis). STARS.

Kusaka, S., Hyodo, F., Yumoto, T., & Nakatsukasa, M. (2010). Carbon and nitrogen stable isotope analysis on the diet of jomon populations from two coastal regions of Japan. *Journal of Archaeological Science*, *37*(8), 1968–1977. https://doi.org/10.1016/j.jas.2010.03.002

Larsen, C. S. (2015). *Bioarchaeology: Interpreting behavior from the human skeleton* (2nd ed.). Cambridge University Press. Retrieved from https://doi.org/10.1017/CBO9781139020398.

Lewis, M. E. (2006). *The bioarchaeology of children: Perspectives from biological and forensic anthropology*. Cambridge University Press. Retrieved from https://doi.org/10.1017/CBO9780511542473.

Longin, R. (1971). New method of collagen extraction for radiocarbon dating. *Nature*, *230*, 241–242. https://doi.org/10.1038/230241a0

MacFarland, T. W., & Yates, J. M. (2016). *Introduction to nonparametric statistics for the Biological Sciences using R* (1st ed.). Springer Cham. Retrieved from https://doi.org/10.1007/978-3-319-30634-6.

McFadden, C., & Oxenham, M. F. (2020). A paleoepidemiological approach to the osteological paradox: Investigating stress, frailty and resilience through CRIBRA orbitalia. *American Journal of Physical Anthropology*, *173*(2), 205–217. https://doi.org/10.1002/ajpa.24091

Michell, S. (2018). *Animals of the Cloud Forest: Isotopic Variation of Archaeological Faunal Remains from Kuelap, Peru* (thesis). STARS.

Muccio, Z., & Jackson, G. P. (2009). Isotope ratio mass spectrometry. *Analyst*, *134*(2), 213–222. https://doi.org/https://doi.org/10.1039/B808232D

Nagaoka, T., Seki, Y., Uzawa, K., Takigami, M., & Morales Chocano, D. (2017). Prevalence of Cribra Orbitalia in Pacopampa during the formative period in Peru. *Anatomical Science International*, *93*(2), 254–261. https://doi.org/10.1007/s12565-017-0404-z

Narváez, A. (1988). Kuélap: una ciudad fortificada en los Andes nor-orientales de Amazonas, Perú. *Arquitectura y Arqueología*, 115-142.

Ogawa, N. O., Nagata, T., Kitazato, H., & Ohkouchi, N. (2010). Ultra-sensitive elemental analyzer/ isotope ratio mass spectrometer for stable nitrogen and carbon isotope analyses. In *Earth, life, and Isotopes* (pp. 339–353). Kyoto University Press.

Ortner, D. J. (2003). *Identification of pathological conditions in human skeletal remains* (2nd ed.). Academic Press.

Ortner, D. J. (2011). What skeletons tell us. the story of human paleopathology. *Virchows Archiv*, *459*(3), 247–254. https://doi.org/10.1007/s00428-011-1122-x

Oxenham, M., & Cavill, I. (2010). Porotic hyperostosis and Cribra Orbitalia: The erythropoietic response to iron-deficiency anaemia. *Anthropological Science*, *118*(1), 199–200. https://doi.org/10.1537/ase.100302

Reitsema, L., & Holder, S. (2018). Stable isotope analysis and the study of human stress, disease, and Nutrition. *Bioarchaeology International*, *2*(2), 63–74. https://doi.org/10.5744/bi.2018.1018

Reitsema, L. J. (2013). Beyond diet reconstruction: Stable isotope applications to human physiology, health, and nutrition. *American Journal of Human Biology*, *25*(4), 445-456. https://doi.org/10.1002/ajhb.22398

Scaffidi, B. K. (2020). Spatial paleopathology: A geographic approach to the etiology of cribrotic lesions in the prehistoric Andes. *International Journal of Paleopathology*, *29*, 102-116. https://doi.org/10.1016/j.ijpp.2019.07.002

Snoddy, A. M., Buckley, H. R., Elliott, G. E., Standen, V. G., Arriaza, B. T., & Halcrow, S. E. (2018). Macroscopic features of scurvy in human skeletal remains: A literature synthesis and diagnostic guide. *American Journal of Physical Anthropology*, *167*(4), 876–895. https://doi.org/10.1002/ajpa.23699

Steyn, M., Voeller, S., Botha, D., & Ross, A. H. (2016). Cribra Orbitalia: Prevalence in contemporary populations. *Clinical Anatomy*, *29*(7), 823–830. https://doi.org/10.1002/ca.22734

Sutton, M. Q. (2020). *Bioarchaeology: An introduction to the archaeology and anthropology of the dead* (1st ed.). Taylor & Francis Group. Retrieved from https://doi.org/10.4324/9781351061117.

Szpak, P., White, C. D., Longstaffe, F. J., Millaire, J.-F., & Vásquez Sánchez, V. F. (2013). Carbon and nitrogen isotopic survey of northern Peruvian plants: Baselines for Paleodietary and paleoecological studies. *PLoS ONE*, *8*(1). https://doi.org/10.1371/journal.pone.0053763

Toyne, J. M. (2015). Tibial Surgery in ancient Peru. *International Journal of Paleopathology*, *8*, 29–35. https://doi.org/10.1016/j.ijpp.2014.09.002

Toyne, J. M., & Narváez Vargas, A. (2017). Un análisis bioarqueológico de los entierros en Kuélap, UN Acercamiento a Los patrones de Morfología y vida. *Boletín De Arqueología PUCP*, (23), 159–186. https://doi.org/10.18800/boletindearqueologiapucp.201702.005

Toyne, J. M., & Turner, B. L. (2020). Linking isotope analysis and paleopathology: An Andean perspective. *International Journal of Paleopathology*, *29*, 117–127. https://doi.org/10.1016/j.ijpp.2019.11.002

Toyne, J. M., Church, W. B., Luis Coronado Tello, J., & Morales Gamarra, R. (2016). Exploring imperial expansion using an isotopic analysis of paleodietary and paleomobility indicators in Chachapoyas, Peru. *American Journal of Physical Anthropology*, *162*(1), 51–72. https://doi.org/10.1002/ajpa.23085

Toyne, J. M., Michell, S., Denierio, M., & Narváez Vargas, L. A. (2020). Examining pre-Hispanic human and animal isotopic variation at Kuelap in the eastern montane of Peru. *Quaternary International*, *548*, 57–70. https://doi.org/10.1016/j.quaint.2020.03.046

Tran, V. (2016). *Patterns in Dental Health: Investigating Dental Disease at the Archaeological Site of Kuelap in Chachapoyas, Peru* (thesis). STARS.

Turner, B. L., & Armelagos, G. J. (2012). Diet, residential origin, and pathology at Machu Picchu, Peru. *American Journal of Physical Anthropology*, *149*(1), 71–83. https://doi.org/10.1002/ajpa.22096

VanValkenburgh, P., Cushman, K. C., Butters, L. J., Vega, C. R., Roberts, C. B., Kepler, C., & Kellner, J. (2020). Lasers without lost cities: Using drone lidar to capture architectural complexity at Kuelap, Amazonas, Peru. *Journal of Field Archaeology*, *45*(1), 75–88. https://doi.org/10.1080/00934690.2020.1713287

Walker, P. L., Bathurst, R. R., Richman, R., Gjerdrum, T., & Andrushko, V. A. (2009). The causes of porotic hyperostosis and Cribra Orbitalia: A reappraisal of the iron-deficiency-anemia hypothesis. *American Journal of Physical Anthropology*, *139*(2), 109–125. https://doi.org/10.1002/ajpa.21031

Wayne, W. L. (2017). *Mann Whitney U Test (Wilcoxon Rank Sum Test)*. Boston University School of Public Health. https://sphweb.bumc.bu.edu/otlt/mph-modules/bs/bs704_nonparametric/bs704_nonparametric4.html

Welcker, H. (1888). Cribra orbitalia, ein ethnologisch-diagnostisches mermal am schadel mehrere mensch-rassen. *Archiv Fur Anthropologie*, *17*, 1-18.

Williams, C. T., Buck, C. L., Sears, J., & Kitaysky, A. S. (2007). Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. *Oecologia*, *153*(1), 11–18. https://doi.org/10.1007/s00442-007-0717-z

Wood, J. W., Milner, G. R., Harpending, H. C., Weiss, K. M., Cohen, M. N., Eisenberg, L. E., Hutchinson, D. L., Jankauskas, R., Cesnys, G., Katzenberg, M. A., Lukacs, J. R., McGrath, J. W., Roth, E. A., Ubelaker, D. H., & Wilkinson, R. G. (1992). The osteological paradox: Problems of inferring prehistoric health from skeletal samples . *Current Anthropology*, *33*(4), 343–370. https://doi.org/10.1086/204084

Zariņa, G., Sholts, S. B., Tichinin, A., Rudovica, V., Vīksna, A., Engīzere, A., Muižnieks, V., Bartelink, E. J., & Wärmländer, S. K. T. S. (2016). Cribra Orbitalia as a potential indicator of childhood stress: Evidence from paleopathology, stable C, N, and O isotopes, and trace element concentrations in children from a 17 th □18 th century cemetery in jŀkabpils, Latvia. *Journal of Trace Elements in Medicine and Biology*, *38*, 131–137. https://doi.org/10.1016/j.jtemb.2016.05.008

Zdilla, M. J., Nestor, N. S., Rothschild, B. M., & Lambert, H. W. (2021). Cribra Orbitalia is correlated with the meningo-orbital foramen and is vascular and developmental in nature. *The Anatomical Record*, *305*(7), 1629–1671. https://doi.org/10.1002/ar.24825