The Early Childhood Diet of Adult Individuals with Evidence of Metabolic Diseases from Commingled Remains at Tell Hisban

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ABSTRACT

In the 19th century, many unknown agropastoralists were buried at Tell Hisban in Jordan. Many of these individuals have a high frequency of rickets (60% between birth and 2 years) during infancy and childhood. Further research of the assemblage discovered that many of the adults had evidence of interglobular dentin (IGD), indicating that many individuals of the population survived at least one IGD-forming condition, possibly rickets, during childhood. Although childhood rickets typically derive from a lack of sunlight, which synthesizes the amount of vitamin D necessary for normal bodily functioning, the timing and duration of breastfeeding can also impact the risk for vitamin D deficiency in infancy. Carbon and nitrogen stable isotopic analysis of incremental dental dentin samples from 7 adult individuals with and without evidence of IGD created a timeline of their childhood diet to identify dietary changes

during early life. Indication of the weaning process was difficult to analyze due to the size of incremental samples, but fluctuations in isotopic ratios showed individuals experienced significant amounts of stress due to climatic shifts and tissue catabolism. Future research will focus on the stable isotope analysis of the infant teeth of the Hisban sample to provide more insight into the weaning process and early diet

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CHAPTER 1: INTRODUCTION

In the Late 19th century, an unidentified population utilized a storeroom in Tell Hisban, Jordan, to bury at least 52 individuals, many of whom were young children who died between birth and 2 years of age (Perry & Edwards, 2021; Walker, 2001). Subsequent research found a high prevalence of metabolic diseases among individuals within the assemblage, including rickets. Rickets, a metabolic disease, can result from low levels of ultraviolet (UV) radiation and lacking vitamin D-rich dietary sources, including breast milk, during infancy. While no active skeletal rickets were present in individuals over the age of 2 years, many non-infants exhibited evidence of childhood rickets in their dentin in the form of interglobular dentin (IGD) (Perry et al., 2021).

Research has suggested that the timing of the weaning process can significantly impact an infant's chances of developing metabolic diseases, including rickets, due to high nutrient demands between six and 18 months of age (Ventadas et al., 2020; Waters-Rist et al., 2018). At six months of age, infants require higher amounts of iron than that provided in breast milk alone (Wharton, 1989; Stantis et al., 2020; Kendall et al., 2011). Thus, the timing and duration of the weaning process and the types of foods introduced during this transition play a significant role in determining an infant's overall health and survival.

The purpose of this project is to study the duration of weaning and breastfeeding practices of non-infant individuals who survived infancy with evidence and without evidence of childhood rickets to learn whether the duration in which a child is breastfed and then weaned impacts the development of rickets. Additionally, this research compares the timing of rickets episodes to the duration of breastfeeding and weaning to determine if rickets occurred during weaning, after weaning, or while being solely breastfed. Understanding the weaning history and early childhood diet of non-infants who survived rickets in infancy can provide insight into why infantile rickets were so prevalent at this site. Eventually, the same isotopic and histological analyses will be conducted on infants dying with and without rickets to understand further why they did not survive beyond infancy.

This study utilizes stable isotope analysis to evaluate the ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes of incremental dentin samples in seven non-infant upper and lower 1st molars. Dentin does not remodel after formation and thus contains isotopes absorbed during childhood dental formation (Beaumont, 2018). The first molar was selected because it begins to form around the time of birth and finishes around 9 years of age. Since the human body digests and records protein intake, analyzing $\delta\ ^{13}C$ and $\delta^{15}N$ of the incremental dental stables can estimate each individual's early life dietary timeline. Interglobular dentin microscopic analysis has already been conducted on each of the adult individuals (Perry et al., 2021). This study will compare the timing of IGD to the duration of breastfeeding, the timing of the weaning process, and the food groups consumed by each individual. Finally, this study will examine the overall diet of the individuals and focus on sources of stress to make inferences about the population's life history. The potential links between diet and weaning duration in infancy and childhood and the development of rickets suggest there will be a difference in isotope ratios between individuals with and without evidence of IGD.

CHAPTER 2: BACKGROUND

Introduction

The archaeological site of Tell Hisban has a 3000-year history of settlement ranging from the early Iron Age (1200 to 550 BCE) to the end of the Late Ottoman period (1789 to 1918 CE. Excavations from 1998 to 2004 uncovered the commingled remains of at least 52 individuals (20 adults, 32 subadults) within a 14th-century Mamluk structure dated to the Late Ottoman period based on material cultural evidence (Walker, 2001). Many of the infants and children included in this assemblage suffered from metabolic disease, with 40% of infants under 24 months dying from active scurvy and 60% dying with active rickets (Perry & Edwards, 2021). In contrast, other Late Ottoman period Jordanian and Israeli archaeological sites have an average frequency of rickets between 0 and 5%, and scurvy is rarely recorded (Perry & Edwards, 2020). The cause of this high prevalence of metabolic diseases at Hisban is still unknown, particularly in the case of rickets.

Vitamin D levels in early infancy often represent vitamin D absorbed *in utero* for the first 8 weeks to 12 weeks and even 16 weeks after birth (Atiq et al. 1998; Pehlivan et al. 2003). Therefore, maternal vitamin D deficiency (VDD) during pregnancy can result in low vitamin D levels in the first 2-4 months after birth. While breastmilk provides essential macro- and micronutrients necessary for growth, a clean water source, and protection against environmental pathogens (Katzenberg et al., 1996; Smith et al., 2023; King et al., 2018; Eerkens et al., 2011), it alone does not provide adequate levels of vitamin D for a growing infant, even with sufficient maternal vitamin D levels. As a result, the American Academy of Pediatrics and most clinicians today recommend vitamin D supplementation along with adequate UVB exposure to prevent VDD in infants and children (Wagner et al. 2008).

Therefore, the increased risk of VDD in early infancy at Hisban may indicate maternal VDD was a problem. This research aims to explore the possible impact of the maternal diet during breastfeeding, the foods used to supplement breastmilk during weaning, and the timing of weaning in individuals, some of whom had rickets in childhood but survived. Incremental stable carbon and nitrogen isotope data from the dentin will be used to track childhood diet and weaning. Childhood rickets was identified through the presence of interglobular dentin, or IGD, which has been linked to VDD, in the same teeth used for isotope analysis. Aligning the ages of dietary shifts and weaning timing with ages of IGD formation can identify possible links between diet and weaning and vitamin D deficiency at Hisban. In addition, these patterns will be compared between individuals with and without evidence of IGD to identify the interactions between metabolic disease and childhood nutrition in this community.

Vitamin D Deficiency

Humans primarily synthesize active vitamin D3 from previtamin D3 in the keratinocytes in the skin through solar UVB exposure; however, vitamin D can also be obtained from other sources such as diet (Wolfowitz & Gilchrest, 2006). In food, vitamin D is found in two forms: vitamin D2 and D3, or cholecalciferol. Foods such as mushrooms and yeast contain Vitamin D2, whereas fatty fish, fish liver oils, and egg yolks are abundant with Vitamin D3 (Jouanne et al., 2021). After obtaining vitamin D by sunlight exposure or diet, it travels to the liver via the bloodstream, and it turns into serum 25-hydroxyvitamin D [25(OH)D] (serum 25(OH)D). Both forms of vitamin D turn into serum 25(OH)D in the liver, but vitamin D3 produces more serum 25(OH)D more efficiently (Trang et al. 1998). In other words, the natural process of synthesizing vitamin D through sunlight exposure or by consuming animal products is a more effective way of obtaining substantial vitamin D levels than consuming plant-based foods. Next, the serum

25(OH)D passes into the kidneys, where it undergoes hydroxylation to become its active form, 1,25-dihydroxyvitamin D [1,25(OH)2D].

Vitamin D plays an active role in regulating calcium and phosphorus levels, which maintain bone density (Lips et al., 2014; Holick et al., 2007). Not only can a lack of vitamin D result in bone mineral deficiency, but it can also cause fatigue, joint pain, muscle pain, and an increased risk of heart disease and certain cancers (Holick et al., 2011). A deficiency in vitamin D is typically defined as having serum 25(OH)D levels below 25 nmol/L and an insufficiency as serum 25(OH)D levels between 25 nmol/L and 50 nmol/L (Lips et al. 2014). Clinical research has documented that the skeletal manifestations of a vitamin D deficiency (rickets or osteomalacia) only occur in extreme cases (Holick, 2006; Grant et al., 2005). Without sufficient vitamin D, calcium and phosphorus levels drop, compromising the structural integrity of bone (Snoddy et al., 2016; Brickley & Ives, 2008). In the growing bones of children, biomechanical pressures such as walking and crawling may make weight-bearing long bones, such as femora and tibiae, bow at the shaft and flare at the metaphysis. Additionally, inhibited bone mineralization causes abnormal bone loss in the form of porosity, especially at the epiphyses (Brickley & Ives, 2008). When sufficient vitamin D levels return, new bone growth appears on the diaphysis of long bones. Since reparative bone growth can mask previous cases of skeletal rickets, the prevalence of skeletal rickets in archaeological populations is only visible if an individual dies during or shortly after the event.

At Hisban, Perry, and Edwards (2021) observed upper and lower limb long bone flaring and bowing among the subadult assemblage (38% of the long bones showed bowing and 46.2% of the long bones showed flaring). They also observed porosity, roughing, and new bone formation on long bone metaphysis. Approximately 60% of infants of various ages between six months and two years died of active rickets. As noted above, this is much higher than the prevalence seen at other contemporary Israeli and Jordanian sites (Perry & Edwards, 2020). After two years of age, no children have signs of active skeletal rickets, and only two older subadults and two adults have retained evidence of long-bone bowing from an earlier bout of rickets. Perry and Edwards (2021) attribute this decrease in rickets to older children possibly having higher rates of sunlight exposure. When children reach the age of two, they gain more independence, and they can walk, run, speak simple phrases, and socialize with other children (CDC, 2023). Therefore, the Hisban children by this age could have had enough sunlight exposure due to these activities to prevent rickets. According to Perry and Edwards (2021), skeletal rickets in this sample must be connected to specific circumstances only seen between birth and two years of age.

Because UVB radiation from sunlight is the driving force behind vitamin D synthesis, rickets is often associated with populations that receive inadequate exposure to sunlight (Zhang et al., 2016; Lockau & Atkinson, 2017). One of the most notable locations is the United Kingdom, where high cloud coverage and long winters increase rickets prevalence even today (Zhang et al., 2016). During the Industrial Revolution, when smog covered London, rickets' prevalence became so common among children that it became known as "English Disease" (Zhang et al., 2016; Lockau & Atkinson, 2017). Some populations that live in areas with low UVB radiation levels will be able to avoid vitamin D deficiencies by consuming large quantities of fish, which is high in vitamin D3, such as a coastal population in Norway (Lips et al. 2014). While cases of rickets in the Middle East are rare, factors such as cultural barriers and genetic variations can influence an individual's vitamin D levels (Mishal, 2001; Sedrani et al., 1983; Alagöl et al., 2000; Khuri-Bulos et al., 2014; Slater et al., 2010)

As noted above, the only cases of childhood rickets that typically can be identified in an archaeological skeletal assemblage are those children who died during or shortly after skeletal rickets formed. In order to uncover hidden episodes of rickets at Hisban, Perry and colleagues (2021) used microscopic analysis of dentin to identify episodes of childhood rickets in individuals who survived childhood and had no outward signs of residual rickets. Vitamin D deficiency during childhood, while the teeth are forming, can result in mineralization defects within the dental dentin, referred to as interglobular dentin (IGD) (D'Ortenzio et al., 2016). When an individual is well-nourished, the dental tissue appears smooth because the calcospherites, or spheres with calcium salts, are entirely fused. If the individual is not receiving enough vitamin D in their diet, the calcospherites will fail to fuse, creating holes in the dentin (D'Ortenzio et al., 2016). Under a polarizing microscope, these holes will present as bubbles or black spaces in thin sections of the dentine. IGD is classified into three grades: Grade 1 (mild, affecting less than 25% of the dentin), Grade 2 (moderate, affecting between 25% and 50%), and Grade 3 (severe, affecting more than 75%). The individuals in this study exhibit Grade 1 or 2 IGD; Grade 3 IGD is generally associated with broader health challenges beyond vitamin D deficiency (D'Ortenzio et al., 2016).

Although vitamin D deficiency is frequently cited in the archaeological record as the primary cause of IGD, studies such as those by Snoddy et al. (2021) suggest other contributing factors. Snoddy and colleagues (2021) indicate that IGD may also result from normal tooth development due to odontoblast retraction within dentine tubules. The presence of IGD is not evenly distributed and may be influenced by regional variation. Furthermore, Snoddy and colleagues (2023) have observed that IGD severity does not necessarily correlate with visible skeletal changes typically associated with rickets. These articles highlight the importance of considering other factors when conducting IGD studies.

Perry and colleagues (2021) found that 64% of the non-infant sample, which included 17 adults, two adolescents, and one older child, displayed evidence of at least one episode of IGD.

The age at which an individual formed IGD varied from the prenatal period to five years of age. The age-related pattern of rickets prevalence in infants based on the presence of active skeletal rickets diverges from the ages seen in IGD in older individuals who survived childhood rickets. It appears that having rickets between 3 months and 2 years of age may contribute more to mortality than having rickets between 2 and 5 years (Figure 1). In addition, no active skeletal rickets are seen in children dying after 2 years of age. Two factors became clear from this study: one is that not only were there individuals in the community who survived childhood rickets, but rickets can occur after two years of age, and second, not all individuals in the community had childhood rickets or at least periods of vitamin D deficiency that resulted in IGD formation.

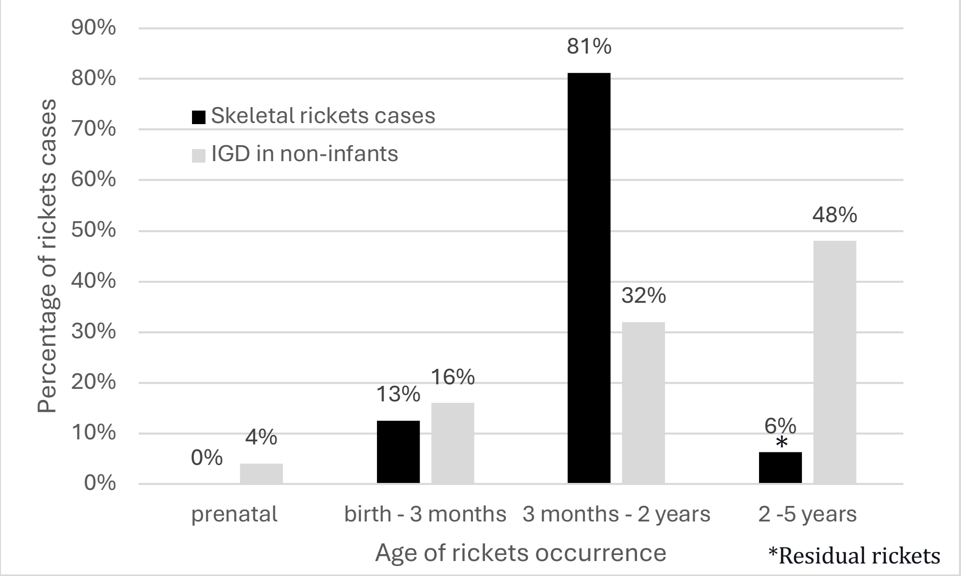


Figure 1: Age distribution of rickets cases at Hisban occurring before five years of age in the deceased children (skeletal rickets) and the non-infant survivors (IGD). The infant counts are based on Minimum Number of Individuals (MNI) for each age category and the non-infant counts on IGD that occurred during that age group. If IGD is formed across two age categories, it will be counted within each.

What is the source of childhood rickets at Hisban?

The cause of rickets at Hisban can be linked to three factors, none of which are mutually exclusive: 1) inadequate UVB radiation, 2) genetic polymorphism inhibiting vitamin D synthesis or bone mineral metabolism, and 3) inadequate dietary vitamin D.

Inhibiting UVB radiation

Although Jordan receives high levels of UVB radiation, vitamin D deficiencies remain common, possibly due to sun-limiting factors such as clothing, which can significantly reduce vitamin D synthesis. Clothing that fully covers most of the body is commonly worn by women, men, and children in rural tribal areas of Jordan for both climatic and cultural reasons. Ethnographic studies of traditional Bedouin communities in Israel, the Egyptian Sinai desert, and Jordan found that mothers often swaddle their infants and keep them inside tents or shelters for at least the first 40 days after birth, potentially inhibiting vitamin D synthesis and contributing to skeletal rickets (Abu-Rabia, 2010).

A modern study conducted in the United Arab Emirates found that children with low vitamin D levels often wear full or nearly full body coverage outdoors (Mishal et al., 2001). Similarly, Adul-Razzak and colleagues found that many vitamin D-deficient infants in Jordan had less than 30 minutes of sun exposure per day. When they were outdoors, they were fully covered. Furthermore, studies have shown a link between women’s clothing coverage and their newborns' vitamin D levels (Mishal, 2001; Khuri-Bulos et al., 2014). Khuri-Bulos and colleagues (2014) attributed low serum 25(OH)D levels in infants to their mothers' clothing choices. Many mothers reported wearing a hijab or niqab, which covers most or all of the face, neck, and arms—areas that typically receive the most sunlight exposure. These studies suggest that sun-limiting barriers, such as clothing, can significantly reduce UV exposure and play a key role in the prevalence of vitamin D deficiency among children in modern Jordan.

If this was a traditional practice amongst Bedouin women in Late Ottoman period Jordan, one would expect to see high rickets frequencies in other skeletal assemblages in the region. Therefore, while limited UVB radiation exposure may have contributed to vitamin D deficiency in the Hisban infants and children, it was likely not the only cause. Patterns in IGD formation at certain ages, such as when a child begins or completes the weaning process, may indicate a change in infant behavior that resulted in increased or decreased UVB exposure.

Genetic factors leading to vitamin D deficiency

Another factor that can influence an individual's vitamin D status is genetics. Currently, there are 35 genes associated with vitamin D deficiency (Sepulveda-Villages et al., 2020). For example, cytochrome P450 2R1 gene (CYP2R1) helps encode the enzyme that converts the serum 25(OH)D into its active form 1,25-dihydroxyvitamin D [1,25(OH)2D], which helps the body maintain the right balance of calcium and phosphorus (Slater et al., 2017; Shea et al., 2009; Engleman et al., 2013). Variations of the CYP2R1 can negatively affect vitamin D levels; two variants of the CYP2R1 are found significantly in Middle Eastern populations: rs10500804 and rs12794714 (Tomei et al., 2020). Additionally, vitamin D receptor (VDR) gene mutations have been shown to adversely affect vitamin D levels. The VDR gene encodes the receptors the body uses to respond to vitamin D and secrete calcium and phosphorus to produce bone. A medical study analyzed the individuals with the VDR gene variant and found that even with additional dietary vitamin D supplements, the individuals still had a vitamin D deficiency (Slater et al., 2017).

Genetics could provide an alternative hypothesis for the high prevalence of rickets at Hisban. Some genetic polymorphisms causing hypophosphatemia-related skeletal rickets are X-linked recessive, meaning that males with an X chromosome with the mutation will show symptoms. Still, a female has to have both X chromosomes with the mutation to develop rickets (Ackah and Immel, 2023). Therefore, this form will be more common among male children. In addition, sex-specific hormones can potentially impact survival rates. A recent study found that higher testosterone levels were associated with higher serum 25(OH)D levels, regardless of age or body mass index (BMI) (Wehr et al. 2010). In addition, they found that since testosterone fluctuates seasonally, being lower in the winter and higher in the summer, this can also affect vitamin D levels in men. Therefore, while both male and female individuals at Hisban may have suffered from rickets, being born male during the winter may have increased the risk of unfavorable survival outcomes due to these genetic factors.

Among the Hisban assemblage previously analyzed for IGD, two out of seven females, three out of three males, and seven out of nine individuals of ambiguous sex had IGD (Perry et al. 2021). Although almost half of the individuals in this sample had an ambiguous sex estimation based on cranial morphology, males may appear to be more likely to develop and survive rickets during childhood than females.

Dietary sources and vitamin D deficiency

Vitamin D deficiency can be influenced by vitamin D levels in dietary sources. While in utero and during infancy, the mother's vitamin D levels serve as the infant's primary source (Aghajarafari, 2013; Pehlivan et al., 2003; Hanun et al., 2005; Gordon et al., 2008). As a result, a deficiency in an infant during the period of maternal pregnancy and lactation may be related more to the vitamin D levels of the mother. Pregnant and lactating women and infants have high vitamin

D requirements and thus may be susceptible to vitamin D deficiency when the mother needs to provide for herself and her baby (Table 1). As a result of these increased requirements, vitamin D deficiencies are common among pregnant women, and even women with adequate vitamin D levels during pregnancy may develop a vitamin D deficiency by their third trimester (Jouanne et al., 2021). In addition, studies have found that breastmilk alone cannot provide sufficient levels of vitamin D for the growing infant (Mellati et al., 2015). The effects of a vitamin D deficiency is exacerbated if the woman wears full-coverage clothing or is in her third trimester during the winter or early spring in the northern hemisphere, when UVB radiation is low (Jouanne et al., 2021). In addition, pregnancy complications such as premature birth may mean the fetus receives an inadequate amount of vitamin D in utero, putting it on track to develop rickets (Chen, 1994; Chen et al., 2012). Two of the individuals in this study, an 8-9-year-old child (Skull #132), had prenatal rickets, and another (B.22.20 mandible) had IGD forming starting at birth. This suggests that the individuals' mothers were vitamin D deficient while pregnant.

Table 1: Daily Dietary Vitamin D Intake Recommendation (National Institute of Health, 2022; Holick, 2010)

Life Stage	**Vitamin D Dosage (*International Units)***
Birth to 12 months	400 IU
Children 1 to 13 years	600 IU
Teens 14 to 18 years	600 IU
Adults 19 to 70 years	600 IU
Older adults 71 years and older	800 IU

Pregnant and breastfeeding teens and women	600 IU

Maternal calcium deficiency during pregnancy, particularly in the last three months of gestation, can also contribute to rickets during infancy (Volk et al., 2022; Lips, 2012; Chen, 1994; Chen et al., 2012) Dietary calcium may play an essential role in the manifestation of rickets because calcium and vitamin D have a symbiotic relationship in the human body (Volk et al. 2022). Vitamin D promotes calcium absorption by influencing the secretion of parathyroid hormone (PTH). In cases of low calcium but adequate vitamin D levels, vitamin D will influence excess PTH secretion to help maintain bodily calcium levels. In the absence of vitamin D, the PTH decreases secretion, and calcium levels also drop, which results in decreased bone density. Many clinical studies have found that providing both calcium and vitamin D supplements can help prevent bone fractures; having both supplements taken together yields better results than either nutrient taken by itself (Lips, 2012). For example, one study conducted in France found that calcium and vitamin D supplementation decreases fractures, while another study only used vitamin D supplementation and found no difference in fracture prevention (Lips, 2012; Arnaud et al., 1992; Grant et al., 2005). However, Lips (2012) notes that these results could have been influenced by other factors such as baseline calcium intake, vitamin D status, supplement dosages, age, and gender can influence the results.

The timing of the shift from relying solely on breastmilk to increased incorporation of solid foods can impact the age of rickets development if maternal deficiency is an issue. Breast milk provides an infant with essential macro and micronutrients, a clean water source, and protection against infectious and metabolic diseases (Abu-Rabia, 2007; Pike & Milligan, 2010; Moffatt et al., p 101). However, after six months of age, breastmilk can no longer supply rapidly developing infants with all their nutritional requirements. Thus, infants should receive supplementary foods alongside breast milk, typically starting at six months of age, although this varies individually by mother-infant dyad and by culture (Tsutaya and Yoneda, 2014; Stantis et al., 2020).

The timing of the weaning process can vary based on the needs of the infant and mother, environmental factors, and cultural norms (Gardner et al., 2018). However, the onset and duration of weaning can significantly influence health, particularly when deviations from optimal timing occur. Early weaning, for instance, can exacerbate nutritional deficiencies such as scurvy, anemia, and rickets (Brickley et al., 2014). One study conducted on a nineteenth-century population in the Netherlands found that infants who were weaned before six months and on unpasteurized cow's milk significantly contributed to their rickets development, demonstrating the importance of the weaning process and complementary foods (Waters-Rist et al., 2018).

Furthermore, it may increase the infant's susceptibility to infectious diseases, especially if solid foods are introduced from unsanitary environments at a stage when the immune system is still underdeveloped. Conversely, exclusive breastfeeding beyond six months may lead to inadequate protein and caloric intake, as infants require more than breastmilk alone to meet their nutritional needs after this period (Kendall et al., 2021). Thus, both early and delayed weaning can pose nutritional challenges, potentially leading to conditions like rickets. In relation to rickets, early weaning may result in the infant missing key nutrients, such as vitamin D, particularly if the diet lacks supplementation, as breastmilk alone may not provide sufficient amounts. On the other hand, delayed weaning can also deprive the infant of essential nutrients in solid foods, such as vitamin D and calcium, exacerbating the risk of developing rickets.

Vitamin D or calcium deficiency during pregnancy increases mortality risks in infancy and childhood. These deficiencies have long-term negative impacts on a fetus, such as a weakened immune system, kidney complications, cancer, cardiovascular disease, and a higher risk of developing type 1 and 2 diabetes (Jouanne et al., 2021; Chen, 1994; Chen et al., 2012). Even after birth, these deficiencies will still cause complications. Besides rickets, children with vitamin D deficiency are at a higher risk of developing an irregular heartbeat, hyperparathyroidism, and seizures (Jouanne et al., 2021; Chen, 1994; Chen et al., 2012). While the clinical literature is still debating whether pregnant women should take additional vitamin D supplements, ensuring proper nutrition for the infant's rapid growth and development after birth is crucial.

For this study, we utilize incremental stable isotope analysis to compare the timing of childhood vitamin D deficiencies between individuals with and without rickets. The age of IGD formation in some individuals may indicate that maternal deficiency in vitamin D began during pregnancy. A maternal deficiency would increase the risk of rickets when a fetus or infant relies solely on the mother for nutrition. We hypothesize that individuals with interglobular dentin (IGD), an indicator of rickets, likely began the weaning process later in infancy than those who did not develop IGD.

Stable isotope data offers a valuable means to investigate whether weaning practices and dietary composition contributed to the high prevalence of rickets observed at Hisban. Additionally, these data allow us to explore early childhood diet's potential sex-based differences in nutrition, which can further inform our understanding of genetic predispositions and UVB radiation exposure.

Stable Isotope Analysis

Stable isotope analysis, as a methodological approach, has allowed archaeologists to investigate the life history of past populations (Sulzman, 2007). Stable isotopes are elements with the same number of protons and electrons but different neutrons; they are non-radioactive, remain constant, and do not decay (Sulzman, 2007). While the chemical properties of stable isotopes stay the same, their varying neutron quantities change their mass, influencing how they react during physical reactions (Sulzman, 2007). In natural biochemical processes, such as photosynthesis, these mass differences result in "fractionation," which is when the heavier isotope is rejected more frequently because plants utilize the lighter isotopes (Malainey, 2010). Each time stable isotopes undergo a biological, chemical, or physical reaction (photosynthesis, digestion, etc.), they undergo fractionation, which can be traced throughout an organism's history. In terms of diet, human tissue records the isotopic composition of food and water digested (Brown T. & Brown K. 2011 pg. 82). By using a mass spectrometer, stable isotopic analysis can be conducted on these tissues and then studied to identify what food groups an individual was consuming. For this study, stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis will be employed to examine the early dietary composition of seven adults from the Hisban sample.

Both carbon and nitrogen isotopes are expressed in parts per mil (‰), and stable isotope ratios are reported using a lowercase delta symbol (δ) and are calculated as follows (Schwarcz & Schoeninger, 1991).

$$\delta = \left[\frac{R_{Sample} - R_{Standard}}{R_{Standard}} - 1\right] x\ 1000$$

Carbon Stable Isotopes

Following oxygen, carbon is the second most abundant element in the human body and is found in all other living organisms (Malainey, 2010). Three carbon isotopes occur in the Earth's atmosphere: ^{12}C (stable), ^{13}C(stable), and ^{14}C(radioactive). Stable isotope analysis of carbon measures the ratio of ^{12}C to ^{13}C (δ^{13}C) in organic material, including plants. These ratios vary due to the three possible photosynthetic pathways utilized for plants to absorb atmospheric carbon: the Calvin Cycle pathway (C_3), the Hatch-Slack pathway (C_4), or the Crassulacean Acid Metabolism pathway (CAM), as shown in the table below (Malainey, 2010 pg. 178).

Table 2: Carbon Isotope Pathways: This table shows the carbon isotope pathways values as well as what regions and plants typically utilize what pathway. The exact value of the pathway depends on environmental conditions such as temperature, humidity, and ultraviolet radiation exposure.

CARBON ISOTOPE PATHWAYS			
	Calvin Cycle Pathway C_3	**Crassulacean Acid Metabolism CAM**	**Hatch-Slack Pathway C_4**
Climate	Temperate	Arid	Tropical
Average Value (parts per million)	-26‰	Values fall between C_3 and C_4 pathways	-12.5‰
Value Range (parts per million)	-24‰ to - 36‰	- 10‰ to - 22‰	-16‰ to - 9‰

Common foods	Potatoes, wheat, barley, and rice	Cacti, yucca, and aloe	Sugarcane, millet, and maize
		(Brown T. & Brown K., 2011 pg. 82 - 83)	

Plants prefer lighter isotopes over heavier isotopes, so when atmospheric carbon is absorbed, there is always an initial enrichment of lighter isotopes (-7‰). In plants that use the C_3 pathway, there is a secondary enrichment when the CO_2 molecules containing ^{12}C are converted into glucose during photosynthesis. No secondary enrichment exists for plants that utilize the C_4 pathway as they absorb all the CO_2 (Brown T. & Brown K., 2011 pg. 82 - 83). Using carbon isotopes, we can identify the plant materials consumed by the population at Hisban, either directly or indirectly, through the animals in their diet.

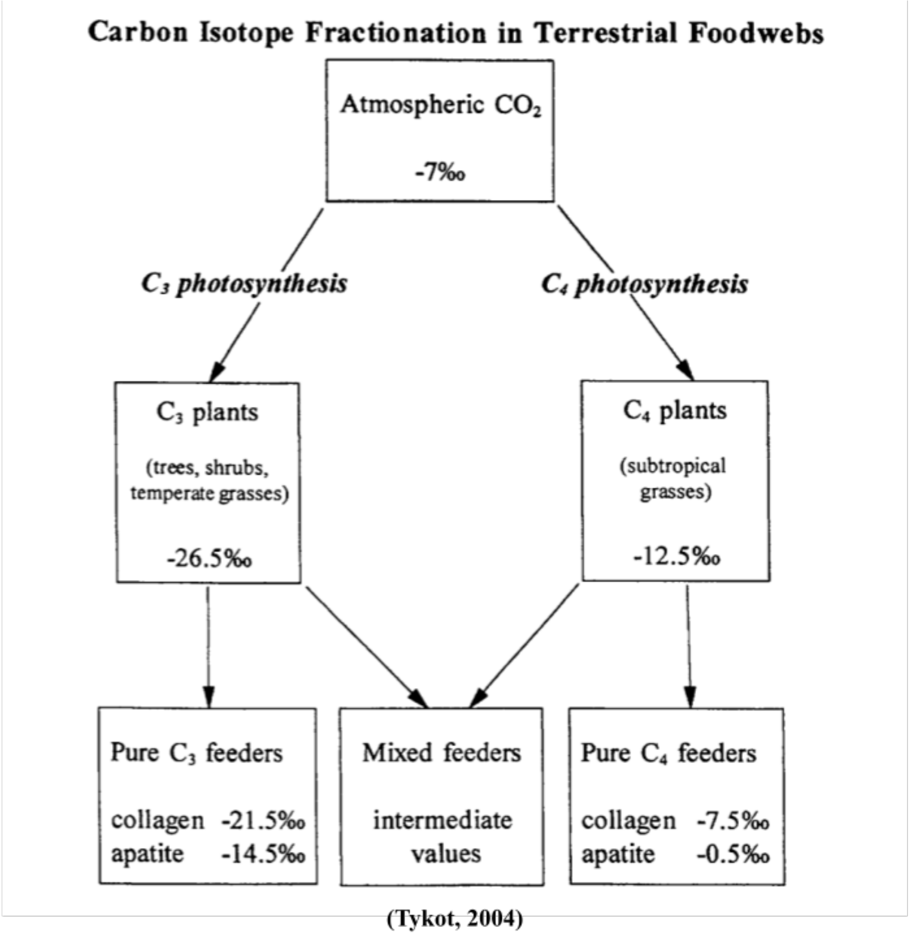


Figure 2: Carbon isotope fractionation in terrestrial food webs (Tykot, 2004)

Nitrogen Stable Isotopes

Nitrogen is one of the most abundant elements in the earth's atmosphere and is required for an organism to synthesize amino acids. Nitrogen exists in the atmosphere as nitrogen-14 (^{14}N) and nitrogen-15 (^{15}N), comprising 78% of the atmosphere. Atmospheric nitrogen cannot be absorbed directly by plants but is converted into compounds by the nitrogen cycle. The nitrogen cycle is a process where plants obtain nitrogen from the soil with N_2-fixing bacteria. These plants have varying ^{15}N values because of the association between moisture, soil pH, and soil microbes (Franche, 2009). Fractionation occurs through each stage of the food web, where ^{14}N, the lighter isotope and the isotope preferred by plants, is absorbed, and ^{15}N, the heavier isotope, remains. The ratio of ^{14}N and ^{15}N or the $\delta^{15}N$ will increase by approximately 3‰ to 5‰ with each level in the food chain (DeNiro, 1985; Gregoricka & Judd, 2016).

At birth, the nitrogen values of a mother and her infant may be similar due to nutrient transfer via the placenta and umbilical cord. However, an infant's nitrogen values may be elevated if the mother experiences stress during pregnancy (Chinique et al., 2020). During breastfeeding, infants essentially consume the tissue of the mother or other caregiver, supplying the breastmilk, so their nitrogen isotopic values are often 3 to 5‰ higher than their caregiver's. (Eerkens & Bartelink, 2013). Directly after birth, an infant's $\delta^{15}N$ levels increase because their animal protein source shifts from the placenta to breastmilk. As children transition from breast milk to solid foods, their nitrogen isotope values usually decrease by 2 to 4‰ throughout the weaning period (Eerkens & Bartelink, 2013; Fuller et al., 2013). The rate of the $\delta^{15}N$ decrease will reflect the rate at which the breast milk to solid food shift occurs. Children fed plant-based foods, such as porridge, while weaning may have lower $\delta^{15}N$ values than children fed meat or dairy products (Eerkens & Bartelink, 2013).

Studies have noted that $\delta^{15}N$ and $\delta^{13}C$ values respond to stress experienced by an individual, whether from catabolism (the breakdown of bodily tissues) or other factors like water availability (Beaumont et al., 2018; Vaiglova, 2020). During periods of catabolism, which may be caused by various forms of stress, such as undernutrition, disease, or even bone fractures, $\delta^{15}N$ tends to increase in relation to $\delta^{13}C$. This was documented in a case study on inmates of the Kilkenny Union Workhouse during the Great Irish Famine (1845–1852 A.D.). Beaumont and Montgomery (2016) used the remains of workhouse inmates dating to the 19th century, during the Great Irish Famine, and found that the workers entered a catabolic state due to prolonged undernutrition. Additionally, water availability can be reflected in $\delta^{13}C$, as the proportions of C_3 and C_4 plants in a region indicate its water supply. In areas with more water, C_3 plants, which require higher water levels, are more prevalent, resulting in more negative $\delta^{13}C$ values. Conversely, in drought conditions or regions with scarce water, more drought-resistant C_4 plants may be consumed, leading to less negative $\delta^{13}C$ values (Vaiglova, 2020). Fluctuations in less negative $\delta^{13}C$ values may thus indicate periods of environmental stress or other factors.

By using $\delta^{15}N$ and $\delta^{13}C$ isotopes, we can investigate the weaning trends of adult individuals and make inferences about their environmental water availability. Here, we expect their nitrogen isotope ratios to drop around six months of age. Additionally, we anticipate that higher nitrogen ratios, indicating states of catabolism, will coincide with periods of less negative $\delta^{13}C$ values, which may reflect reduced water availability and potential evidence of environmental stress. We also expect higher nitrogen values indicating states or catabolism coincide with periods of less negative $\delta^{13}C$ due to less water availability and possible evidence of environmental stress.

Incremental Dentin Sampling

Tracking shifts in diet and trophic level within an individual is determined by sampling tissues forming at different ages. Previous archaeological studies have used carbon and nitrogen stable isotope analysis of bone collagen from infants of various ages to derive population-level weaning trends (Tsutaya & Yoneda, 2013; Schurr, 1997). However, using bone collagen and aggregating samples from different individuals dying during infancy results in many significant limitations.

First, bones have different turnover rates by age and activity level, and thus, bone collagen from different individuals will reflect different periods of isotopic absorption before death. As a result, stable isotopes examined from children dying at different ages reflect different periods before their death, creating "noise" in assessing dietary change and limiting our ability to make inferences about environmental stressors affecting a single individual (Tsutaya & Yoneda, 2013). Another limitation of using bone collagen to track childhood dietary change is the impact of growth stunting on bone formation. During periods of nutritional stress, tissues like dentin may continue to form and absorb isotopes, whereas bone growth may slow or even cease, reducing isotopic absorption. Therefore, bone collagen is unreliable for tracking isotopic changes associated over a period of time, such as weaning or if the infant is under nutritional stress that results in stunting.

Kendall and colleagues (2021) address these challenges by emphasizing the importance of the osteological paradox when conducting breastfeeding studies. First, they highlight that infants in these samples may or may not accurately represent the living population's health and environmental conditions, as those who did not survive were often the most ill. Kendall et al. (2021) also discuss selective mortality; without complete life histories, bioarcheologists risk misinterpreting early weaning as a cause of death when a child who was weaned early may have died from unrelated health issues. Since metabolic diseases typically result from undernutrition, the isotopic data from the Hisban assemblage should be assessed in dentin instead of bone. In summary, bone collagen is not the ideal tissue sample for this study, and instead, another human tissue should be used.

Dentin does not remodel after formation; therefore, it contains isotopes absorbed during childhood dental growth. Thus, the teeth of individuals of any age can be analyzed for childhood diet using dentin (Beaumont et al. 2018). Taking sequential samples following the growth trajectory of dentin can create a timeline for an individual's early childhood diet. Since dentin formation is canalized, the age at which each increment is formed can be estimated. Sampling dentin starts from the dentin-enamel junction (DEJ), moves inward toward the pulp chamber, and then down along the root (Figure 3). Each ca. 1 mm sample represents six months of the individual's life (Czermak et al., 2020). Typically, a first molar can produce between 8 and 20 serial samples, averaging diet at 0.5 to 1-year intervals (Eerkens et al., 2011). Therefore, incremental analysis provides a timeline of the dietary change across childhood. The available ethnographic data of Bedouins in Jordan and Israel can provide some expectations of the foods consumed and the likely ages of weaning in the Hisban community.

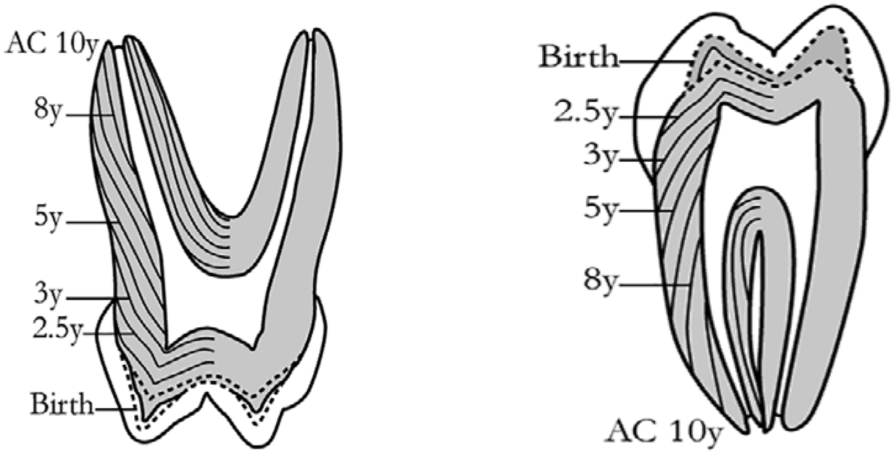


Figure 2: Upper permanent first molar (left) and lower permanent first molar (right). Both images show the growth lines of teeth (Brickley, 2020)

Diet, Breastfeeding, and Weaning Patterns among the Bedouin

Dietary, breastfeeding, and weaning practices are often influenced by a population's cultural and environmental factors. While the true tribal identity of the population buried at Hisban is unknown, the lack of material evidence associated with the graves and historical documentation suggests that they were Bedouin (Walker, 2001; Conder 1889: 108; de Saulcy, 1872: 279; Prag, 1991; Walker & LaBianca, 2003 & 2005). Therefore, ethnographic and historical data on Bedouins' infant diet and weaning practices in this region can be used to build expectations for our study.

The livelihoods of Bedouin are often defined by their herds of sheep and goats and occasionally camels and the crops they cultivate. The Bedouin rarely slaughtered their goats and sheep; instead, they used these animals to produce dairy products such as milk, cheese, and yogurt (Hobbs, 1989; Groen et al., 1964; Abu-Rabia, 1999). The milking season would last five months in good pasture areas and three months in bad pasture areas. During summer months, women of the tribes would milk their livestock to create cheese and butter that would last them the rest of the year (Abu-Rabia, 1999). The Bedouin of this area would herd their animals to springs in the summer and then, in the winter, migrate to the Jordan Valley or other arable regions to grow cereals, such as wheat, barley, and millet (Burckhardt, 1831; Groen et al., 1964; Abu-Saad et al., 2001). Bedouin tribes in Jordan would trade with other tribes and residents of nearby towns (such as as-Salt, in the case of the Hisban Bedouin) to receive other crops such as cucumbers, lentils, tomatoes, radishes, oranges, olives, apricots, and dates (Groen et al., 1964; Abu-Saad et al., 2001; Palmer, 2002). These foods are not considered high in vitamin D (Table 3). As mentioned earlier, calcium may help reduce the effects of a vitamin D deficiency; some of the foods the Bedouin were coming to were high in calcium, which could potentially mitigate a poor vitamin D diet.

Table 3: Vitamin D and calcium levels of foods traditionally consumed by Bedouins in the region (US Department of Agriculture, 2024)

Food Product	**Amount of Vitamin D out of 100 grams**	**Calcium**	**Percentage of Vitamin D 400 IU**
Goat Milk	52 IU	135 mg	13%
Goat Cheese	20 IU	298 mg	5%
Sheep milk	8 IU	473 mg	2%

Camel milk	100 IU	220 mg	25%
Wheat	0 IU	38 mg	0%
Barley	41 IU	36 mg	10%
Millet	0 IU	9 mg	0%
Cucumber	0 IU	16 mg	0%
Lentils	0 IU	62 mg	0%
Tomatoes	0 IU	10 mg	0%
Radishes	0 IU	25 mg	0%
Oranges	0 IU	38 mg	0%
Olives	0 IU	1 mg	0%
Apricots	0 IU	13 mg	0%
Dates	0 IU	64 mg	0%

Since much of an infant's nutrition comes from breastmilk, a peak in rickets before the introduction of breastmilk supplementation could suggest that the vitamin D deficiency originated from the mother. Additionally, because weaning represents a significant life stage, it may signal a shift in childcare practices that either increase or reduce the child's exposure to UVB radiation. Individual preferences and a population's social and cultural norms influence breastfeeding and weaning patterns. Among pastoral tribes, such as the Bedouin, breastfeeding is favored as the best source of nutrients for infants, believing that the longer a child is breastfed, the stronger they will become (Abu-Rabia, 2007). In Bedouin communities, gender also plays a role in weaning duration due to the perceived economic value of boys (Abu-Rabia, 2007; Al-Ani, 1980). Mothers with infant daughters may aim for a shorter interbirth interval, hoping to conceive a son, which can lead to earlier weaning of girls (Abu-Rabia, 2002, 2010). For example, Bedouin in Sinai and Libya may breastfeed boys for one to three years, while girls may be weaned starting as early as six months. Similarly, in a 20th-century semi-pastoral community near Bethlehem, boys were breastfed until 2.5 years old and girls until 1.5 years old (Abu-Rabia, 2007, 2010). Isotopic data from 19th-century adult Bedouin of Khirbat al-Mudayna, located 21 miles south of Hisban, indicates that breastfeeding lasted until six months, followed by gradual weaning until two years of age (Gregoricka & Judd, 2016).

Changes in community mobility may also impact weaning practices, leading to an earlier transition from breastmilk to solid foods as women's household responsibilities increase with the shift to a more sedentary lifestyle (Abu-Rabia, 2002, 2010). As a result, some mothers introduced complementary foods and started weaning as early as two months postpartum to reduce their workload. Conversely, women who received additional help with household tasks may prioritize an extended breastfeeding period (Abu-Rabia, 2002, 2010).

A study of Bedouin women in the Negev in the 1980s found that the period of exclusive breastfeeding (or breastfeeding along with formula and/or animal milk) decreases with lower levels of postpartum social support and higher parity with some introducing solid foods as early as 2 months of age (Forman et al. 1990; Naggan et al. 1991). The type of housing also impacted the length of exclusive breastfeeding, with women in tents rather than more permanent structures breastfeeding for at least 6 months of age (Naggan et al. 1991). In addition, infants born during the rainy season were more likely to be breastfed longer than those in the dry season (Naggan et al.

1991). All women in the study had introduced solid food by 12 months of age, with breastfeeding and/or bottle feeding continuing along with solid foods for the past 18 months (Forman et al. 1990).

This study investigates early childhood diet, breastfeeding, and weaning patterns and their potential influence on childhood rickets. If the mother is deficient in vitamin D, her breast milk will also lack this nutrient; in such cases, transitioning to solid foods may help alleviate the deficiency. Conversely, if the mother has sufficient vitamin D levels but the infant is fed foods low in vitamin D and calcium, weaning too early may contribute to a deficiency. This scenario could indicate broader nutritional issues related to breast milk and the solid foods consumed during weaning. Nutritional deficiencies like anemia and scurvy, which can co-occur with rickets, may further exacerbate a vitamin D deficiency. If no clear relationship is seen between weaning and rickets, it may be possible that other factors, such as genetic polymorphism or shifts in UVB radiation, played a role. Stable isotope analysis will be used to examine the early isotopic values of non-infant individuals in the assemblage, focusing on their weaning patterns to explore these factors. This phase will concentrate on those who survived past two years of age, including those who survived childhood rickets, to avoid "mortality bias" by excluding those who did not survive infancy due to their frailty. A later stage of the study will analyze isotopic evidence from infants who died with and without rickets to better understand the health risks faced by those who did not reach adulthood. Comparing IGD formation timing with ages of weaning will identify possible links to infant dietary sources as a factor for rickets and potential shifts in childcare practices associated with weaning that resulted in increased or decreased UVB radiation exposure.

The storeroom sample at Hisban is unique and worth studying for a few reasons. First, the prevalence of rickets at Hisban, 60%, is unusually high compared to rates at other Jordanian and Israeli sites, which range from 0 to 5%, indicating that the population at Hisban may have been exposed to unique circumstances (Perry & Edwards, 2021). Secondly, the significant number of infants remaining with metabolic disease may indicate a population under stress (DeWitte et al., 2015; Brothwell, 1987). For the first six months of life, infants are thought to be protected from environmental pathogens and metabolic disease as their immune system is directly linked to their mothers (Gowland, 2015). If a population has such a high frequency of metabolic disease, especially among infants, then this could indicate a population experiencing stress, as the mother may be stressed. Children are usually the first victims of environmental stressors such as social stress, famine, and disease (King et al., 2018). Therefore, understanding the high prevalence of rickets at Hisban, particularly the early life history of those who lived past infancy, could provide valuable insights into the broader social, environmental, or nutritional stressors affecting this community and may provide answers to this high vitamin D prevalence.

CHAPTER 3: MATERIALS AND METHODS

A minimum of 1 child, two adolescents, and 20 adult individuals in the Hisban sample underwent histological analysis for IGD identification (Perry et al. 2021). Here, six adults and one adolescent were selected for incremental isotope analysis. Preservation of the Hisban sample, including the teeth, was excellent. The age of the adolescent was estimated based on dental mineralization and eruption, according to AlQahtani and colleagues (2010). Sex estimation of the adults relied on cranial morphology following Buisksta and Ubelaker (1994). The individuals were sampled based on the presence of a first molar, which forms from around birth to 9 years of age, encompassing the pre-, peri-, and post-weaning periods, and whether they had previously undergone histological analysis for IGD observation (Perry et al. 2021) (Table 4). We selected four individuals without evidence of IGD and three individuals with IGD that formed during the hypothesized weaning period (1 to 3 years).

Table 4: List of samples selected for this study along with the results of previous IGD analysis

Individual	**ID**	**Age**	**Sex**	**Evidence of IGD**	**Tooth**
A	H98 FLDL Sq2 Loc7 #103	Adult	Indeterminate	Not Present	RM_1
B	H No ID Misc Skull #216	Adult	Female	Not Present	LM^1
C	H No ID Misc Skull #214	18-20 yrs	Female	Not Present	LM^1
D	H98 FLDL Sq2 Loc3 Bur #22 Mand. #19	Adult	Indeterminate	Not present	LM_1
E	H98 FLDL Sq2 Loc3 Bur#22 Mand.#20	Adult	Male	Present Prenatal - 1 years	LM_1

F	H04 Hisban FLDL Sq7 Loc3 Pail#23 Indv.#2	15 yrs ± 36 months	Intermediate	Present Birth - 2.5 years	LM_1
G	H Skull #3	Adult	Male	Present 1-3 years	RM^1

Staple Isotope Analysis Preparation

Each of the first molars selected for stable isotope analysis had already been photographed and thin-sectioned for histological analysis of IGD. For IGD analysis, the teeth had been sectioned mesiodistally through the buccal side of the tooth (Perry et al., 2021), leaving a slightly larger portion for incremental sampling that includes most of the lingual side of the tooth portion. The lingual portions were removed from the embedding material (2.22:1 ratio of EpoThin Hardener and resin from Buehler) by soaking them in acetone for 36-48 hours. Once removed from the resin, the lingual portion was scrubbed to remove any excess resin. In some cases, the buccal portion was also used to obtain at least 10 milligrams of dentine. Next, the tooth halves were demineralized in 0.5 m HCL solution for 7 to 10 days, changing the solution every 1 to 2 days. The demineralized teeth were rinsed three times with deionized water.

At first, we used Czermak and colleagues' (2020) hole punch method to take incremental samples; however, the resulting weight of each sample did not reach the needed 0.10 grams. Instead, we used a scalpel to cut 1-millimeter incremental samples from the DEJ to the root following the growth lines (Figure 3). If a sample was less than 10 milligrams, it was combined with the sample above or below. Here, between 7 and 14 serial samples of each tooth were collected from each tooth, labeled in numerical sequence from crown to root apex, and the bottom of each increment was measured from the top of the amelodentinal junction. Next, the samples were transferred to 1.5 micro-tubes and labeled in numerical sequence from the crown to the root apex.

The isotope analyses were conducted in the Laboratory for Archaeological Science and Technology at the University of South Florida (LAST) under the direction of Dr. Robert Tykott. At LAST, the samples were analyzed using a continuous flow Delta+XL with Carlo-Erba NA2500 EA for the collagen samples and a ThermoFisher MAT253 isotope ratio mass spectrometer coupled to a GasBench-II + continuous-flow interface. Standards are regularly analyzed so that data may be compared between different laboratories. The carbon and nitrogen isotope samples were reported ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in percent per mil (‰) and compared against Vienna Pee Dee Belemnite (VPDB) and Ambient Inhalable Reservoir (AIR) standards. The standard error (σ) of the stable isotope ratio mass spectrometry analysis was ± 0.15‰ for $\delta^{13}C$ and ± 0.18‰ $\delta^{15}N$.

Age Estimation

The age at each dentin increment formed was calculated following the procedure from Beaumont and Montgomery (2015), which incorporates known rates of dental growth from alQahtani and colleagues (2010). The first molar begins forming around birth (age = 0) at the amelodentinal junction. Around 3.5 years of age, the crown of the M1 finishes forming at the cementum-enamel junction (CEJ). The root continues to develop, and its apex closes around 9.5 years, marking a fully developed first molar (alQahtani et al. 2010). The number of years of crown formation (3.5) was divided by number of crown increments, and the span of root formation (6 years) was divided by the number of crown increments to generate age ranges of formation of each increment. The midpoint of the age range for each increment was used for plotting the isotope ratios in Excel and for statistical analyses in JMP (version 17.0). The ages of

IGD formation are depicted on the graphs showing the changes in isotope ratios with age by shaded boxes.

CHAPTER 4: RESULTS

Overall Sample Results

All 59 of the Hisban dentin samples met the criteria for bone collagen preservation (0.51% of 10 milligrams of material) and were analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes (Ambrose, 1990; DeNiro, 1985). The individuals in this sample consumed a mostly C_3 diet with additional C_4 plants, as shown below in Figure 4. Three individuals (B, C, and G) in the sample displayed periods of greater C_4 inputs between or around the ages of 4 and 6 years. The overall $\delta^{13}C$ analysis results ranged from -19.8‰ to -13.4‰ with an average of -17.8‰. As shown in Table 5, the average $\delta^{13}C$ value of individuals with no IGD was -17.3‰ and -18.4‰ with IGD.

The nitrogen results were more uniform than the $\delta^{13}C$ results (Figure 5). Here, the results stay between 8 and 12‰ throughout birth and 10 years of age. Individuals C and D start out much higher than the sample, and while Individual C stays high throughout life, Individual D drops below 8.0‰. The $\delta^{15}N$ values ranged from 7.8‰ to 19.2‰ with an average of 11.3‰. The average $\delta^{15}N$ value of individuals with no IGD was 11.7‰, whereas the individuals with IGD had an average of 10.9‰.

Table 5: Overall results of stable isotope analysis, including by the presence or absence of IGD

	$\delta^{13}C$ Mean	$\delta^{13}C$ σ	$\delta^{15}N$ Mean	$\delta^{15}N$ σ
IGD	-18.5‰	0.9	10.7‰	1.9
No IGD	-16.7‰	1.3	12.4‰	2.5
Entire Sample	-17.8‰	1.36	11.3‰	2.24

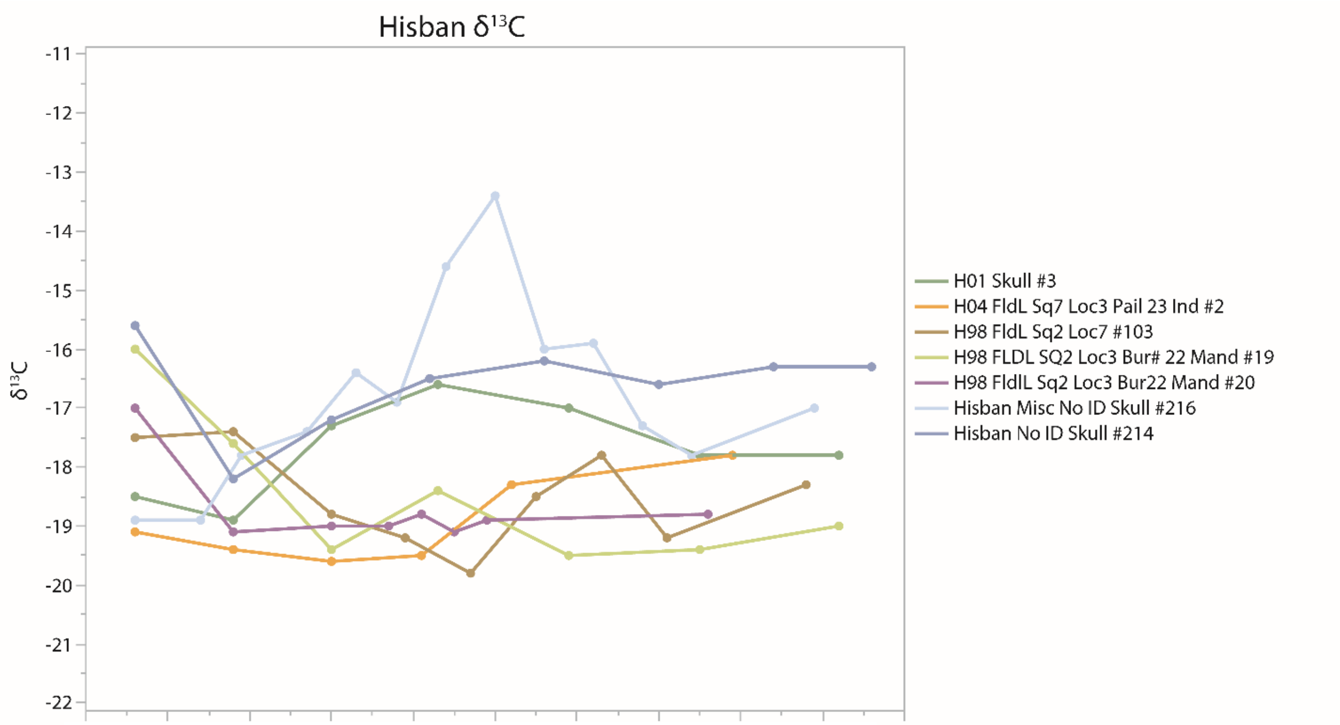


Figure 3: Results of $\delta^{13}C$ analysis of all sampled individuals

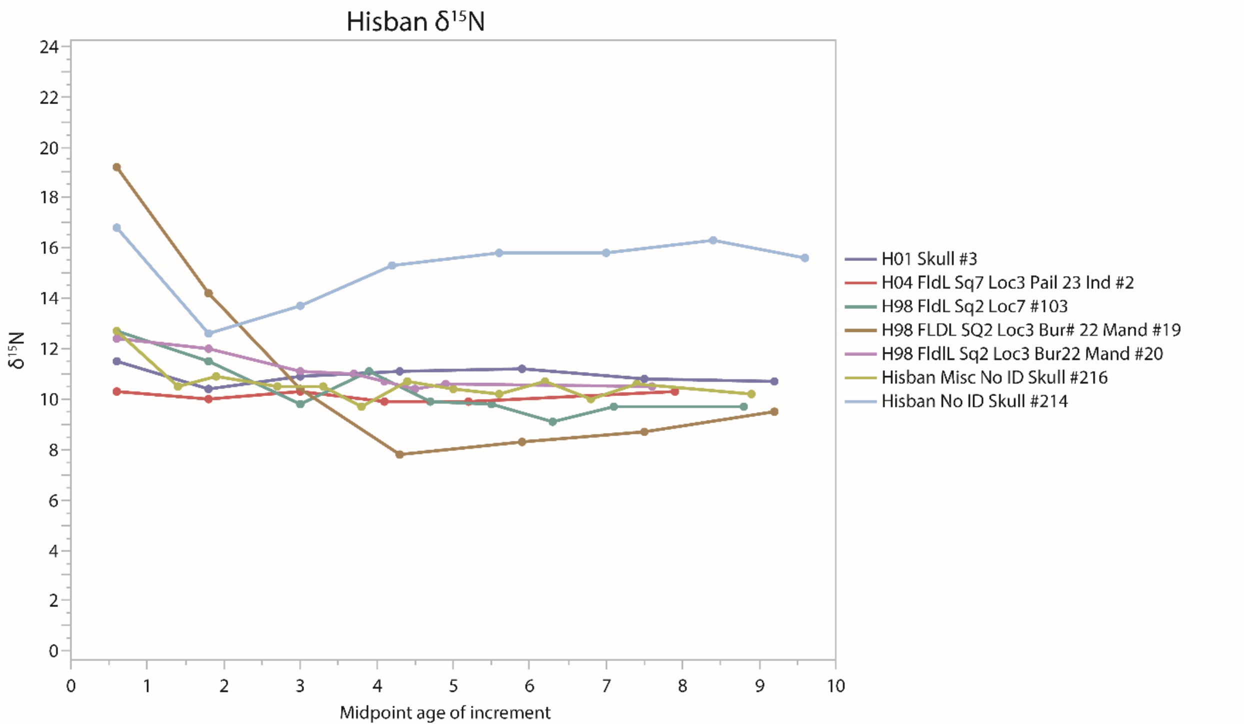


Figure 4: Results of $\delta^{15}N$ analysis of all sampled individuals

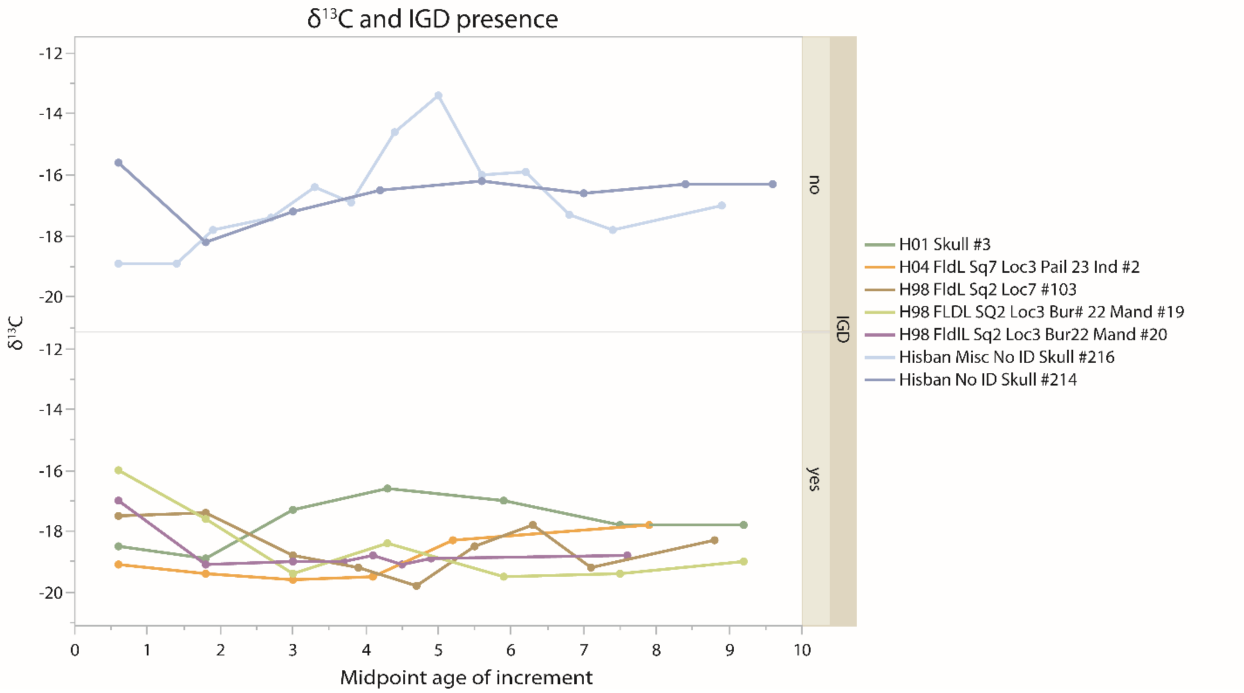


Figure 5: $\delta^{13}C$ results in individuals with (bottom) and without (top) evidence of IGD

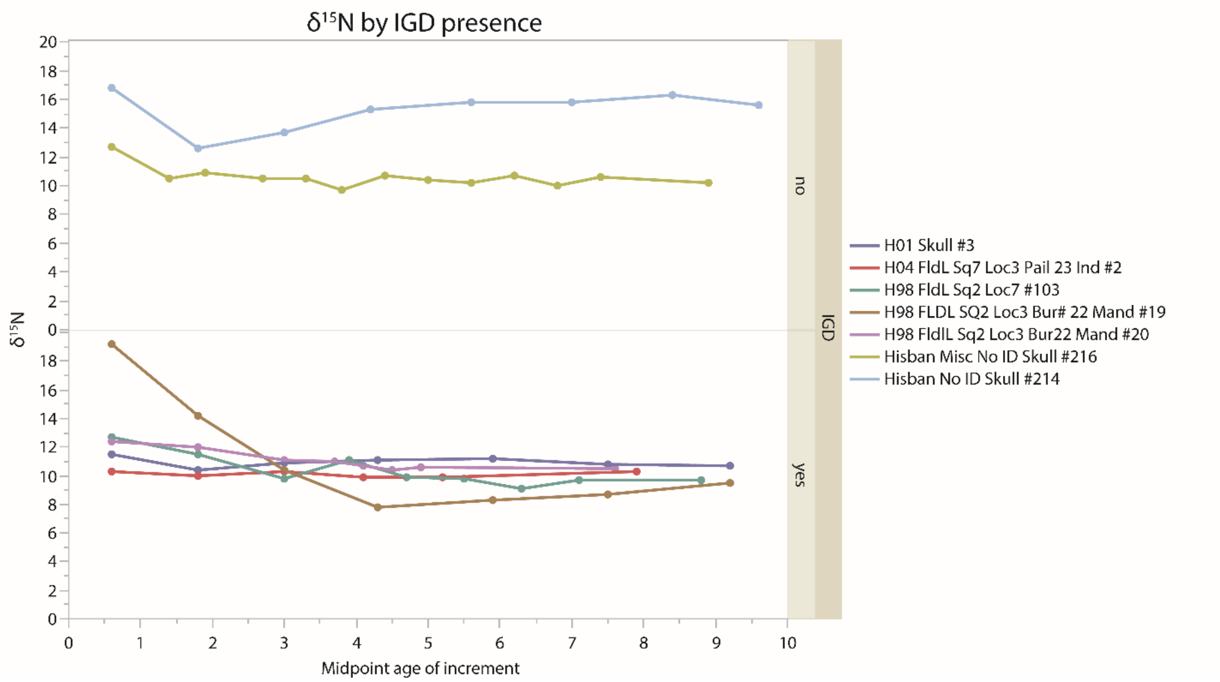


Figure 6: δ^{15}N results in individuals with (bottom) and without (top) evidence of IGD

The Wilcoxon two-sample test indicates that the medians of δ^{13}C (Z=4.66489, $p<0.0001$) and δ^{15}N (Z=2.26696, p=0.0234) significantly differ between individuals with IGD and those without IGD. Additionally, Levene's test indicates that individuals without IGD have greater variation in both their δ^{13}C (F=28.9337, df+1, $p<0.0001$) and δ^{15}N (F=9.3374, df=1, p=0.0034) results, presented above in Figures 6 and 7.

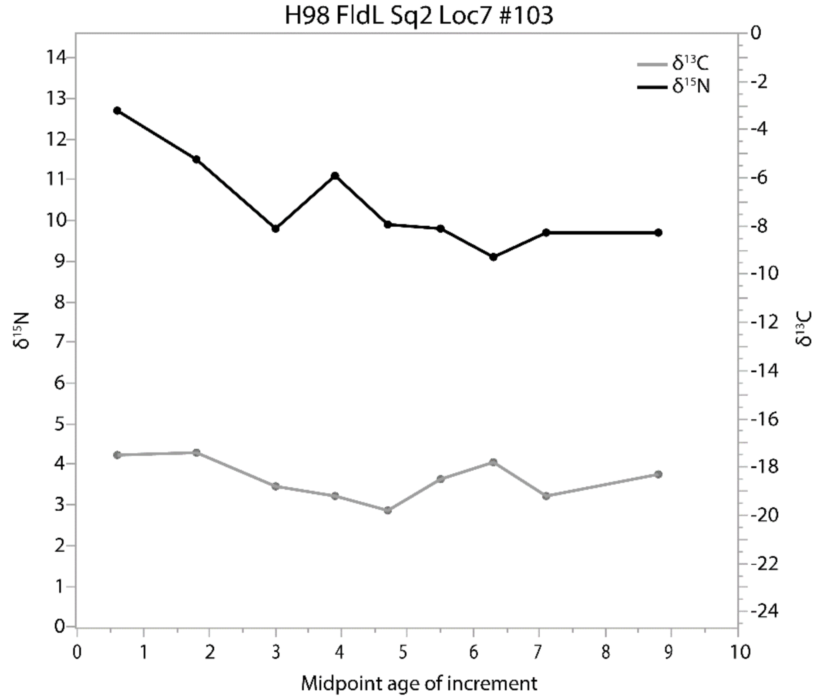


Figure 7: Results of $\delta^{13}C$ and $\delta^{15}N$ analysis of incremental dentin samples of Individual A (H98 FldL Sq2 Loc7 #103) who has no evidence of IGD

<u>*Individual A (H98 Fldl Sq2 Loc 7 # 103)*</u>

Individual A is an adult whose sex is indeterminate and has no evidence of IGD; nine samples were incrementally taken from their lower right first molar (RM_1). The results of the $\delta^{13}C$ analysis ranged from -19.8‰ to -17.4‰ with an average of -18.5‰. The $\delta^{15}N$ analysis results ranged from 9.1‰ to 12.7‰ with an average of 10.4‰ (Figure 8).

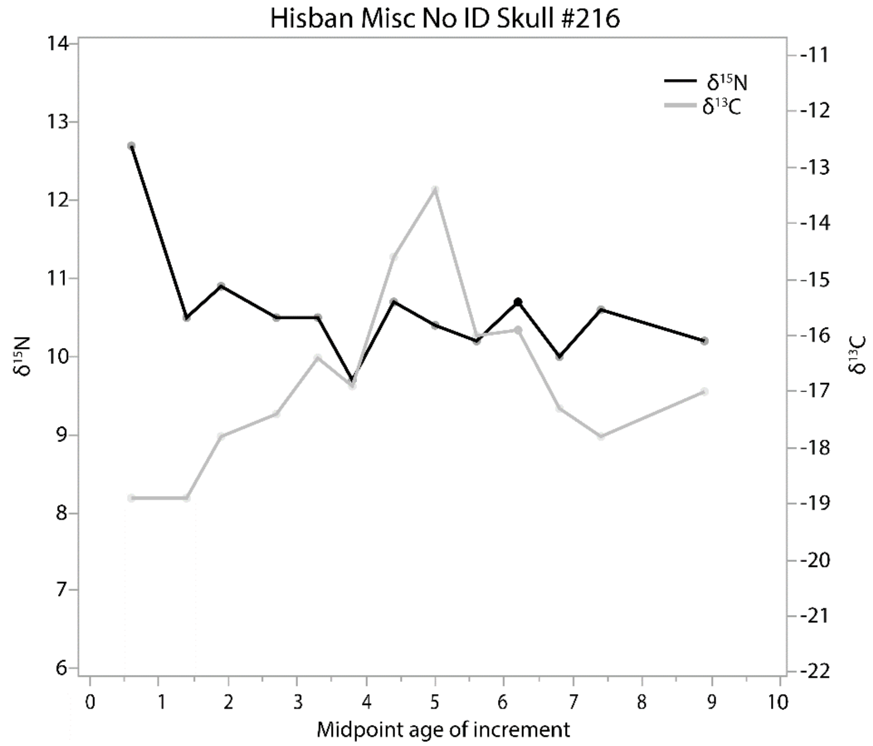


Figure 8: Results of $\delta^{13}C$ and $\delta^{15}N$ analysis in relation to age estimation of Individual B's first molar dentin

<u>*Individual B (Hisban Misc NoID Skull# 216)*</u>

Individual B is an adult female with no evidence of IGD; thirteen incremental samples were taken from the upper left first Molar (LM^1). The results of the $\delta^{13}C$ analysis ranged from -13.4‰ to -18.9‰ with an average of –16.8‰. Compared to the rest of the sample, Individual B is an anomaly because their $\delta^{13}C$ values had drastic fluctuations (Figure 9). Individual B's $\delta^{13}C$ values increase by 3.5‰ over one year. The results of the $\delta^{15}N$ analysis ranged from 9.7‰ to 12.7‰ with an average of 10.6‰.

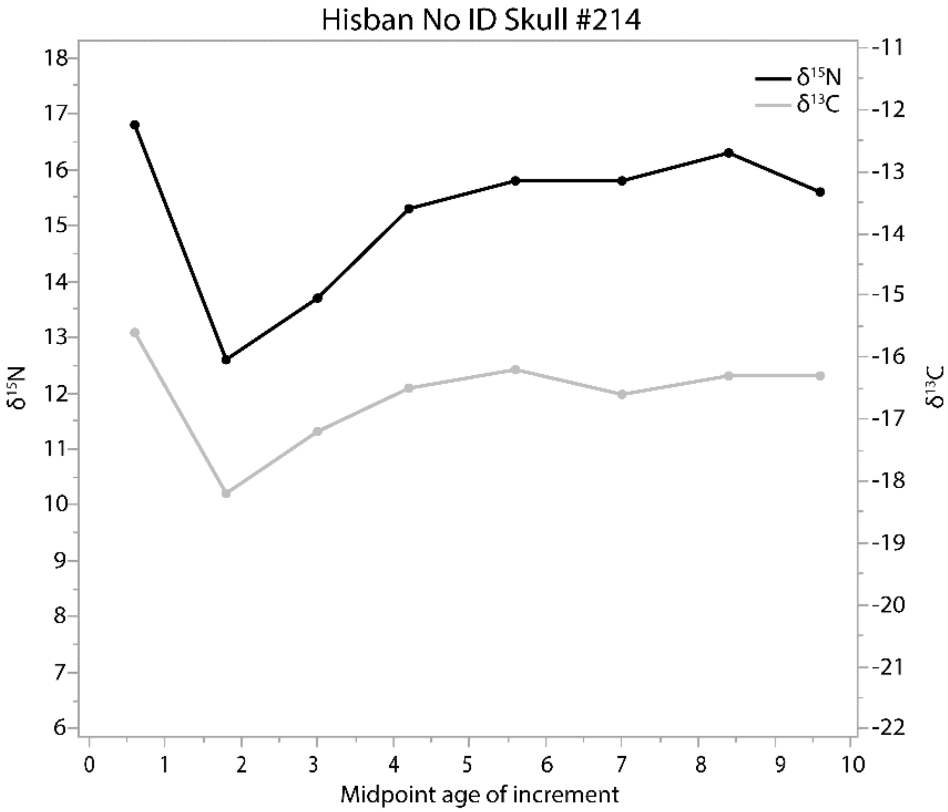


Figure 9: Results of $\delta^{13}C$ and $\delta^{15}N$ analysis in relation to age estimation of Individual C's first molar dentin

<u>*Individual C (Hisban No ID Skull # 214)*</u>

Individual C is an adult female, aged between 18 and 20 years, with no evidence of IGD; seven incremental samples were taken from their upper left first molar (LM^1). The results of the $\delta^{13}C$ analysis ranged from -18.2‰ to -15.6‰ with an average of – 16.6‰. The results of the $\delta^{15}N$ analysis ranged from 12.6‰ to 16.8‰ with an average of 15.2‰ (Figure 10).

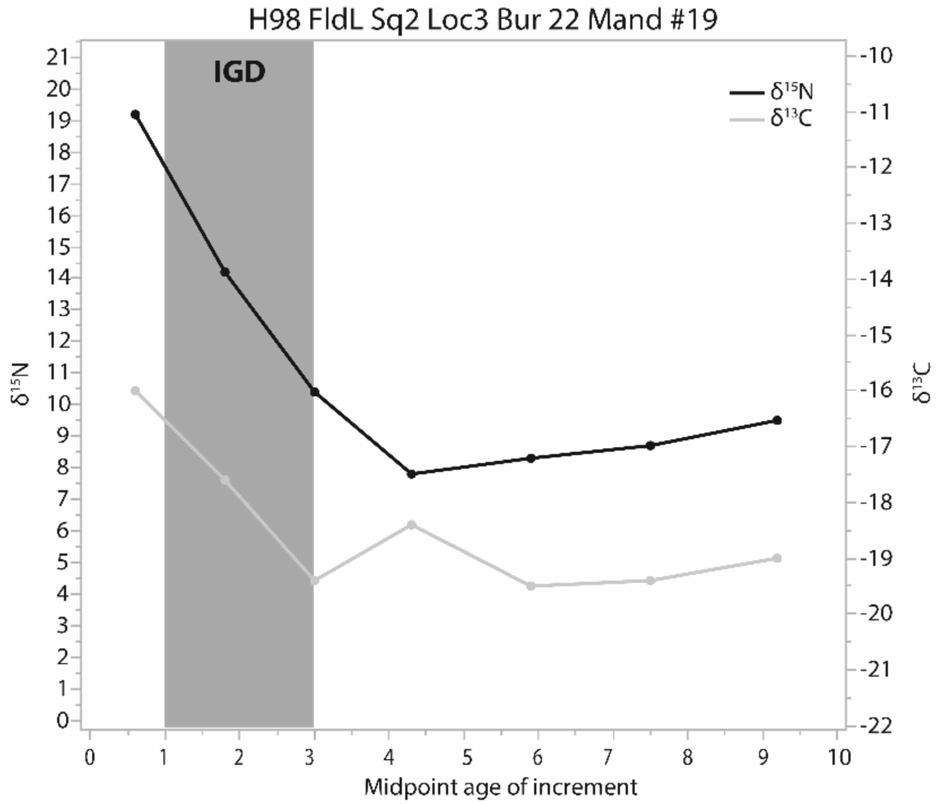


Figure 10: Results of $\delta^{13}C$ and $\delta^{15}N$ analysis in relation to age estimation of Individual D's first molar dentin

<u>*Individual D (H98 FLDL Sq2 Loc3 Bur#22 Mandible #19)*</u>

Individual D is an adult whose sex is indeterminate with evidence of IGD between one and three years. Seven incremental samples were taken from the lower left first molar (LM_1). The results of the $\delta^{13}C$ analysis ranged from –19.5‰ to –16.0‰, with an average of –18.5‰. The results of the $\delta^{15}N$ analysis ranged from 7.8‰ to 19.2‰ with an average of 11.2‰ (Figure 11).

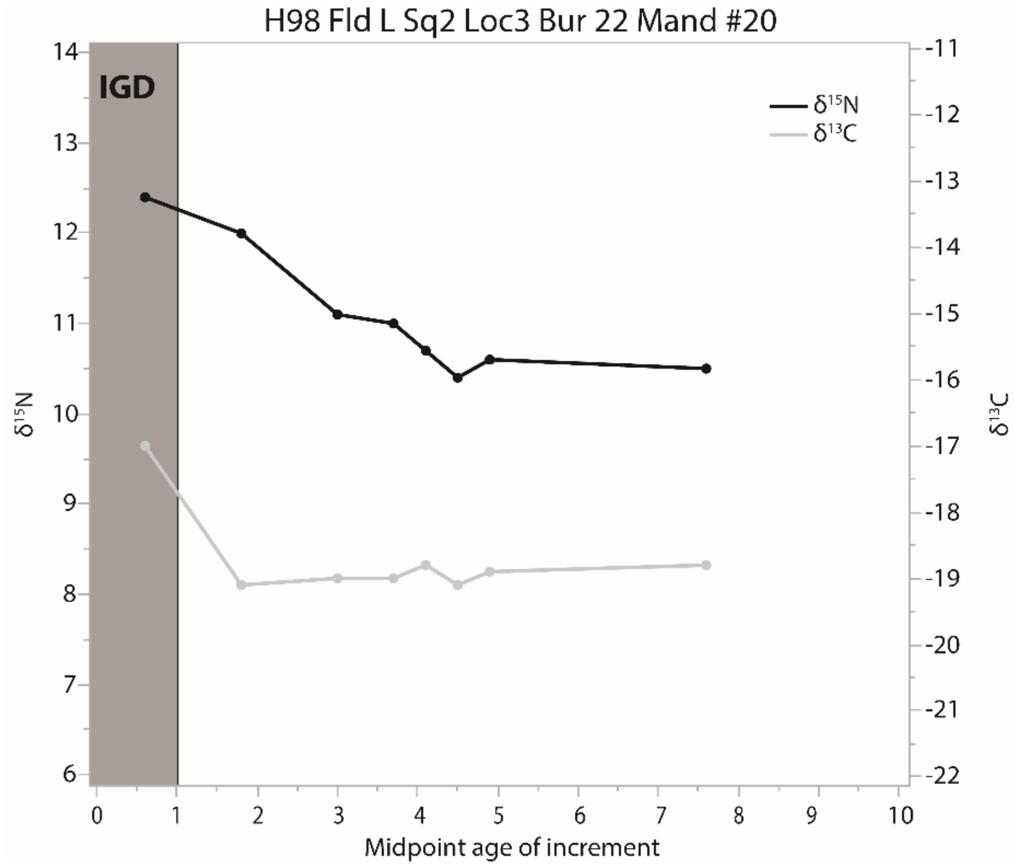


Figure 11: Results of $\delta^{13}C$ and $\delta^{15}N$ analysis in relation to age estimation of Individual E's first molar dentin. The gray box indicates evidence of IGD in Individual E's first molar.

<u>Individual E (H98 FldL Sq2 Loc 3 Burial #22 Mandible #20)</u>

Individual E is an adult male with evidence of IGD between prenatal and one year of age. Eight incremental samples were taken from the lower left first molar (LM_1). The $\delta^{13}C$ analysis results ranged from -19.1‰ to -17.1‰, with an average of -18.7‰, 0.9‰. The $\delta^{15}N$ analysis results ranged from 10.4‰ to 12.4‰ with an average of 11.1‰ (Figure 12).

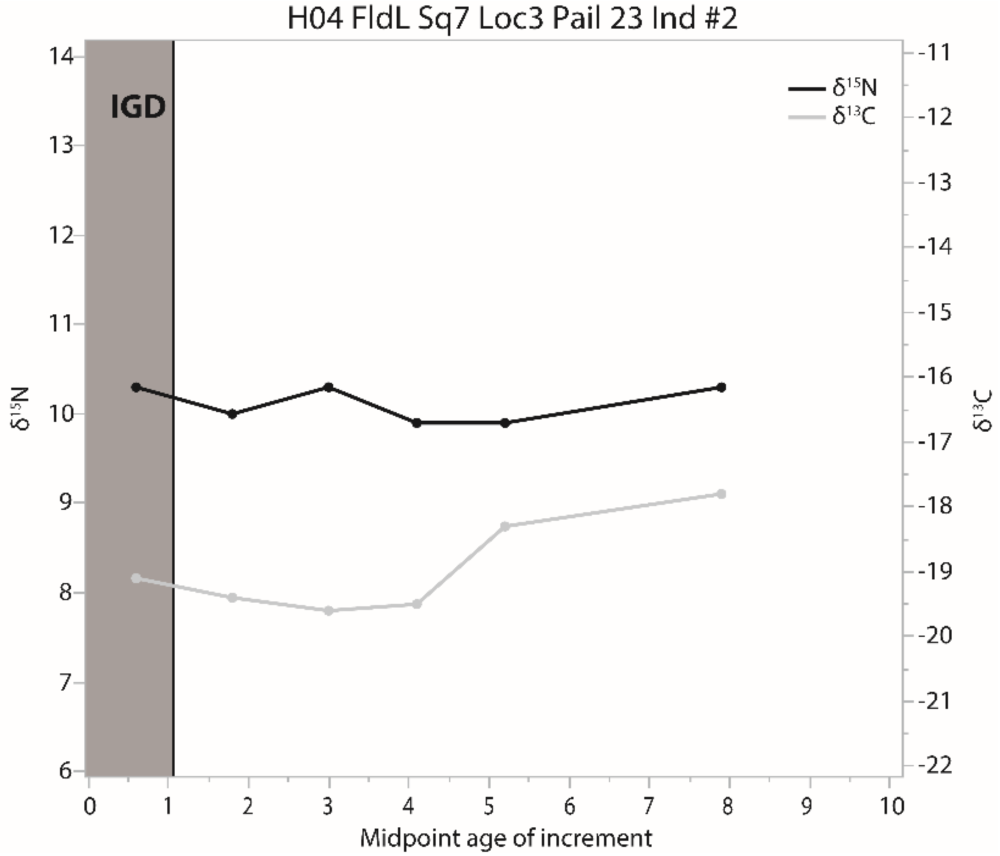


Figure 12: Results of δ^{13}C and δ^{15}N analysis in relation to age estimation of Individual F's first molar dentin. The grey box shows evidence of IGD in Individual F's first molar.

Individual F (H04 FldL Sq7 Loc3 Pail 23 Ind. #2)

Individual F is an adolescent aged around 15 years ± 36 months with evidence of IGD between birth and one year. Six incremental samples were taken from their lower left first molar (LM$_1$). The δ13C analysis results ranged from –19.6‰ to –17.8‰ with an average of –18.9‰. The results of the δ^{15}N analysis ranged from 9.9 to 10.3‰, with an average of 10.1‰ (Figure 13).

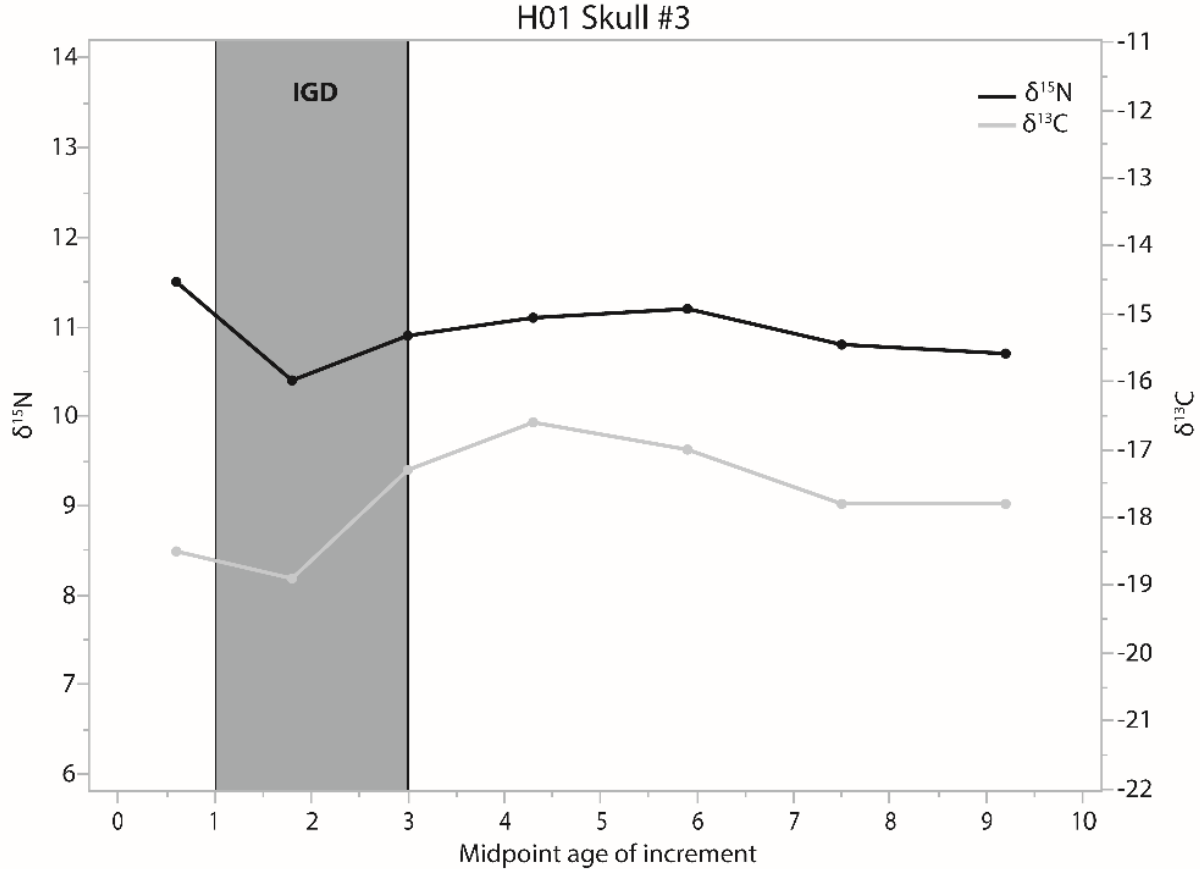


Figure 13: Results of $\delta^{13}C$ and $\delta^{15}N$ analysis in relation to age estimation of Individual G's first molar dentin. The grey box demonstrates evidence of IGD in Individual G's first molar.

<u>*Individual G H01 Skull #3*</u>

Individual G is an adult male with evidence of IGD between 1 and three years of age. Nine incremental samples were taken from the lower right first molar (RM_1). The results of the $\delta^{13}C$ analysis ranged from -18.9‰ to -16.6‰ with an average of -17.6‰. The $\delta^{15}N$ analysis results ranged from 10.1‰ to 11.5‰ with an average of 10.8‰ (Figure 14).

CHAPTER 5: DISCUSSION

Overall dietary and weaning patterns

The results of the $\delta^{13}C$ analysis (mean $\delta^{13}C$ = -17.8±1.4‰) revealed that the Hisban population primarily consumed a C_3 diet, with some C_4 contributions consumed either directly or indirectly through animal protein such as sheep, goats, and potentially cattle who grazed on C_4 plants. The $\delta^{15}N$ values (mean $\delta^{15}N$ = 11.3±2.2‰) suggest a significant intake of animal protein and environmental stress factors such as living in an arid climate (Vaiglova et al., 2020). Isotopic studies from similar ecological regions indicate that these values diverge slightly from previous studies. A Late Ottoman site, Khirbat al-Mudayna, 40 km south of Hisban, yielded a $\delta^{13}C$ mean of -15.6 ±1.5‰ and $\delta^{15}N$ mean of 12.7‰ ±1.9 (Gregoricka & Judd, 2016). The children at Hisban seemed to consume more C_4 sources and had a slightly lower protein component. Another site 30 km north of Hisban, Ya'amūn, dated to an earlier period, results yielded much higher $\delta^{13}C$ (Bronze Age mean: -18.8 ±0.3‰, Byzantine mean: -19.1 ±0.3‰) and lower $\delta^{15}N$ values (Bronze age mean: 8.8 ±0.7‰, Byzantine mean: 8.1 ±0.6‰) than either Hisban or Khirbat al-Mudayna (Sandias & Muldner, 2014).

Beaumont and colleagues (2018) found that stunted growth from stress or malnutrition leads to slower bone turnover, preventing accurate dietary capture in bone collagen, unlike continuously forming dentine. Consequently, the potential incomparability of bone and dentin collagen values means our results may not be comparable to those of Sandias and Muldner (2014) and Gregoricka and Judd (2016). Additionally, the ages of the individuals captured in our sample and the Gregoricka and Judd (2016) study are different. Our study analyzes the childhood diet of adult individuals, and their study analyzes children and adult diets. The higher isotope ratios in the Late Ottoman communities, Hisban and Khirbat al-Mudayna, could result from greater water stress experienced by nomadic communities.

As previously discussed, studies have identified this weaning-related isotopic shifts in permanent teeth as a -2 to -4‰ drop in $\delta^{15}N$ isotope values (Tsutaya & Yoneda, 2015; Jay, 2009; Eerkens et al., 2011). While studies have utilized this method, it is important to consider the variability of the weaning process, with its timing and duration influenced by each individual's unique needs (Fuller et al., 2006; King et al., 2017). Information on the beginning and end of the weaning process at Hisban is less clear, as inferring weaning practices based on isotopic shifts relies on analyzing small increments of dentin in the tooth crown and root. In deciduous teeth, 1mm increments in crown dentin reflect around 0.2 years of development. In permanent teeth used in this study, each 1mm of crown dentin can equate to over 1.2 years. Therefore, if weaning begins at 0.5 years, the first increment would include a tiny portion of the prenatal period, the sole breastfeeding period, and the period when solid foods began to be added (see Figure 14). The second increment may capture the weaning period, marked by a shift in isotope ratios as breastfeeding ceases. Therefore, the isotope results in this study may not clearly indicate the age at which weaning began. In addition, pinpointing the exact end age of weaning may be obscured by isotopic mixing within increments spanning both during and post-weaning periods, although a decline in $\delta^{15}N$ between the first two increments would reflect different stages of the weaning process. However, it is also necessary to consider that not every isotopic shift in childhood reflects the weaning process or dietary change alone. Environmental factors such as water availability and transhumance patterns may also influence $\delta^{15}N$ and $\delta^{13}C$ isotopic patterns, particularly in this context.

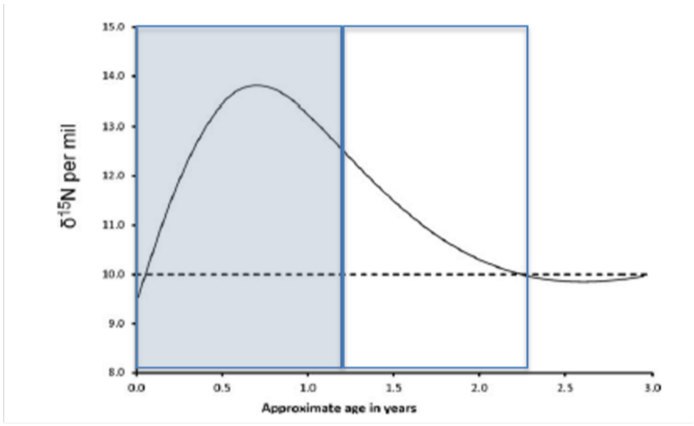


Figure 14: The typical postnatal pattern of δ^{15}N if weaning begins at 0.5 years of age. At birth, the infant only has absorbed nitrogen isotopes in utero that are similar to the mother's values. During breastfeeding, the infant's δ^{15}N increases gradually as its tissues absorb δ^{15}N from breastmilk. The values then decline with decreased breastmilk and increased solid foods during weaning (from Beaumont et al. 2015) to return to expected adult values. The shaded box indicates the ages during which the first 1mm increment of dental enamel in the permanent first molar formed. The unshaded box represents the next increment, which averages isotopes absorbed between 1.2 and 2.4 years

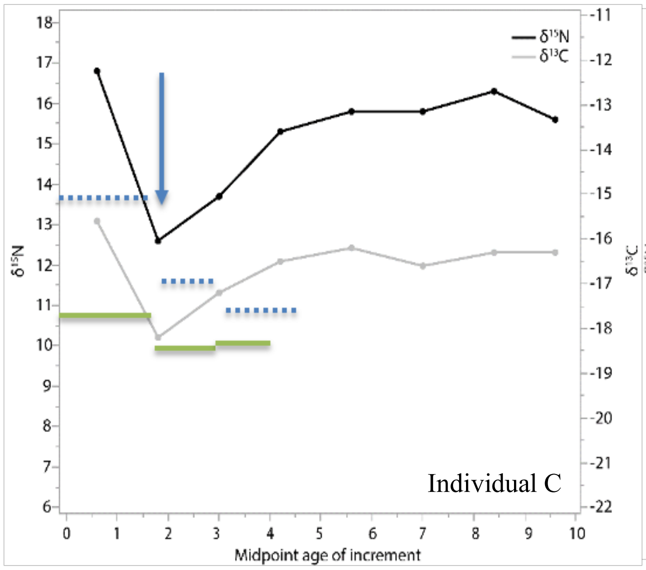
Environmental fluctuations due to transhumance practices and yearly weather patterns would also impact δ^{15}N and δ^{13}C. For example, the proportion of contracted diffused vegetation in their diets due to water availability in arid environments can impact isotope ratios. Contracted vegetation exhibits higher δ^{15}N values than diffused vegetation due to biomass accumulation in drainage channels. As organic material decomposes, it releases nitrogen gas, which enriches the soil and is absorbed by plants (Vaiglova et al., 2020; Reid et al., 2018; Heaton, 1987). Diffused vegetation growing on flatlands or exposed ridges has lower δ^{15}N values because these soils release less nitrogen gas. Environmental water availability also influences carbon fractionation rates in plants; the δ^{13}C values of C_3 plants become more negative under higher water availability, whereas the δ^{13}C values of C_4 plants become less negative during water stress (Vaiglova et al., 2020; Hartman & Danin, 2010; Reid et al., 2018; Farquhar et al., 1989).

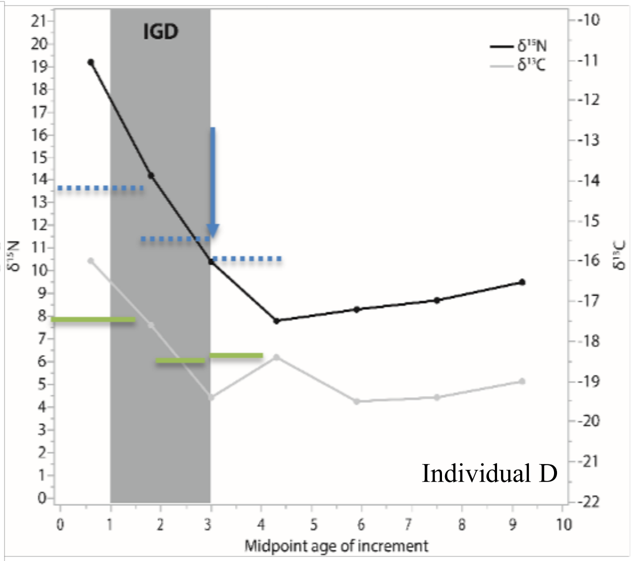
This variation in isotopic values between contracted and diffused vegetation is reflected in the diets of domesticated animals, such as sheep and goats, which consume both types of vegetation depending on availability (Habte et al., 2022; Tulu, 2023; Lu, 1988). During periods of water stress, contracted vegetation may be sparse, whereas diffused vegetation may be more available. Nitrogen and carbon isotope ratios in animals and humans reflect this dietary balance, providing insights into environmental conditions during longer-term climatic shifts (Vaiglova et al., 2020), but in this case, seasonal variations may not be reflected due to the developmental period of each dentin increment discussed above. These isotopic patterns reflect environmental conditions and provide a framework for interpreting broader climatic changes and their impact on human health, including the prevalence of rickets.

Tracking the shifts in δ^{15}N in relation to those in δ^{13}C will, by the individual, highlight the environmental, dietary, and physiological impacts on isotope values. Researchers such as Beaumont and colleagues (2015, 2018) note that dietary shifts due to weaning or transitioning to a new environment usually produce covariation in δ^{13}C and δ^{15}N across childhood. A rise in δ^{15}N and a decline in δ^{13}C, producing negative covariation, may indicate prolonged stress from factors like injury, illness, or malnutrition that disrupts metabolic balance, resulting in tissue catabolism (Brickley et al., 2015; Beaumont & Montgomery, 2016; Beaumont et al., 2015, 2018;). On the other hand, a decline in δ^{15}N with little-to-no changes in δ^{13}C may represent periods of growth. (Feuillâtre et al. 2022; Kendall et al., 2020). Recognizing these isotopic shifts and the interplay between the δ^{15}N and δ^{13}C values can provide insight into the relationship between stress, diet, and early childhood.

As noted in the background, ethnographic studies of Bedouin communities in the Near East have noted that breastmilk remains part of an infant's diet from anywhere from 6 months up to two years, but the actual duration depends on the needs and decisions of the mother (Abu Rabia, 2007; Joseph, 2013). How much social support the mother has, what season the infant is born, and the health of the mother all influence the weaning process's duration (Naggan et al., 1991). This pattern of weaning beginning at 6 months and ending past 18 months is paralleled in isotopic studies on bone collagen of the Bedouin community at Khirbet al-Mudayna, which indicate that the weaning process began at 6 months of age and ended around 2 years (Gregoricka & Judd, 2016). Therefore, we expect the isotopic values from Hisban to reflect that seen in Figure 15 if the dietary sources of the infant's solid food are isotopically the same as that seen in the adults and the climate and local environment remained stable.

Some individuals display the expected weaning-related slight decline in both $\delta^{13}C$ and $\delta^{15}N$ between the first and second increment, including Individuals E, F, G, and possibly A. Two individuals, C and D, show a much more significant than expected decline, possibly indicating other factors impacting their isotopic values and the weaning process (Figure 15). These two individuals have $\delta^{13}C$and $\delta^{15}N$ values from their first year of life, which are much higher than the remaining individuals in this study. By their second year, Individual C has reached their lowest level of $\delta^{13}C$and $\delta^{15}N$ values, with a slightly elevated $\delta^{15}N$ compared to the average. On the contrary, Individual D reaches its lowest levels between 3 and 4 years, which at their lowest are





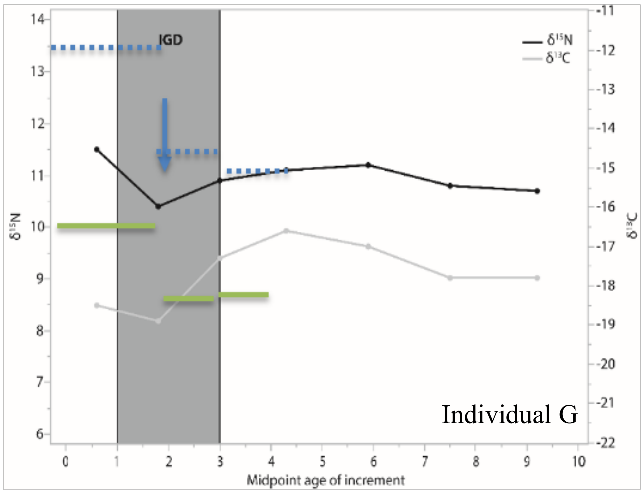
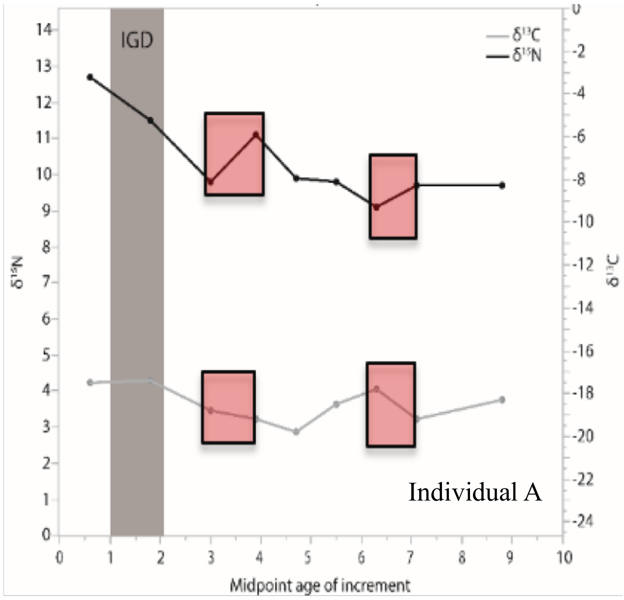


Figure 15: Individual C (upper left), individual D (upper right), and Individual G (lower left). The lowest levels of δ¹³C and δ¹⁵N are indicated by the arrows, and the δ¹⁵N in the first three increments is indicated by the blue dotted line, and the average δ¹³C of the first three increments is indicated by the solid green line.

below the average isotope ratio values. This pattern may indicate that, in addition to the breastfeeding and weaning signature, these individuals spent their earliest years in a climate more arid and largely supported C_4 sources before moving to an area less arid and with more C_3 sources, particularly in the case of Individual D. While Individual D, by 9 years of age, has isotopic ratios similar to the other individuals. Individual C's values increase again in a covarying manner and remain high throughout their childhood, which may be due to returning to a relatively arid region with more C_4 sources. Individual G has a similar pattern of $\delta^{13}C$ and $\delta^{15}N$



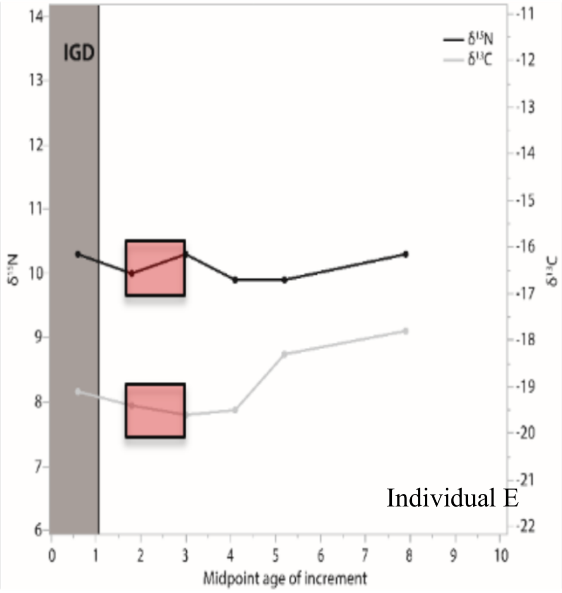
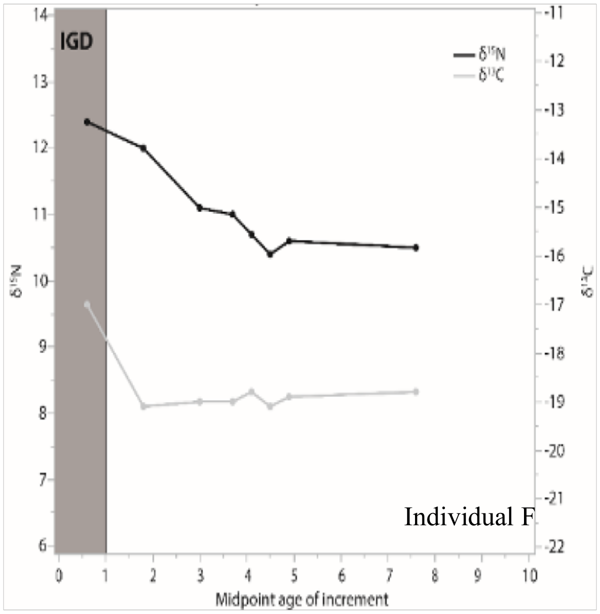


Figure 16: Individual A (top left), Individual E (top right), Individual F (bottom right). Periods of negative covariance are shown with the red boxes on Individual A and E.



covariance as Individual C, with a sharp decline between the first two increments and a gradual increase from 2 until about 4.5, followed by a slight decline. However, individual G's values align more with the average.

Individuals A, E, and F have δ^{13}Cand δ^{15}N values that remain relatively stable throughout their childhood. As shown in Figure 16, A and E have slight declines between the first and second increments, indicative of normal weaning. Individuals A and F have some negative covariance periods between δ^{13}C and δ^{15}N. These increases in δ^{15}N concomitant with decreases in δ^{13}C, possibly indicating physiological stress. Individual F has negative covariance between 2 and 3 years, following their period of IGD between birth and 1 year of age. Additionally, this pattern is shown between 3 and 4 years in Individual D (Figure 15). This pattern could also occur with decreased animal protein consumption and stable or increased C_4 resources.

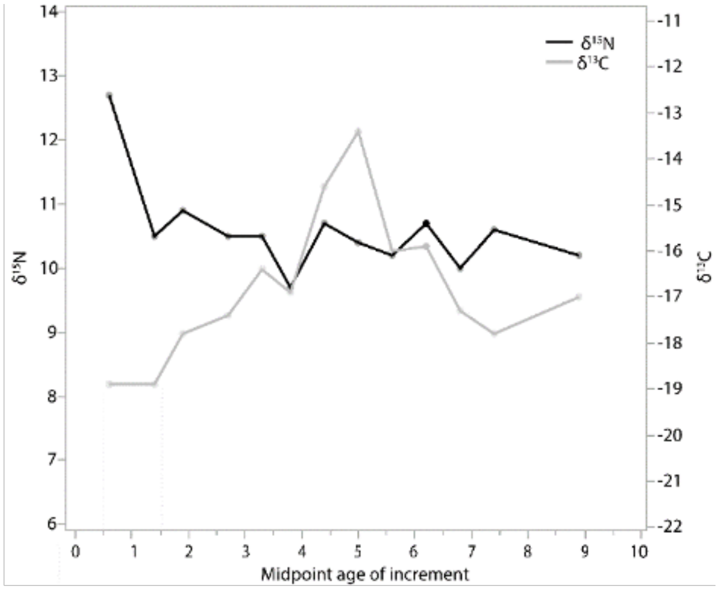


Figure 17: Individual B showing the dramatic fluctuations in δ13C and lack of consistent covariation in δ13C and δ15N. this pattern may result from a combination of weaning and seasonal or longer-term changes in aridity and plant sources.

Individual B's results yield very little covariance throughout their childhood and instead have dramatic fluctuations in δ^{13}C and δ^{15}N values compared to the other individuals in the sample (Figure 17). Between two and five years of age, there is an increase in C_4 sources and then a decline between five and seven and half years, with only a nominal change in δ^{15}N. Additionally, there is no parallel decline in δ^{13}C with the 2.5‰ decline in δ^{15}Nduring years one to two. Much of Individual B's pattern is most likely related to drastic fluctuations of C_4 plants in their diet due to climatic or migration shifts from drier to wetter years or locations.

Isotopic patterns and IGD

Five individuals included in this study have IGD in their first molar: Individuals A, D, E, F, and G. The δ^{13}Cvalue averaged -16.7‰ ± 1.3 for individuals without IGD and -18.5‰ ± 0.9 for those with IGD. Similarly, δ^{15}N values averaged 12.4‰ ± 2.5 for individuals without IGD and 10.4‰ ± 1.9 for those with IGD. Individuals E and F have evidence of IGD between birth and 1 year of age, indicating that the mother was vitamin D deficient (Perry et al., 2021). Although breastmilk typically does not provide sufficient vitamin D for the infant, even if the mother has adequate levels of vitamin D, maternal vitamin D deficiencies can still impact the infant (Mellati et al., 2015). Therefore, if the mother has VDD, complications such as malnutrition, hypothyroidism, and even psychological stress can hinder breast milk production (Gatti et al., 2015; WHO, 2023; 2016; Hamdam et al., 2012). As a result, the inability to produce adequate breast milk can make these mothers more likely to initiate the weaning process earlier than mothers with sufficient vitamin D (Adebi et al., 2018; Pope & Mazmanian, 2016).

Individual A has IGD forming between 1 and 2 years of age, where their nitrogen isotope ratio is decreasing, and the carbon isotope ratio remains stable. Individuals D and G have IGD forming between 1 and 3 years of age, but their isotopic patterns diverge. Individual D has a dramatic decline in δ^{13}C and δ^{15}N that may be attributed to spending their first 3 or 4 years in a more arid environment or consuming more animal products or more C_4 sources, and their solid foods contain increasingly more C_3 sources and less animal protein components over time.

With the exception of Individual D, the individuals with IGD have an isotopic pattern reflecting the expected weaning pattern and a stable diet with only minor fluctuations in the amount of C_4 sources consumed. Individuals without IGD, including Individuals B and C, display more dramatic dietary and sometimes climatic shifts during childhood. In general, both had lower $\delta^{13}C$ and higher $\delta^{15}N$, which would indicate, in addition to any possible weaning isotopic shifts, they had more C_4 contributions to their diet and either consumed more animal products or lived in a more arid region compared to those with IGD. While this may point to diet as a factor in IGD, and thus VDD, in childhood, it is more likely that it reflects time spent in a slightly different climate with different plant resources available for humans and their herd animals.

There does not appear to be a relationship between negative isotopic covariance indicative of malnutrition or other factors related to this pattern and the development of IGD. Individual A has IGD forming between 1 and 2 years of age and a negative covariance possibly indicative of stress from 3 to 4 and from 6.5 to 7.5 years. Individual F has IGD forming between 0 and 1 years of age and isotopic evidence of stress from 2 to 3 years of age. The pattern of IGD formation followed by possible catabolism a year or so later may indicate that early-life IGD, and thus VDD, can have longer-term effects after VDD resolves. However, not all individuals with IGD have signs of stress-related isotopic negative covariance; thus, this relationship is tentative.

Environmental Factors

Our study underscores the importance of considering environmental factors in dietary studies. This variation highlights the need to adapt dietary studies to account for the unique circumstances of specific populations. In arid regions like Jordan, rainfall directly influences food availability and migration patterns, potentially increasing the risk of nutritional deficiencies during critical developmental periods, such as early childhood. Desert regions exhibit distinct carbon and nitrogen isotopic patterns shaped by vegetation growth influenced by rainfall (Hartman & Danin, 2010; Tulu, 2023). The Jordanian landscape has two types of vegetation: diffused and contracted (Le Houérou, 2005). Diffuse vegetation, composed of dwarf shrubs and perennial grasses, requires less water and includes a mix of C_3 and C_4 plants. In contrast, contracted vegetation, which depends on an annual rainfall of at least 100 mm, primarily comprises C_3 plants, such as long grasses, which flourish in drainage channels, collecting runoff during the winter rains.

While the identity of the Hisban community represented by these burials is unclear, ethnohistorical and archaeological evidence suggests they were agropastoralists, some or all of whom may have practiced transhumance. (Perry and Edwards, 2020; LaBianca, 1990; Abujaber, 1989; Rogan, 1999). Historical travelers report tribes in the area of Hisban growing "maize" (a British English term used during the period to refer to wheat and barley) that the Hisban Bedouin could have either grown for a larger established farmstead or traded with other communities. Isotopic shifts could, therefore, reflect either seasonal shifts due to migration or climate or both or the dispersal of the group for more extended periods of time. Unfortunately, the time represented in one dentin increment would be too long to reflect seasonal transhumance or temperature and precipitation patterns. Thus, we can only suggest that seasonal patterns resulted in some shifts while individuals' environments are more consistent. If these individuals were nomadic, these dietary shifts could have reflected longer-term residence in regions with different climates and plant resources (Carroll, 2006; Russell, 1988). The shift to more C_4 sources in

Individuals B, C, and D may indicate a different residence during childhood, either due to economic and climatic pressures or because they may have been affiliated with a different tribal lineage with different tribal lands.

Shifts in residential environments seem to have been beneficial in terms of the development of IGD. In general, the individuals who developed IGD have more homogeneity in both their $\delta^{13}C$ and $\delta^{15}N$ than those who had none. Further isotopic studies of human mobility using $\delta^{18}O$ and $^{87}Sr/^{86}Sr$ in dental enamel can illuminate whether or not these shifts in $\delta^{13}C$ and $\delta^{15}N$ actually reflect exposure to different environments and different dietary sources, but it is possible that the nomadic lifestyle of one subsample of individuals meant they spent more time exposed to UVB radiation and potentially dietary sources that helped prevent VDD.

In addition, there may be a potential temporal effect on the isotopic patterns seen in this community. Between the 18th and 19th centuries, Hisban transitioned from a seasonal outpost with small cultivation areas to a region surrounded by extensive farmsteads. During this period, crops like wheat became increasingly popular for cultivation, reflecting broader regional agricultural trends (LaBianca, 1990; Walker & LaBianca, 2003). Perhaps isotopic shifts in individuals C and B show evidence of this cultural transition.

Longer-term climatic shifts due to historical climatic events like volcanic eruptions may have exacerbated these conditions for some individuals. For instance, during the 19th century, 19 volcanoes in the lower hemisphere erupted at various times. Each eruption released sulfur and ash into the atmosphere, blocking UVB rays and resulting in "long winters" with global temperature drops and unpredictable climates. Notable examples include the eruptions of Mt. Tambora (1815) and Mt. Krakatoa (1883), followed by recorded famines in the northern hemisphere (Schaller et al., 2009). These environmental stressors likely compounded the challenges of maintaining sufficient vitamin D levels through sunlight exposure or diet, particularly for vulnerable populations like those at Hisban. These findings highlight the compounded effects of environmental stress, maternal health, and cultural shifts on the nutritional challenges faced by the Hisban population.

Why did these individuals survive?

While the individuals with IGD in this study represent those who survived episodes of childhood rickets, many of the infants in the burial assemblage did not. The isotopic data presented here does not indicate why these individuals survived into later childhood and adulthood. However, isotopic analysis of incremental dentin as well as IGD in infants who died may shed light on their different early life histories that resulted in infant mortality.

A possible reason why the individuals in this study survived childhood rickets while others did not could be alternate sources of calcium during breastfeeding. The average mean $\delta^{15}N$ value of the sample was 11.3±2.2‰, including the step nitrogen inclines at the beginning of life, but then leveled off to 8‰. Many stable isotope studies have attributed C_4 signatures in individuals to indirect consumptions of C_4 plants through goat or sheep protein consumption since C_4 plants are one of the main components of goat and sheep diets (Gregoricka & Judd, 2016; Sandias & Muldner, 2014; Lu, 1988). As a result of their consumption of C_4 plants along with the 1‰ increase in milk compared to its source (Fuller et al. 2006), goat and/or sheep milk can reflect a more C_4 signature than human milk. In addition, goat and sheep milk has higher amounts of calcium than breast milk or cattle milk [goat milk (130 mg/100 g of milk), sheep milk (197.5 mg/100 g of milk), cattle milk (112 mg/100 g of milk), breast milk (26.1 mg/100 g of milk)] (Nguyen, 2022). As reflected in the background section, Vitamin D and calcium have a symbiotic relationship. If an individual's calcium or vitamin D levels drop, the bone matrix could be compromised (Lips et al., 2014; Holick et al., 2007). According to some research, calcium supplements may help improve bone matrix integrity when Vitamin D is low (Snoddy et al., 2023). The individuals in this sample may have had greater access to or consumed more animal milk than those in the Hisban assemblage who did not survive. Isotopic analysis of the infants at Hisban tracking their diet before death would illuminate this hypothesis.

In addition, the infants in the Hisban sample may have died during a period of climatic stress more profound than that experienced by the non-infants. The timing of birth may have determined survival. Individuals who survived childhood metabolic diseases may have been born at the end of these climatic shifts, when environmental conditions began stabilizing, allowing for better nutrition. In addition, birth during periods of higher UVB radiation, such as the spring and summer months, can reduce the likelihood of developing VDD. As a result, they survived till 2 years of age. By this stage, children would have spent more time outdoors, receiving higher levels of UVB radiation essential for vitamin D synthesis. In contrast, those born during the middle or onset of these periods likely faced harsher environmental challenges and limited resources, which made their nutritional deficiencies more extreme. As a result, they would be less likely to survive past 2 years of age.

Summary

The site of Hisban is unique because, compared to nearby sites, it has an unusually high rate of juvenile rickets (Perry & Edwards, 2020). Isotopic analysis also revealed that these individuals consumed slightly more C_4 foods and had a high protein intake. While dentine incremental resolution of permanent teeth limits the specific time frame of the weaning process, it also shows seasonal variation and water availability. These individuals experienced periods of climate change either from migration, weather, or transhumance change. These results highlight the complex interaction between environment, cultural practices, and physiological stress in shaping early childhood health and survival at Hisban.

CHAPTER 6: CONCLUSION

Climatic shifts like famine and drought can severely stress historic populations, with children often the first affected. In the case of the Hisban individuals, it seems that changes to the lived environment, either through migration or longer-term climatic change, impacted most of the isotope ratio patterns rather than weaning practices alone. Here, individuals with childhoods spent in more environmentally diverse contexts were less likely to develop IGD, and hence rickets, than those who remained in more homogeneous regions across childhood.

A few individuals experienced potential periods of nutritional stress based on the negative covariance patterns of their $\delta^{13}C$ and $\delta^{15}N$, but this was not necessarily linked to the development of IGD. The infants in the sample demonstrate scurvy was a consistent issue alongside rickets, but evidence of vitamin C or vitamin D deficiencies was not noted in the non-infant individuals studied here. The skeleton does not retain any known signs of childhood scurvy after bone reparation, but we can assume that some of the individuals who survived infancy also suffered from scurvy during that period. According to Schattman & colleagues (2016), a co-occurrence of rickets and scurvy is often seen in young, undernourished juveniles between three months and five years of age, and in their case, scurvy was the more prominently developed condition. Determining whether rickets or scurvy is the primary and secondary disease can be difficult and, in the case of Hisban, is unclear, particularly since we are unable to link together crania with scorbutic lesions with postcranial with their rachitic changes. For the most part, once children reach the age of two when they gain slightly more mobile independence and potentially have broader food sources, their nutritional deficiencies are alleviated.

While the Hisban population has been deceased for close to 200 years, their struggles are still relevant today. When explaining the Hisban project to the public, they often respond with confusion to the diagnosis of rickets. Rickets is a disease primarily caused by a lack of sunlight, and Jordan experiences loss of sun throughout the year, so how can a population living in an environment constantly exposed to UVB radiation have a vitamin D deficiency? The Hisban sample shows that rickets can occur even in environments with ideal levels of UVB radiation. During the first few months of an infant's life, their nutrients are entirely reliant on their mothers, and if their mother has vitamin D deficiency, they will be as well. Climatic shifts can heighten the risk factor of rickets, increase the stress on the mother, and result in diminished food sources. According to the National Institute of Health, today, 1 in every 200,000 children under 15 has vitamin D deficiency symptoms. With climate change creating unstable weather patterns, drought, and forced climatic immigration, these numbers will likely increase over the next century.

Future Research

While incremental stable isotope analysis of dentine has proven valuable in dietary studies, it is not without limitations. A significant drawback is its temporal resolution. Although dental development is relatively consistent across humans, there is still variation among populations (Dean, 2009; Eerkens et al., 2011). The method used in this study assumes crown formation is completed around 3.5 years of age, with root development concluding at approximately 9.5 years. Each incremental sample represents a period of six to eight months based on these averages. However, this broad averaging can obscure short-term variations in weaning practices or dietary transitions, limiting the method's ability to capture abrupt changes such as cessation of breastmilk, further complicating interpretations of weaning practices. Using incremental samples of deciduous teeth, where 1mm dentine increments of the crown reflect around 0.2 years, might provide more accurate data about the start and end of the weaning process. However, this is not possible with adults who have since lost their deciduous teeth. The next stage of research for the Hisban sample will be to collect stable isotope data on the infant teeth of the Hisban sample, which may allow insight into the weaning process. It will also provide additional context to the broader implication of rickets survival rates by comparing the childhood diets of the adults who survived infantile rickets to the juveniles who did not survive.

Limitations

While conducting this study, it is essential to note that the dead may or may not represent the living members of a community (DeWitte et al., 2015; Brothwell, 1987). All members of a mortality sample are there because they did not survive past a certain age, resulting in a "mortality bias" in interpreting their morbidity and mortality patterns. This research focuses on the individuals who survived infancy to better understand how and why some individuals may or may not have developed childhood rickets and why some may or may not have died while they had this condition. Additionally, this sample comes from commingled remains, which prevents linking together lesions seen across the entire skeleton of an individual to better understand comorbidities.

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APPENDIX

Incremental stable isotope collected data.

USF #	SAMPLE	**δ^{13}C**	**δ^{15}N**	C: N	C%	N%	LABEL
42402	H1032a	**-16.4**	13.1	3.3	44.7	15.6	H98 FldL Sq2 Loc7 #103
42402	H1032a	**-18.5**	12.2	3.3	40.5	14.3	H98 FldL Sq2 Loc7 #103
42403	H1032b	-17.6	**10.9**	3.3	22.5	7.9	H98 FldL Sq2 Loc7 #103
42403	H1032b	-17.1	**12.2**	3.2	22.0	8.0	H98 FldL Sq2 Loc7 #103
42404	H1032c	-19.1	9.2	3.4	28.6	9.8	H98 FldL Sq2 Loc7 #103
42404	H1032c	-18.5	10.3	3.3	34.5	12.4	H98 FldL Sq2 Loc7 #103
42405	H1032d	**-20.1**	10.6	**3.7**	24.7	7.8	H98 FldL Sq2 Loc7 #103
42405	H1032d	**-18.2**	11.7	3.2	25.4	9.2	H98 FldL Sq2 Loc7 #103
42406	H1032e	**-20.8**	9.8	**3.9**	32.2	9.7	H98 FldL Sq2 Loc7 #103
42406	H1032e	**-18.8**	10.0	3.3	26.2	9.3	H98 FldL Sq2 Loc7 #103
42407	H1032f	-18.2	9.7	3.3	29.4	10.4	H98 FldL Sq2 Loc7 #103
42407	H1032f	-18.8	9.9	3.2	26.4	9.6	H98 FldL Sq2 Loc7 #103
42408	H1032h	**-17.1**	9.6	3.2	30.1	10.9	H98 FldL Sq2 Loc7 #103
42408	H1032h	**-18.4**	8.7	3.4	25.6	8.7	H98 FldL Sq2 Loc7 #103
42409	H1032g	-19.0	9.5	3.4	22.3	7.7	H98 FldL Sq2 Loc7 #103
42409	H1032g	-19.4	9.9	3.4	31.4	10.8	H98 FldL Sq2 Loc7 #103
42410	H1032i	-18.3	9.7	3.3	32.8	11.5	H98 FldL Sq2 Loc7 #103
42410	H1032i	-18.4	9.8	3.4	37.4	13.0	H98 FldL Sq2 Loc7 #103
42411	H2002A	-16.5	**13.2**	3.4	31.8	11.1	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42411	H2002A	-17.6	**11.6**	3.3	33.3	11.7	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42412	H2002B	-18.9	12.3	3.3	27.2	9.7	H98 FldlL Sq2 Loc3 Bur22 Mand #20

42412	H2002B	-19.3	11.7	3.4	31.4	10.8	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42413	H2002C	-18.9	11.1	3.2	30.6	11.1	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42413	H2002C	-19.1	11.1	3.2	36.4	13.2	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42414	H2002D	-19.1	10.6	3.3	24.7	8.7	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42414	H2002D	-18.8	11.4	3.4	26.0	9.1	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42415	H2002E	-18.7	10.5	3.2	28.8	10.4	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42415	H2002E	-18.9	10.9	3.2	26.7	9.6	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42416	H2002F	-19.1	10.2	3.3	25.6	9.2	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42416	H2002F	-19.1	10.7	3.3	29.0	10.3	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42417	H2002G	-18.9	10.8	3.3	32.9	11.7	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42417	H2002G	-18.9	10.4	3.3	36.9	13.2	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42418	H2002H	-18.7	10.4	3.3	35.8	12.8	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42418	H2002H	-18.9	10.6	3.3	35.4	12.5	H98 FldlL Sq2 Loc3 Bur22 Mand #20
42419	H2162a	-19.2	13.1	3.5	21.5	7.1	Hisban Misc No ID Skull #216
42419	H2162a	-18.6	12.3	3.2	24.6	8.9	Hisban Misc No ID Skull #216
42420	H2162b	-18.8	10.0	3.3	19.3	6.8	Hisban Misc No ID Skull #216
42420	H2162b	-18.9	11.0	3.3	24.8	8.8	Hisban Misc No ID Skull #216
42421	H2162c	-18.0	**11.9**	3.4	22.1	7.7	Hisban Misc No ID Skull #216
42421	H2162c	-17.5	**9.8**	3.2	19.6	7.1	Hisban Misc No ID Skull #216
42422	H2162d	**-16.0**	10.2	3.3	18.9	6.8	Hisban Misc No ID Skull #216
42422	H2162d	**-18.9**	10.8	3.3	18.6	6.5	Hisban Misc No ID Skull #216
42423	H2162e	**-18.4**	10.4	3.2	19.1	6.9	Hisban Misc No ID Skull #216
42423	H2162e	**-14.5**	10.6	3.3	20.2	7.2	Hisban Misc No ID Skull #216
42424	H2162f	**-18.8**	9.2	3.5	15.1	5.0	Hisban Misc No ID Skull #216
42424	H2162f	**-15.1**	10.1	3.3	22.7	8.0	Hisban Misc No ID Skull #216
42425	H2162g	-14.4	10.7	3.2	19.5	7.1	Hisban Misc No ID Skull #216
42425	H2162g	-14.8	10.7	3.3	26.1	9.4	Hisban Misc No ID Skull #216
42426	H2162h	-13.3	10.4	3.2	23.0	8.4	Hisban Misc No ID Skull #216
42426	H2162h	-13.6	10.5	3.2	23.2	8.5	Hisban Misc No ID Skull #216

42427	H2162i	**-15.1**	10.7	3.3	20.6	7.2	Hisban Misc No ID Skull #216
42427	H2162i	**-16.9**	9.6	3.3	18.8	6.8	Hisban Misc No ID Skull #216
42428	H2162j	**-16.8**	10.5	3.4	21.1	7.3	Hisban Misc No ID Skull #216
42428	H2162j	**-15.1**	10.9	3.3	23.1	8.1	Hisban Misc No ID Skull #216
42429	H2162k	-17.4	10.1	3.4	22.1	7.7	Hisban Misc No ID Skull #216
42429	H2162k	-17.1	9.9	3.3	22.6	8.0	Hisban Misc No ID Skull #216
42430	H2162l	-17.8	10.4	3.2	33.1	11.9	Hisban Misc No ID Skull #216
42430	H2162l	-17.8	10.8	3.2	20.2	7.4	Hisban Misc No ID Skull #216
42431	H2162m	-16.8	10.3	3.2	24.0	8.6	Hisban Misc No ID Skull #216
42431	H2162m	-17.1	10.0	3.4	24.5	8.5	Hisban Misc No ID Skull #216
42432	H232a	-19.2	10.3	3.3	39.1	13.7	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42432	H232a	-19.0	10.3	3.3	38.4	13.4	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42433	H232b	-19.4	10.0	3.5	38.1	12.9	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42433	H232b	-19.3	9.9	3.4	39.8	13.6	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42434	H232c	**-20.4**	10.0	3.6	42.0	13.5	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42434	H232c	**-18.8**	10.7	3.4	42.6	14.8	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42435	H232d	-19.4	9.6	3.4	40.5	14.0	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42435	H232d	-19.6	10.2	3.3	46.6	16.5	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42436	H232e	-18.5	9.8	3.3	37.3	13.2	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42436	H232e	-18.2	10.0	3.3	40.1	14.4	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42437	H232f	-18.0	10.1	3.3	33.0	11.7	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42437	H232f	-17.6	10.4	3.4	32.5	11.2	H04 FldL Sq7 Loc3 Pail 23 Ind #2
42438	H2142a	**-16.2**	16.3	3.2	29.3	10.7	Hisban No ID Skull #214
42438	H2142a	**-15.1**	17.4	3.2	31.8	11.7	Hisban No ID Skull #214
42439	H2142b	-18.5	12.9	3.6	31.0	10.2	Hisban No ID Skull #214
42439	H2142b	-17.8	12.2	3.2	32.0	11.6	Hisban No ID Skull #214
42440	H2142c	-17.1	14.1	3.2	28.2	10.4	Hisban No ID Skull #214
42440	H2142c	-17.4	13.4	3.2	29.1	10.6	Hisban No ID Skull #214
42441	H2142d	-16.5	15.6	3.3	29.2	10.5	Hisban No ID Skull #214

42441	H2142d	-16.6	14.9	3.4	34.6	11.9	Hisban No ID Skull #214
42442	H2142e	-16.2	15.7	3.2	25.4	9.1	Hisban No ID Skull #214
42442	H2142e	-16.2	15.8	3.2	27.9	10.1	Hisban No ID Skull #214
42443	H2142f	-16.3	15.9	3.3	28.0	10.1	Hisban No ID Skull #214
42443	H2142f	-17.0	15.7	3.3	30.3	10.7	Hisban No ID Skull #214
42444	H2142g	-16.1	16.1	3.2	35.7	13.0	Hisban No ID Skull #214
42444	H2142g	-16.5	16.4	3.2	42.9	15.5	Hisban No ID Skull #214
42445	H2142h	-15.9	15.2	3.2	35.3	12.8	Hisban No ID Skull #214
42445	H2142h	-16.7	15.9	3.3	41.9	15.0	Hisban No ID Skull #214
42446	H3002a	-18.5	12.0	3.3	30.3	10.9	H01 Skull #3
42446	H3002a	-18.4	11.1	3.2	29.1	10.6	H01 Skull #3
42447	H3002b	-18.9	10.4	3.3	35.5	12.4	H01 Skull #3
42447	H3002b	-18.8	10.3	3.4	42.2	14.7	H01 Skull #3
42448	H3002c	-17.1	11.3	3.2	25.5	9.2	H01 Skull #3
42448	H3002c	-17.5	10.4	3.3	25.9	9.3	H01 Skull #3
42449	H3002d	-16.6	11.1	3.2	23.1	8.5	H01 Skull #3
42449	H3002d						H01 Skull #3
42450	H3002e	-16.6	11.3	3.2	25.2	9.3	H01 Skull #3
42450	H3002e	-17.5	11.0	3.2	25.6	9.3	H01 Skull #3
42451	H3002f	-17.6	10.9	3.2	25.0	9.1	H01 Skull #3
42451	H3002f	-18.0	10.6	3.2	38.8	14.1	H01 Skull #3
42452	H3002g	-17.8	10.8	3.2	20.0	7.3	H01 Skull #3
42452	H3002g	-17.8	10.6	3.3	34.7	12.3	H01 Skull #3
42453	H2162n	-16.8	10.0	3.2	20.9	7.7	H01 Skull #3
42453	H2162n	-17.3	10.2	3.3	26.0	9.3	H01 Skull #3
42454	H2202a	-16.0	19.4	3.5	43.3	14.6	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42454	H2202a	-15.9	19.1	3.2	39.7	14.5	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42455	H2202b	-17.8	13.9	3.3	40.1	14.1	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42455	H2202b	-17.4	14.5	3.3	35.6	12.5	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19

42456	H2202c	-19.8	**9.2**	3.5	42.2	14.0	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42456	H2202c	-19.0	**11.6**	3.3	46.3	16.5	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42457	H2202d	-18.8	7.3	3.6	28.4	9.1	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42457	H2202d	-18.1	8.3	3.2	29.4	10.7	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42458	H2202e	-19.6	8.7	3.2	36.1	13.0	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42458	H2202e	-19.4	8.0	3.2	33.9	12.2	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42459	H2202f	-18.8	8.7	3.2	34.7	12.6	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42459	H2202f	-19.9	8.8	3.2	35.9	12.9	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42460	H2202g	**-19.6**	9.1	3.3	43.0	15.0	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19
42460	H2202g	**-18.3**	9.9	3.3	42.6	15.1	H98 FLDL SQ2 Loc3 Bur# 22 Mand #19