STABLE ISOTOPE ANALYSIS OF CHILDHOOD DIET AT 1ST CENTURY B.C./A.D. PETRA, JORDAN

by

Mallory Provan

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Director of Thesis: Dr. Megan Perry

Major Department: Anthropology

Investigations of diet and disease of the population of the Nabataean capital city Petra have found that the adult residents suffered little from chronic infections or malnutrition and had a diet consisting primarily of C_3 sources. However, little is known of childhood health and nutrition in the population since few remains of children have been recovered from excavated tombs. This study uses stable carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) of dental enamel apatite in first molars (n=31), first premolars (n=20), and second molars (n=29) along with $\delta^{13}C$ of bone apatite (adults n=27, subadults n=4) to explore childhood diet and weaning patterns in a 1st century B.C/A.D. sample from Petra. To assess dietary shifts by age related to weaning, the $\delta^{13}C_{ap}$ bone and enamel results were grouped into five different age ranges based on age of tissue formation: 0.0–2.5 years, 2.6–4.5 years, 4.6–7.0 years, 7.1–19.9 years, and 20.0+ years. The first three categories reflect the ages of dental formation in the first molar (M1), first premolar (PM1), and second molar (M2), respectively. Dietary and trophic level changes, particularly those associated with weaning, should result in a decrease in mean $\delta^{18}O_{ap}$ values and $\delta^{13}C_{ap}$ values should increase between 0.0–2.5 years and 2.6–4.5 years then decrease between 2.6–4.5 and 4.6–7.0 as the adult diet would have been adopted fully after weaning is completed.

In the Petra sample, the differences in $\delta^{13}C_{ap}$ between age groups mostly followed the expected pattern with mean $\delta^{13}C$ values increasing from 0.0–2.5 ($\bar{x}$=-11.6 ± 0.4‰) and 2.6–4.5 years of age ($\bar{x}$=-11.3 ± 0.5 ‰) (Mann-Whitney U Z=2.949, p=0.003), remaining stable through 4.6–7.0 years ($\bar{x}$=-11.3 ± 1.1‰), and then decreasing dramatically after 7.1 years to fall close to the adult $\delta^{13}C_{ap}$ value. In addition, the three youngest age categories (i.e., below 7.1 years of age) had values significantly higher than the values of 7.1–19.9 years ($\bar{x}$=-12.9 ± 0.4‰) and 20+ years ($\bar{x}$=-12.9 ± 1.0‰) age groups (p<0.001 in each case). Mean $\delta^{18}O_{ap}$ values initially decrease between M1 and PM1 before increasing in M2 value, which does not follow the expected pattern. These results may indicate that C_4 foods were used as supplementary resources during weaning and that weaning was likely completed after three years of age or a specialized childhood diet was implemented for a period after weaning but before a fully adult diet was adopted. Some confounding factors may include the use of non-human milk during weaning, an early introduction to environmental water, and seasonality in groundwater $\delta^{18}O$ values. The influence of immigration on the $\delta^{18}O$ values was applicable to only one individual.

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Mallory Provan

APPROVED BY:

DIRECTOR OF THESIS: ____
Megan Perry, PhD

COMMITTEE MEMBER: ____
Robert H. Tykot, PhD

COMMITTEE MEMBER: ____
James Loudon, PhD

COMMITTEE MEMBER: ____
Laura Mazow, PhD

CHAIR OF THE DEPARTMENT
OF ANTHROPOLOGY: ____
Randolph Daniel, PhD

DEAN OF THE
GRADUATE SCHOOL: ____
Paul J. Gemperline, PhD

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CHAPTER 1: Introduction

Childhood health and nutritional values are often used by bioarchaeologists as a measure of overall population adaptability to its environment (Goodman and Armelagos 1989; Lewis 2007). Fetal and infant nutrition levels and infectious disease burdens can have a lasting impact later in an individual's life and even possibly into later generations (Gluckman et al. 2005; Jang and Serra 2014). A particularly vulnerable period for growth and health maintenance in childhood is the shift in dietary sources and altered immune system functioning that occurs during weaning. This project illuminates the nature of the childhood diet and the timing of the weaning period in a 1st century A.D. population of non-elites in Petra through analysis of stable isotope carbon and oxygen isotope ratios (δ^{13}C and δ^{18}O) in dental enamel apatite.

The city of Petra was a large urban center during the 1st century B.C. through 4th century A.D. The individuals investigated in this study come from the Petra North Ridge Project (PNRP), which consists of tombs that contain the remains of individuals thought to be non-elite city residents. Preliminary investigations of diet and disease (Canipe 2014; Appleton 2015) found that the non-elite adult residents of Petra suffered little from chronic infections or malnutrition and had a diet well-balanced between animal protein and C_3 plants such as wheat, barley, fruits, and legumes. However, little is known of childhood health and nutrition in this sample, since very few remains of children were recovered from the tombs. In this study, the use of dental enamel in older children and adult individuals provides a reflection of childhood diet, since dental enamel does not remodel once it has mineralized during childhood dental development. Studying childhood diet is beneficial for understanding the morbidity and mortality of subadult individuals as childhood health can greatly affect population growth and decline. In addition, this study helps shed light on the timing of the weaning period as a means to better understand childhood patterns of disease and stress.

Stable carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotope data can provide important information on shifts in childhood diet from the breastfeeding through weaning and post-weaning periods. In this study, isotopic analyses from dental enamel apatite of three different permanent tooth classes, representing different ages during childhood, tracks dietary changes that occur during infancy and childhood, including shifts during the weaning process. Comparing average $\delta^{13}C$ and $\delta^{18}O$ observed in different tooth classes that develop at different ages can identify approximately when weaning tended to occur in this society. In addition, the $\delta^{13}C$ value itself can illuminate the dietary composition of solid foods consumed during and after weaning.

Determining the age of weaning in a population can illuminate cultural ideas about growth and development as well as the role of weaning on nutrition and infection. At Petra, weaning can provide insights into cultural adoption of Greco-Roman ideas on health and weaning during a period of increased interaction with the Roman Empire. This research is also beneficial in exploring childhood health in Petra, which could not otherwise be determined due to the dearth of infant and subadult remains at the site.

CHAPTER 2: Background

Historical Background

Petra, originally called *Rekem* by the Nabataeans (Retso 1999), is located in the eastern highlands of the Jordan Rift Valley (Figure 1). The geographic origin of the Nabataeans and the date that their tribal kingdom was established at Petra remains uncertain. The Greek Historian Diodorus in the 4th century A.D. describes the Nabataeans as nomads, giving the impression that at the time the Nabataeans lacked permanent dwellings (Schmid 2002). Over the following centuries, the Nabataeans gained wealth through trading incense and spices between the Mediterranean and South Arabia and Petra became a major center for trade, collecting taxes from caravans passing through the city (Fiema 2003). The increased interaction with international polities and intensified trade likely stimulated the increased sedentism of Nabataeans (Schmid 2002), who began building permanent structures at Petra in the 3rd to 2nd centuries B.C. (Graf et al. 2005; Graf et al. 2007).

Figure 1. Map of modern Jordan showing the location of Petra.

Petra experienced a dramatic increase in monumental private and civic construction through the 1st century B.C. to the 1st century A.D. (Al-Muheisen 2007; Schmid 2002). Structures built included temples, an amphitheater, a pool and garden complex, monumental tombs, and elaborate villas. The Nabataeans also fully established their own style of ceramics and coinage around this time (Schmid 2002). In addition, decorative façades were carved around tomb entrances that appear to serve two purposes: they reflected the architectural designs of the rest of the city, including houses and villas, and they demonstrated the social and economic status of the interred (Schmid 2002). At the same time, smaller, less visible tombs were being carved into exposed bedrock for less politically- and socially-important citizens.

Due to the semi-arid to arid climate of the region, Petra's impressive hydraulic and water catchment systems were necessary to sustain agricultural fields to support both semi-nomadic and sedentary populations in the city and its environs (Al-Muheisen 2007; Beckers et al. 2013;

Ortloff 2005). With more people settling in Petra during this period (Wenning 2007), there grew increased pressure on local agricultural sources, and it is unclear how this impacted access to resources within the city. One way to explore possible differences in resource acquisition is through the composition of individuals' diets at Petra. The lack of historical documentation describing the diets of Nabataean adults and children means that researchers have relied primarily on bioarchaeological and archaeological data, such as stable isotope analysis of human and faunal remains and paleobotanical and zooarchaeological evidence Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis of human bone collagen and apatite has shown that the adult population had a high intake of C_3 plants such as wheat, barley, and fruits, along with meat from animals also consuming C_3 plants (Appleton 2015). Additional evidence of the types of food that were accessible to the Nabataeans comes from paleobotanical and zooarchaeological studies. Paleobotanical evidence from archaeological excavations within Petra have included a variety of crops including cereals, fruits, and vegetables (Ramsay and Bedal 2015). Zooarchaeological data show that animal protein mostly came from domestic animals such as pigs, cattle, sheep, and chickens (Studer 2007).

Most of the information on population health and diet comes from adult individuals at Petra. Child nutrition and disease, however, can be more sensitive reflections of overall population health. This is partly due to the impact of childhood morbidity and mortality on population dynamics as well as the high mortality risk of this subgroup when populations are stressed (Goodman and Armelagos 1989). Very few skeletal remains of infants and children have been recovered from the Petra tombs. Fortunately, adults retain the effects of childhood malnutrition and physiological stress in their dental enamel, which forms and mineralizes during childhood and remains unchanged throughout the remainder of their lives. These variables include hypoplastic defects in the enamel and stable isotope values that are recorded while they are children during enamel formation. In particular, comparing carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) isotopes of apatite in dental enamel from different teeth can reveal the dietary composition of that individual during and after weaning (Wright and Schwarcz 1998), one of the most vulnerable periods of childhood (Lewis 2007). Adult individuals, of course, represent only those children who survived childhood and lived into adulthood, thus information on those who perished during childhood will be missing in this assessment. However, evidence of childhood frailty in the form of early weaning or improper supplementation of breastfeeding could identify a certain level of resiliency in the population in terms of surviving childhood stress.

In addition, determining when weaning occurred can shed light on childhood rearing practices in Petra. The Roman annexation of Petra took place in 106 A.D. (Schmid 2002), but the impact of the ensuing "Romanization" (as described by Mattingly, 2004) on Nabataean culture is negligible as the Nabataeans had already incorporated many aspects of the Roman and Hellenistic ethos. This incorporation can be seen in the anthropomorphization of the Nabataean deities as well as Nabataean architectural style and ritual activities (Bedal 2002; Schmid 2002).

An unexplored area of influence is the adoption by the Nabataeans of Hellenistic ideals of health and medicine prior to Roman annexation. Certain precepts regarding breastfeeding, weaning, and proper nutrition of infants survive in Greek and Latin texts by Galen, Soranus, and Oribasius that date from the 2nd century A.D. to 4th century A.D. (Lascaratos and Poulakou-Rebelakou 2003; Prowse et al. 2008). While these ancient physicians documented practices postdating the 1st century B.C. to 1st century A.D. (the date of the cemetery explored here), their texts borrow heavily from earlier medical writers that would have been contemporary with this period (Prowse et al. 2008). Many of the practices discussed, such as age of weaning and introduction of soft foods, have important health consequences for the child and the larger community. This transition was considered by these ancient physicians to be a significant period of development for a child (Lascaratos and Poulakou-Rebelakou 2003), a valid conclusion considering that rapid growth during this period requires high levels of nutrition, which results in low physiological tolerance for deficiencies (Bourbou and Garvie-Lok 2009; Lewis 2007). Foods introduced to the infant that lack the appropriate nutrients can lead to increased infant mortality or long-term poor health such as susceptibility to chronic infections (Lewis 2007). Oribasius and Soranus suggested feeding the child milk, honey, spelt, eggs, gruel, and pap (a mixture of flour and bread cooked in water) during the weaning period (Bourbou and Garvie-Lok 2009); exploring the composition of childhood diet during the weaning period using stable isotopes may track the process of weaning and perhaps shed light on whether those foods were used to supplement breastmilk (Fuller et al. 2006a; Wright and Schwarcz 1998). It should also be noted that the Greco-Roman medical texts tended to focus on higher class individuals and variation likely existed within the Roman populations themselves (Prowse et al. 2008); this factor could potentially account for differences between the weaning foods recommended by the texts and what was actually implemented by the non-elite Petraeans.

The lack of childhood diet studies on Nabataean sites other than Petra, such as from contemporaneous agricultural villages or rural/nomadic populations in the region, hinders any comparative urban-rural analysis with. The examination of if the Nabataeans adopted the infant feeding and weaning practices outlined in the Greco-Roman medical texts can be aided by comparing the childhood diet at Petra to other sites within the Greco-Roman realm. Three Roman sites that are relatively contemporaneous with Petra where childhood diet has been studied through stable isotope analyses include the Isola Sacra necropolis in Rome, Italy, (Prowse et al. 2008); the Kellis 2 cemetery in the Dakhleh Oasis, Egypt (Dupras et al. 2001; Dupras and Tocheri 2007); and Queenford Farm cemetery in Dorchester-on-Thames, England (Fuller et al. 2006b). These comparisons reveal evidence of similar practices as well as variability between these locations.

The childhood dietary composition and nutrition at Petra provides important supplemental information on overall population disease patterns and physiological well-being within the city during a period of substantial growth. In addition, following the guidelines of Greco-Roman physicians in terms of when to wean a child and what foods to feed him or her may indicate some knowledge of medical practices from a source outside of the Nabataean culture.

Exploring Childhood Diet

Stable isotopic analyses for dietary reconstruction generally focus on the collagen and mineral components of human bone. Sampling the bones of children of different ages enables the reconstruction of childhood diet before, during, and after weaning. Underrepresentation of infant skeletons at Petra, either due to issues with preservation or differential burial practices by age, limits this method of sampling. However, childhood dietary stable isotope values are retained in the apatite of dental enamel, which reflects dietary composition during childhood enamel formation. Two stable isotope ratios in apatite that can provide information on weaning-related dietary shifts are the ratios between ^{13}C and ^{12}C ($\delta^{13}C$) and between ^{18}O and ^{16}O ($\delta^{18}O$) (Wright and Schwarcz 1998). Carbon isotopes extracted from skeletal tissue vary primarily based in the relative consumption of two plant types that use different photosynthetic pathways: C_3 plants, which include trees and shrubs and the domesticates wheat, barley, rice, wetland grasses, legumes, and tree-bearing fruits; and C_4 plants, which tropical grasses and include the domesticates maize, sorghum, millet, and some pasture grasses (Ambrose and Norr 1993). $\delta^{13}C$ values of C_3 plants range between -33 to -22‰ (per mil) and C_4 plants range from -16 to -9‰ (Smith and Epstein 1971). Based on the 11-12‰ offset between plant values and human enamel carbonate (Krueger and Sullivan 1984), the average values for a human consuming mostly C_3 plants would be -22 to -11‰ and -5 to +2‰ for a largely C_4 plant diet. The arid environment likely contains indigenous CAM (Crassulacean acid metabolism) plants that have a third photosynthetic pathway, but their contribution to $\delta^{13}C$ values is not considered here.

The ratio of ^{13}C and ^{12}C in dental apatite reflects not only the types of plants an organism directly consumes, but also protein or lipid dietary sources of carbon, such as through consumption of herbivores (Ambrose and Norr 1993; Schwarcz and Schoeninger 1991; Tieszen and Fagre 1993). Children who are shifting from breastfeeding, when they are absorbing carbon consumed by their milk source, to a diet supplemented with solid foods may experience a slight shift in $\delta^{13}C$ values (Fuller et al. 2006a; Wright and Schwarcz 1998). In addition, the $\delta^{13}C$ value itself may indicate any animal protein and plant sources used to supplement breastmilk during weaning. Whether an infant receives non-human rather than human breastmilk can be a confounding factor, since milk from a herbivore such as a cow or goat tends to have a lower $\delta^{13}C$ value than that of human breastmilk (Humphrey 2014). A fully "adult" diet (that is, like that consumed by adults) can be informed by $\delta^{13}C$ values from apatite in adult skeletal remains. At Petra, these adult bone values come from an earlier study by Appleton (2015).

Oxygen isotopes in human apatite on the other hand reflect dietary sources of water (Luz et al. 1984). Archaeologists primarily use $\delta^{18}O$ in dental enamel carbonate, which reflects childhood water sources, to assess population mobility by comparing this value to local water sources in the region where the individuals died (Dupras and Schwarcz 2001; Perry et al. 2009; Prowse et al. 2007). However, children subsisting on human breastmilk are at a higher trophic level than those relying on water sources other than milk, and thus have more enriched δ^{18}O values (Wright and Schwarcz 1998). Thus, a shift in reliance from breastmilk to other water sources will result in a downward shift in their δ^{18}O value with age. However, δ^{18}O values can be affected by the consumption of non-human breastmilk, an early introduction to environmental water, or seasonal fluctuations in δ^{18}O enrichment. Grazing animals that have access to a larger variety of water sources could have higher δ^{18}O values than those that are restricted to a single location (Daux et al. 2008), which could mask shifts in values due to weaning. Environmental water introduced early in infancy would minimize any trophic level shift expected from a reduction in breastmilk consumption during weaning (Williams et al. 2005). Additional factors that could impact δ^{18}O values include conditions such as anemia; however, those are related to atmospheric oxygen instead of oxygen from water sources (Epstein and Zeiri 1988).

The study of dietary shifts in childhood usually relies on stable isotope analysis of samples of bone collagen from bone and dental dentine across age classes (e.g, Bourbou et al. 2013; Dupras et al. 2001; Fuller et al. 2006a; Haydock et al. 2013; Howcroft et al. 2012; Humphrey 2014; Mays 2002; Prowse et al. 2008; Reitsema et al. 2016; Richards et al. 2002; Schmidt et al. 2016; Schurr 1997; Wright and Schwarcz 1998). However, as noted above, the Petra sample does not contain a representative sample of subadult remains to assess the transition of stable isotopic values through childhood. Instead, these shifts can be identified in dental enamel from tooth types with different ages of enamel formation. Studies of modern children have found that enamel formation of the first molar occurs from birth to three years of age, first premolars from two to five years of age, and second molars from three to seven years of age

(Massler et al. 1941; Moorees et al. 1963). These teeth thus can provide information on three slightly overlapping periods during childhood growth, that of early weaning, late weaning, and post-weaning.

Comparing differences in $\delta^{13}C_{ap}$ ($\delta^{13}C$ analysis of apatite) and $\delta^{18}O_{ap}$ ($\delta^{18}O$ analysis of apatite) by age to understand ancient childhood diet has been implemented in many cultural contexts. Wright and Schwarcz (1998) compared $\delta^{18}O_{ap}$ and $\delta^{13}C_{ap}$ in the first molars, premolars, and third molars from the prehistoric Guatemalan site of Kaminaljuyú (circa 700 B.C. to 1500 A.D.) to illuminate the cultural practices surrounding infant feeding such as the introduction of supplemental foods and breastfeeding patterns. They discovered that breastmilk was a component of the diet up until five or six years of age based on similarity in $\delta^{18}O_{ap}$ values between first molars and premolars in comparison to the third molars. The $\delta^{13}C_{ap}$ values showed a general increase over time with a more marked difference between first molars and premolars, likely due to the introduction of maize gruel and other C_4 foods at one year of age. Although they admit that the inclusion of other types of stable isotope ratios in addition to $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ can lead to a more precise description, Wright and Schwarcz concluded that $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ on their own can still give an accurate and informative picture of past infant feeding and weaning practices (Wright and Schwarcz 1998).

Williams et al. (2005) conducted $\delta13C_{ap}$, $\delta13C_{co}$, $\delta^{18}O$, and $\delta^{15}N$ isotopic analyses on two Postclassic Maya sites in Belize, Marco Gonzalez and San Pedro. Using bioapatite and collagen from 67 samples, the analysis established that weaning began at one year and ended around three to four years of age. The isotopic values also indicated that maize (a C_4 food) was a common component of the weaning diet. However, the authors did mention that the $\delta^{13}C_{ap}$ analysis was the least informative as the C_4 supplementary food used during weaning had similar values to that of breastmilk.

Dupras and Tocheri (2007) discovered that Roman-period infants at Dakhleh Oasis in Egypt were weaned from around six months to three years based on $\delta^{13}C$ and $\delta^{18}O$ of dental apatite in addition to $\delta^{13}C$ and $\delta^{15}N$ of dental dentin. The pattern of $\delta^{13}C_{ap}$ values across all deciduous and permanent tooth classes (except for the third molar) indicate that C_4 foods, for example pearl millet, or milk from animals subsisting on a C_4 diet, were used as a supplemental food during weaning followed by an introduction of a predominantly C_3 diet similar to the adults.

Britton et al. (2015) used $\delta^{18}O$ from enamel phosphate from individuals perishing at different ages during childhood. They reported enriched $\delta^{18}O_p$ values for individuals under two to three years of age, whereas most individuals between four and twelve years old, subadults, and adults showed values that were consistent with local drinking water. Britton et al. (2015) determined that using $\delta^{18}O$ from phosphate is a good indicator of the cessation of breastfeeding.

The ages between which the children at Petra were weaned is similar to other non-industrialized populations. Modern recommendations include exclusive breastfeeding for the first six months and then implementation of a diet of breastmilk and supplementary foods until two years of age (Kramer and Kakuma 2012). Greco-Roman medical texts on the other hand suggest extension of the weaning diet until the child is three or four years of age (Lascaratos and Poulakou-Rebelakou 2003). Based on these recommendations, I expect at Petra that the first molar would correspond with the pre-weaning period, and the premolars and second molars with the late and post-weaning periods. The $\delta^{13}C_{ap}$ values should increase between first molars and first premolars as supplementary food becomes a larger component of the diet than breastmilk, decrease between first premolars and second molars as the adult diet was fully adopted after weaning was completed (Dupras and Tocheri 2007; Wright and Schwarcz 1998). The $\delta^{18}O_{ap}$ values should gradually decrease between first molars and first premolars and between first premolars and second molars as individuals were weaned off enriched breastmilk and began to consume environmental water (Wright 2013). By comparing the isotopic values on diet from teeth that form in the later stages of weaning and post-weaning with values on adult diet, support for the age that children may have fully transitioned to a diet similar to adults can be obtained.

Dental enamel apatite was used in this study not only because of the dearth of subadult bones in the North Ridge tombs, but also because it is less susceptible to diagenesis, i.e. the physical, chemical, or biological alteration of the bone after burial. Diagensis can affect the isotopic composition of the bone and provide inaccurate isotopic values (Lee-Thorp and van der Merwe 1991; Nelson et al. 1986). Even though bone in general is at risk of contamination, the bones of children are even more susceptible because of their more porous nature (Guy et al. 1997). As the structure of dental enamel is almost entirely inorganic with low porosity and is more highly crystallized, it has less chance of postmortem diagenetic alteration than bone (Koch et al. 1997; Lee-Thorp and van der Merwe 1991; LeGeros 1981; Wang and Cerling 1994).

Brief Overview of Petra North Ridge Project

The analysis of childhood diet at Petra is based on skeletal remains recovered as part of the Petra North Ridge Project (PNRP). The North Ridge is located directly north of the main thoroughfare that runs east-west through the heart of the city (Parker and Perry 2014). The goal of PNRP is to examine the life of the non-elites in Petra and this has been done through the excavation of Nabataean shaft tombs that date circa 1st century B.C.–1st century A.D. and domestic structures from the 1st–4th century A.D. (Parker and Perry 2014). Eight tombs (B.4, B.5, B.6, B.7, B.8, B.9, F.1, and F.2) were explored during the three field seasons in 2012, 2014, and 2016, although only B.4, B.5, B.6, B.7, and F.1 contained skeletal remains. The remains in these tombs were largely fragmented and commingled, making it impossible in most cases to group bones by individual. Previous investigations of skeletal remains from the North Ridge have revealed a mostly adult population that displayed almost no pathological lesions from infectious diseases or malnutrition (Canipe 2014) and had a relatively balanced diet in terms of proteins and grains (mostly deriving from C_3 plants) (Appleton 2015). Paleobotanical and zooarchaeological evidence has confirmed that cereals, fruits, legumes, and vegetables were the primary components of the adult Petraean diet (Ramsay and Bedal 2015), as well as meat from chicken, sheep, and goat (Studer 2007). These findings along with Canipe's (2014) indicate that the low levels of malnutrition seen in the population can be attributed to a diet that fulfilled appropriate requirements of proteins, carbohydrates, and fats.

This project investigates the diet and weaning of children at Petra based on childhood isotopic indicators retained in the dental enamel of adults. While this study only provides information on adults who survived childhood, it can reveal if they experienced and survived stressful events in the past as well as dietary changes. The use of older children and adult remains can also lessen the impact of infant mortality bias that could be caused from non-survivors of an atypical infant diet (Dupras and Tocheri 2007). Analysis of the enamel of first molars, first premolars, and second molars at Petra, which form from birth to three years, two to five years, and three to seven years of age respectively (Hillson 1996), can provide a diachronic perspective that can reflect shifts in diet related to weaning practices. Since the second molar forms between the ages of three to seven years, it was chosen over the third molar used in many of the studies described above since it would better capture the years immediately following the expected weaning period (two to four years based on Soranus and Galen; Lascaratos and Poulakou-Rebelakou 2003). As outlined above, dietary shifts seen through isotopic differences between teeth formed at different ages can enable an approximate identification of when weaning occurred.

Hypotheses

The intent of this research project was to explore the childhood diet and the weaning process of the non-elite Petraeans through stable isotope analyses of $\delta^{13}C$ and $\delta^{18}O$ values from the enamel apatite of a sample of PNRP adults. These data can provide valuable insight into the weaning period and the overall health of the non-elite population of the city. The adoption of non-local cultural practices for weaning can be explored by determining the type of supplementary food given and at what age this was added into the diet. Two hypotheses were examined for this research project.

Null Hypothesis 1: The childhood diet and weaning practices determined through the stable isotope analysis will not be different than those from the Roman populations.

Alternative Hypothesis 1: The childhood diet and weaning practices determined through the stable isotope analysis will be different than those from the Roman populations.

The Greco-Roman medical texts and isotopic studies of other Greco-Roman populations suggested using milk, honey, spelt, eggs, gruel, and pap to supplement breastfeeding (Bourbou and Garvie-Lok 2009; Lascaratos and Poulakou-Rebelakou 2003; Prowse et al. 2008). The age at which the texts recommended beginning the weaning process with the addition of these substances to the diet was six months to one year as long as the child was in good health (Bourbou and Garvie-Lok 2009; Lascaratos and Poulakou-Rebelakou 2003). With the increased interaction with Roman culture, I expected that the non-elite Petraeans might have followed similar weaning practices.

The age that weaning was completed can be supported by comparing the isotopic values of childhood diet with those of adult diet. The previous data from Appleton (2015) provided the adult diet comparison.

Null Hypothesis 2: The stable isotope values on diet from the second molars will be no different than the adult diet.

Alternative Hypothesis 2: The stable isotope values on diet from the second molars will be different than the adult diet.

As the second molars form from three to seven years of age (Hillson 1996), they should display values of diet after the weaning period was completed and so should be similar to those of adults. If the second molars show greater similarity to the first premolar, it would infer that the weaning process extended into the three to seven-year age.

CHAPTER 3: Materials and Methods

Sample Selection

The PNRP sample contains at minimum the remains of 120 individuals. The commingled nature of the sample necessitated careful isotopic sample collection to prevent unintentionally oversampling specific individuals, which would inadvertently skew the results. To minimize the chance of individual oversampling, teeth within a particular archaeological context were selected by: 1) limiting selection to only certain teeth from that context (e.g., all left lower first molars), and/or 2) observing level of wear and taphonomic changes of the teeth to rule out the possibility that two of the same tooth type came from the same individual. This selection process was adhered to as much as possible, but the reality of the sample options required some deviations from this process. Only first molars, first premolars, and second molars that could provide at least 10 mg of powdered enamel were included. Cases of minimal wear were prioritized when possible so that the resulting value represents the average of the entire age range for which enamel formation occurred. The final selection attempted to include an equal sample size across tooth types as well as relatively equal distribution of tooth types between the tombs in order to have as representative a sample as possible.

This study included a total of 80 teeth, including 21 first premolars, 30 first molars, and 29 second molars. Thirty teeth from the 2012 and 2014 excavations had already been carefully selected for isotopic analysis of strontium by Perry and had remaining enamel sufficient for further isotopic analysis. This sample consisted of 24 first molars, five second molars, and one first premolar. The use of these samples resulted in multiple isotopic signatures ($^{87}Sr/^{86}Sr$, $\delta^{13}C_{ap}$, and $\delta^{18}O_{ap}$) for the individuals represented by these teeth, which can aid future studies in providing a more complete picture of mobility of non-elite people of Petra.

In addition, five first premolars and two second molars from the 2012, 2014, and 2016 seasons chosen for cemento-chronological age estimation (Propst 2017) were also chosen for $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ analysis. Although not directly tested, four additional teeth (one premolar, one first molar, and two second molars) had age estimations based on the cemento-chronological study as they were from individuals that had a different tooth included in the study by Propst (2017). Sample selection of teeth from the commingled collection for cemeto-chronological analysis followed the same protocol as the sampling for isotopic analyses. Although the roots of the teeth were previously thin-sectioned for histological analysis of cementum to assess age-at-death (Propst 2017), the enamel crowns remained intact and untouched. The benefit of using this sample is that we can pair the isotopic results with the age-at-death estimates determined. An additional 15 premolars, six first molars, and 22 second molars were selected from the remaining 2012, 2014, and 2016 samples to round out the sample size to 80 teeth. Nine teeth were sampled from subadults and 71 from adults. Ages of the subadults were determined from the stage of tooth development following Moorrees et al. (1963). The subadults with known ages consisted of four individuals aged 3 years ± 12 months, three individuals aged 4 years ± 12 months, one individual aged 7 to 10 years, and one individual aged 8 to 9 years. Nine adult individuals had known ages based on cemento-chronology. The age estimates of these individuals were 28 years, 30 years, 33 years, 34.5 years, 37 years (two teeth from this individual were analyzed), 41.5 years, 45 years, 46.5 years, and 52 years (two teeth from this individual were analyzed).

While most of the different tooth types could not be linked by individual due to the commingled nature of the sample, in eleven cases multiple teeth came from one individual. Nine of these cases were intact, primary burials: two individuals from Tomb B.5, three individuals from Tomb B.6, and four individuals from Tomb B.7. The other two individuals were excavated from a commingled pit in Tomb F.1, and the teeth were identified as belonging to the same person despite the commingled context because they were still in articulation with the maxilla. All individuals with multiple sampled teeth had reached adulthood. Two had undergone the cemento-chronological analysis by Propst (2017) and have more precise age estimations: one individual from Tomb B.7 was estimated to be 52 years old and one individual from Tomb F.1 was estimated to be 37 years old.

The adult $\delta^{13}C_{ap}$ values for this study were derived from Appleton (2015), which included bone samples from 27 adults from Tomb 2, Tomb B.4, and Tomb B.5. In addition, $\delta^{13}C_{ap}$ values from the bones of seven subadults supplemented the dental enamel-derived values. These subadults were aged 36 to 37 weeks, two to three years, 3 to 4 years, 16 to 22 years, two individuals were 18 to 20 years, and the last individual was identified only as a subadult without a more specific age range.

Sample Preparation Methods

The processing of the samples occurred at the University of South Florida Laboratory for Archaeological Science in Tampa, Florida under the direction of Dr. Robert Tykot and following established methods (Tykot 2018). The general protocol for sample preparation and isotopic analysis in this lab is as follows:

The teeth that had not been previously selected for strontium isotope analysis were cleaned using a brush and distilled water. After drying, a drill was implemented to remove the surface of the enamel and then at least 10 mg of enamel powder was extracted from internal enamel. When necessary, a porcelain mortar and pestle was used to grind enamel that came off in larger pieces. The enamel powder samples were weighed and placed in 2% sodium hypochlorite for 24 hours. The samples were then centrifuged so that the powdered enamel was not lost when the solution was poured off and replaced with distilled water. This was repeated four times to ensure that the 2% sodium hypochlorite was removed. This was followed by at least 24 hours in the drying oven. After being weighed again, the samples were left in 1.0 M buffered acetic acid for 24 hours to eliminate organic components and non-biogenic carbonates (Koch et al. 1997). The same centrifuging and pouring off process as before was implemented followed by at least 24 hours in the drying oven and a third round of weighing the sample. The last step was to remove ~1 mg of the sample and place it in a separate vial for isotope analysis. Any powdered enamel left over was stored in case one of the samples needed to be run again and/or for future isotopic analyses.

Once all samples were prepared, they were analyzed using a ThermoFisher MAT253 isotope ratio mass spectrometer coupled to a GasBench-II with a continuous-flow interface. The samples were first turned into gas using 600 µl of 104% phosphoric acid (H_3PO_4) at 25˚C for 24 hrs. The gas produced through this process was compared against a reference standard. For carbon, this standard is Vienna PeeDee Belemnite (V-PDB) (Hoefs 2015; Tykot 2018). The same reference standard was used for oxygen (Werner et al. 2001; Wright and Schwarcz 1998).The precision of stable isotope ratio mass spectrometry analysis for $\delta^{13}C$ is ± 0.1‰ and for $\delta^{18}O$ is ± 0.1‰.

Statistical Methods

A non-parametric Mann-Whitney U test was chosen for the statistical analysis as the data were not normally distributed. This test determined whether there were significant differences in isotopic values for each tooth class (first premolars, first molars, and second molars). A Mann-

Whitney U test was also used for comparing these values to PNRP adult bone apatite values previously reported by Appleton (2015). Finally, a Mann-Whitney U test was implemented to determine the differences between individuals who reached adulthood and those who did not. In addition, Kolmogorov-Smirnoff tests compared carbon and oxygen isotope ratios across age classes between each tomb.

CHAPTER 4: Results

This chapter presents the results from the stable isotope analysis of $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ along with the statistical analyses that were used to identify the significance of differences between tooth classes and skeletal tissues, i.e., dietary changes with age. The raw data from the $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ isotope analysis are reported in Table 1. The values are expressed in parts per mil (‰). The $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values are plotted in Figure 2.

Carbon Isotope Results

The overall dental enamel $\delta^{13}C_{ap}$ values range between -13.3‰ and -6.6‰ and have a mean of -11.4 ± 0.7‰. The $\delta^{13}C_{ap}$ values for first molars range between -12.4‰ and -10.5‰ with a mean of -11.6 ± 0.4‰; first premolars range between -12‰ and -10.5‰ and have a mean of -11.2 ± 0.3‰; and second molars range between -13.3‰ to -6.6‰ and have a mean of -11.3 ± 1.1‰. The adult (20+ years) $\delta^{13}C_{ap}$ values are between -14.0‰ and -10.4‰ with a mean of -12.9 ± 1.0‰ (Appleton 2015).

For statistical comparisons to assess dietary shifts by age related to weaning, the $\delta^{13}C_{ap}$ bone and enamel results were grouped into five age ranges based on age of tissue formation: birth to 2.5 years, 2.6 to 4.5 years, 4.6 to 7.0 years, 7.1 to 19.9 years, and 20.0+ years. The first three categories reflect the ages of dental formation in the first molar, first premolar, and second molar, respectively. The $\delta^{13}C_{ap}$ bone values from subadults with age-at-death estimates between birth and 7.0 years of age were added to the appropriate age category, with the assumption that these values represent the period shortly preceding death. All adult bone $\delta^{13}C_{ap}$ values were grouped into the 20.0+ age category. In addition, some of the bones sampled by Appleton (2015) included subadults dying after 7 years of age but before 20 years of age. These $\delta^{13}C_{ap}$ values were grouped together into an age category bridging the completion of second molar enamel formation and 20.0 years of age.

Studies of the relationship between enamel and bone apatite $\delta^{13}C$ values have not found a significant intra-individual difference (Loftus and Sealy 2012), and thus they can be considered analogous. However, Webb et al. (2014) noted a +4.3 ± 1.2‰ in enamel-bone differences for $\delta^{13}C$ based on ten juveniles. It should also be noted that the length of the delay between a dietary change and its isotopic signature being incorporated into the skeletal tissue can differ depending on tissue type, with bone taking several months to several years and enamel being more accurate to the age since it does not undergo turnover (Tsutaya and Yoneda 2015).

In the Petra sample, the general pattern of $\delta^{13}C_{ap}$ by age indicates an increase in mean value from 0.0–2.5 and 2.6–4.5 years of age, remaining stable through 4.6–7.0 years, and then decreasing dramatically after 7.1 years to fall close to the adult $\delta^{13}C_{ap}$ value (Figure 5). A Mann-Whitney U test confirmed that the increase in mean $\delta^{13}C_{ap}$ values from 0.0–2.5 years and 2.6–4.5 years was significant at the Bonferroni-corrected p-value of 0.01 (Table 3). In addition, the three youngest age categories (i.e., below 7.1 years of age) had values significantly higher than the values of subadult (7.1–19.9 years) and adult (20+ years) age groups.

Oxygen Isotope Results

The overall $\delta^{18}O_{ap}$ values range from -4.7‰ to 1.1‰ with a mean of -1.9 ± 1.0‰. For $\delta^{18}O_{ap}$ values, the first molars range between -3.1‰ and -0.8‰ with a mean of -1.8 ± 0.7‰; the first premolars range between -3.8‰ to -0.5‰ with a mean of -2.2 ± 0.8‰; and the second molars range between -4.7‰ and 1.1‰ with a mean of -1.9 ± 1.3‰. A Mann-Whitney U test also was used to identify any statistically significant differences in $\delta^{18}O_{ap}$ values among the three tooth classes (see Table 4). All the p-values are over the Bonferroni-corrected alpha level of 0.0167, and therefore none of the differences in mean $\delta^{18}O_{ap}$ values among tooth classes are statistically significant.

Table 1. Results of the $\delta^{13}C$ and $\delta^{18}O$ isotope analysis of enamel apatite and the $\delta^{13}C$ isotope of bone apatite (samples designated by MEP#) from the Petra North Ridge Tombs. Bone apatite values (designated by PAP #) are from Appleton (2015).

Sample ID	Context	Database Accession	Sample Type	Age	Cementum Age[1]	Sex	$\delta^{13}C_{ap}$ (‰)	$\delta^{18}O_{ap}$ (‰)
MEP01	B.4:10	B.4:10.a	RM^2	Adult	41.5 years	--	-11.1	-1.7
MEP02	B.4:23	B.4:23a	RPM_1	Adult	--	--	-11.6	-1.5
MEP04	B.4:23 "teeth bag"	B.4:23.c	LM_2	Adult	--	--	-11.3	-2.4
MEP05	B.4:23 "teeth bag"	B.4:23.d	LM_2	Adult	--	--	-11.6	-2.4
MEP06	B.4:23 individual 2	B.4:23.e	RM_2	Adult	--	--	-11.4	-1.9
MEP07	B.5:11	B.5:11.a	LM^2	Adult	--	--	-12.5	-2.4
MEP09	B.5:31	B.5:31.a	LM^2	Adult	46.5 years	Male	-13.3	-4.7
MEP10	B.5:32	B.5:32.h	LM_1	3 years +/- 12 months	--	--	-11.4	-1.0
MEP11	B.5:32	B.5:32.g	RM_1	Adult	--	--	-11.2	-1.2
MEP12	B.5:34 Skull #13	B.5:34.55.a	RPM^1	Adult	--	--	-10.9	-2.4
MEP13	B.5:34	B.5:34.a	RPM^1	Adult	45 years	--	-11.1	-1.8
MEP14	B.5:34 from Mand #20	B.5:34.b	RPM_1	Adult	--	--	-11.2	-1.9
MEP15	B.5:34	B.5:34.47.a	RPM_1	Adult	--	--	-11.1	-2.0
MEP16	B.5:34 Max #20	B.5:34.j	RM^1	Adult	--	--	-11.9	-1.2
MEP17	B.5:34 Mand#11	B.5:34.k	LM_1	Adult	--	--	-11.5	-2.5
MEP18	B.5:34 Max #20	B.5:34.53.a	LM^2	Adult	--	--	-11.3	-2.0
MEP19	B.5:34	B.5:34.l	M_2	Adult	--	--	-11.4	1.1
MEP20	B.5:35	B.5:35.b	RPM_1	Adult	--	--	-11.4	-1.3
MEP21	B.5:35	B.5:35.n	RM^1	Adult	--	--	-11.6	-1.9
MEP22	B.5:35	B.5:35.r	RM^1	Adult	--	--	-11.4	-1.4
MEP23	B.5:35	B.5:35.s	RM^1	3 years +/- 12 months	--	--	-11.5	-2.3
MEP25	B.5:35 from Mand	B.5:35.q	RM_1	Adult	--	Male	-12.4	-0.8
MEP26	B.5:35 Skull 13	B.5:35.55.a	LM^2	Adult	--	--	-11.1	-2.2
MEP27	B.5:35 Skull(?)#27	B.5:35.a	RM^2	Adult	--	--	-11.3	-2.3
MEP28	B.6:6 Layer 6	B.6:6.c	RPM_1	Adult	--	--	-11.5	-1.5
MEP29	B.6:27 Layer 2 #30	B.6:27.b	LPM_1	Adult	--	--	-10.9	-1.6
MEP30	B.6:27 Layer 2 #30	B.6:27.d	RM_1	Adult	--	--	-11.0	-1.6
MEP31	B.6:27 Layer 2 #30	B.6:27.30.a	RM^2	Adult	--	--	-12.0	-1.7

Sample ID	Context	Database Accession	Sample Type	Age	Cementum Age[1]	Sex	$\delta^{13}C_{ap}$ (‰)	$\delta^{18}O_{ap}$ (‰)
MEP32	B.6:28	B.6:28.e	LM^1	3 years +/- 12 months	--	--	-11.7	-1.7
MEP33	B.6:28	B.6:28.h	LM^1	4 years +/- 12 months	--	--	-11.8	-2.8
MEP34	B.6:28	B.6:28.g	RM^1	4 years +/- 12 months	--	--	-11.1	-1.4
MEP36	B.6:28	B.6:28.f	RM_1	7-10 years	--	--	-12.2	-2.4
MEP37	B.6:28	B.6:28.250.c	RM^2	Adult	33 years	--	-11.7	-2.9
MEP38	B.6:31.110	B.6:31.343.110.c	RM_2	Adult	--	--	-10.5	-3.1
MEP39	B.6:34	B.6:34.c	LM_2	Adult	--	--	-11.2	-1.7
MEP40	B.6:39	B.6:39.a	LM_2	Adult	--	--	-12.1	-1.6
MEP41	B.6:42	B.6:42.418.a	LPM_1	Adult	--	--	-11.2	-2.3
MEP42	B.6:42	B.6:42.a	RM_1	Adult	--	--	-11.8	-1.3
MEP43	B.6:42	B.6:42.418.b	LM_2	Adult	--	--	-11.1	-2.6
MEP45	B.6:43	B.6:43.a	RM_1	Adult	--	--	-11.4	-1.7
MEP46	B.6:43	B.6:43.435.c	LM^2	Adult	--	--	-11.9	-1.1
MEP48	B.6:44	B.6:44.447.a	LPM^1	Adult	--	--	-10.5	-2.5
MEP49	B.6:44	B.6:44.446.b	LM^1	Adult	--	--	-11.7	-2.3
MEP50	B.6:44	B.6:44.446.a	LM^2	Adult	--	--	-11.9	-1.5
MEP51	B.6:44	B.6:44.a	LM_2	Adult	--	--	-11.2	-1.7
MEP52	B.7:20	B.7:20.488.c	LPM^1	Adult	--	--	-11.4	-3.8
MEP53	B.7:20	B.7:20.h	LM^1	4 years +/- 12 months	--	--	-11.4	-2.3
MEP54	B.7:20	B.7:20.g	RM_1	Adult	--	--	-11.8	-2.0
MEP55	B.7:25	B.7:25.a	RM^1	Adult	--	--	-12.0	-1.8
MEP57	B.7:25.83	B.7:25.83.a	RM^1	Adult	--	--	-10.5	-1.6
MEP58	B.7:27	B.7:27.a	RPM^1	Adult	--	--	-11.2	-3.0
MEP59	B.7:27.76	B.7:27.76.c	LM_1	Adult	--	--	-11.4	-3.1
MEP60	B.7:27.76	B.7:27.76.a	RM^2	Adult	--	--	-11.3	-3.3
MEP61	B.7:31 Cranium E. End	B.7:31.a	RPM^1	Adult	--	Female	-11.4	-2.1
MEP62	B.7:31 Cranium/Mandible W End	B.7:31.c	LPM_1	Adult	52 years	Male	-10.6	-3.3
MEP63	B.7:31 Skull E. End	B.7:31.l	RM^1	Adult	--	Female	-11.5	-1.3
MEP64	B.7:31	B.7:31.b	LM^2	Adult	--	--	-10.8	-3.2

Sample ID	Context	Database Accession	Sample Type	Age	Cementum Age[1]	Sex	$\delta^{13}C_{ap}$ (‰)	$\delta^{18}O_{ap}$ (‰)
MEP65	B.7:31 Skull W. End	B.7:31.m	RM_2	Adult	52 years	Male	-11.0	-3.1
MEP66	B.7:33 with Skull 1?	B.7:33.j	LM_1	Adult	--	--	-11.1	-3.0
MEP67	B.7:33	B.7:33.499.a	RM_2	Adult	--	--	-10.5	-1.9
MEP68	B.7:34 Mandible 3?	B.7:34.b	LPM_1	Adult	--	Female	-11.3	-2.3
MEP69	B.7:34 Mand#3	B.7:34.a	PM_1	Adult	--	Female	-11.3	-2.2
MEP70	B.7:34 Mand#3	B.7:34.e	M_1	Adult	--	Female	-11.7	-1.9
MEP71	B.7:34	B.7:34.g	RM_1	3 years +/- 12 months	--	--	-11.5	-1.4
MEP72	B.7:34 "Crania"	B.7:34.533.a	RM^2	Adult	--	--	-6.6	1.0
MEP73	B.7:35	B.7:35.a	LPM^1	Adult	--	--	-11.0	-3.2
MEP74	F.1:26.120	F.1:26.120.16.a	LM^1	Adult	--	--	-11.4	-1.4
MEP75	F.1:27.123	F.1:27.123.S1.bb	RM^2	Adult	--	--	-12.1	0.3
MEP76	F.1:28.90	F.1:28.90.S1.bo	LPM^1	Adult	34.5 years	--	-11.1	-2.4
MEP77	F.1:28.94	F.1:28.94.S1.ai	LM_2	Adult	--	--	-11.1	-2.5
MEP78	F.1:28.97	F.1:28.97.69.a	LM^1	Adult	37 years	--	-11.3	-1.3
MEP79	F.1:28.97	F.1:28.97.69.a	LM^2	Adult	37 years	--	-11.4	-3.0
MEP80	F.1:28.102	F.1:28.102.S40.s	RM^1	Adult	--	--	-11.7	-2.9
MEP81	F.1:28.115	F.1:28.115.S34.aa	RPM_1	Adult	28 years	--	-11.6	-0.5
MEP82	F.1:28.115	F.1:28.115.35.f	RM^1	Adult	--	--	-12.3	-0.9
MEP83	F.1:28.115	F.1:28.115.35.f	RM^2	Adult	--	--	-11.5	-0.4
MEP84	F.1:28.115	F.1:128.115.35.n	RPM^1	Adult	30 years	--	-12.0	-0.9
MEP85	F.1:28.141	F.1:28.141.14	RPM_1	Adult	--	--	-11.3	-3.1
MEP86	F.1:30.139	F.1:30.139.S1	LM^2	8-9 years	--	--	-11.7	0.2
MEP87	F.1:30.134 Individual 3	F.1:30.134.S20.s	RM_1	Adult	--	--	-11.6	-0.9
PAP1	B.5:31	--	Long Bone Fragment	Adult	--	Female	-13.7	--
PAP2	B.5:17	B.5:17.CN1-CN7	Humerus	18-20 years	--	Female	-12.7	--
PAP3	B.5:9 Individual 1	B.5:9.CN401	Rib	Adult	--	Female	-13.1	--
PAP4	B.5:12	--	Rib	Adult	--	Male	-13.7	--
PAP5	B.4:18	B.4:18.CN1?	MT3	Adult	--	--	-13.6	--
PAP6	B.4.17	B.4:17.CN1	MT4	Adult	--	Male	-13.1	--
PAP7	B.4:22	B.4:22.CN1	MC3	Adult	--	Male	-13.5	--

Sample ID	Context	Database Accession	Sample Type	Age	Cementum Age[1]	Sex	$\delta^{13}C_{ap}$ (‰)	$\delta^{18}O_{ap}$ (‰)
PAP8	B.4:23 Individual 1	B.4:23.CN201/INDIV-2	MT3	Adult	--	Male	-12.5	--
PAP9	B.4:23 Individual 2	B.4:23.CN1/INDIV-2	MT3	Adult	--	Male	-13.0	--
PAP10	B.4:23 Individual 3	B.4:23.CN1/INDIV-3	MC5	Adult	--	--	-13.2	--
PAP11	B.4:18	--	Tibia	Subadult	--	--	-13.5	--
PAP12	B.4:10 Individual 1	B.4:10.CN1	Humerus	Adult	--	--	-12.8	--
PAP13	B.4:10 Individual 2	B.4:10.CN2	Humerus	Adult	--	Female	-13.6	--
PAP14	B.4:10 Individual 3	B.4:10.CN3	Humerus	Adult	--	--	-13.3	--
PAP15	B.5:15 skull #1	B.5:15.CNa1/SKULL-1	Cranial Fragment	Old Adult	--	Possible Female	-13.5	--
PAP16	B.5:15 skull #2	--	Parietal	Adult	--	Male	-13.6	--
PAP17	B.5:15 skull #4	B.5:15.CNc1/SKULL4+	Parietal	Adult	--	Male	-13.2	--
PAP18	B.5:15 skull #5	B.5:15.CNd1/SKULL-5	Cranial Fragment	Adult	--	Possible Female	-14.0	--
PAP19	B.5:15 skull #6	B.5:15/CNe1/SKULL-6	Cranial Fragment	Adult	--	Male	-13.4	--
PAP20	B.5:15 skull #7	B.5:15.CNf1/SKULL-7	Cranial Fragment	Adult	--	Female	-12.7	--
PAP21	B.5:15 skull #9	B.5:15.CNh1/SKULL-9	Parietal	Adult	--	Possible Female	-13.1	--
PAP22	B.5:15 Skull #10	B.5:15/CNj1/SKULL-10	Cranial Fragment	Adult	--	Possible Male	-12.9	--
PAP23	B.5:9 Skull #11	B.5:9.CN301-CN309/SKULL#11	Parietal	Adult	--	Male	-13.7	--
PAP24	B.5:15 Skeleton #1	--	Rib	Adult	--	Male	-13.2	--
PAP25	Tomb 2 Burial 1	--	Femur	20-24 years	--	Female	-13.2	--
PAP26	Tomb 2 Burial 2	--	Long Bone Fragment	45-49 years	--	--	-10.8	--
PAP27	Tomb 2 Burial 4	--	Femur	25-29 years	--	Male	-11.1	--
PAP28	Tomb 2 Burial 5	--	Femur	50-59 years	--	Female	-11.1	--
PAP29	Tomb 2 Burial 6	--	Femur	35-39 years	--	Female	-10.4	--
PAP30	Tomb 2 Burial 8	--	Femur	18-20 years	--	Female	-12.7	--
PAP31	B.5:15 Subadult	--	Femur	2-3 years	--	--	-12.7	--
PAP32	B.4:13 Subadult	B.4:13.2	Ilium	36-37 weeks	--	--	-12.0	--

Sample ID	Context	Database Accession	Sample Type	Age	Cementum Age[1]	Sex	$\delta^{13}C_{ap}$ (‰)	$\delta^{18}O_{ap}$ (‰)
PAP33	B.4:23 Subadult	--	Clavicle	16-22 years	--	--	-12.8	--
PAP34	B.4:17/22 Subadult	--	C1-7	3-4 years	--	--	-12.7	--

[1] Data for cementum age is from Propst (2017)

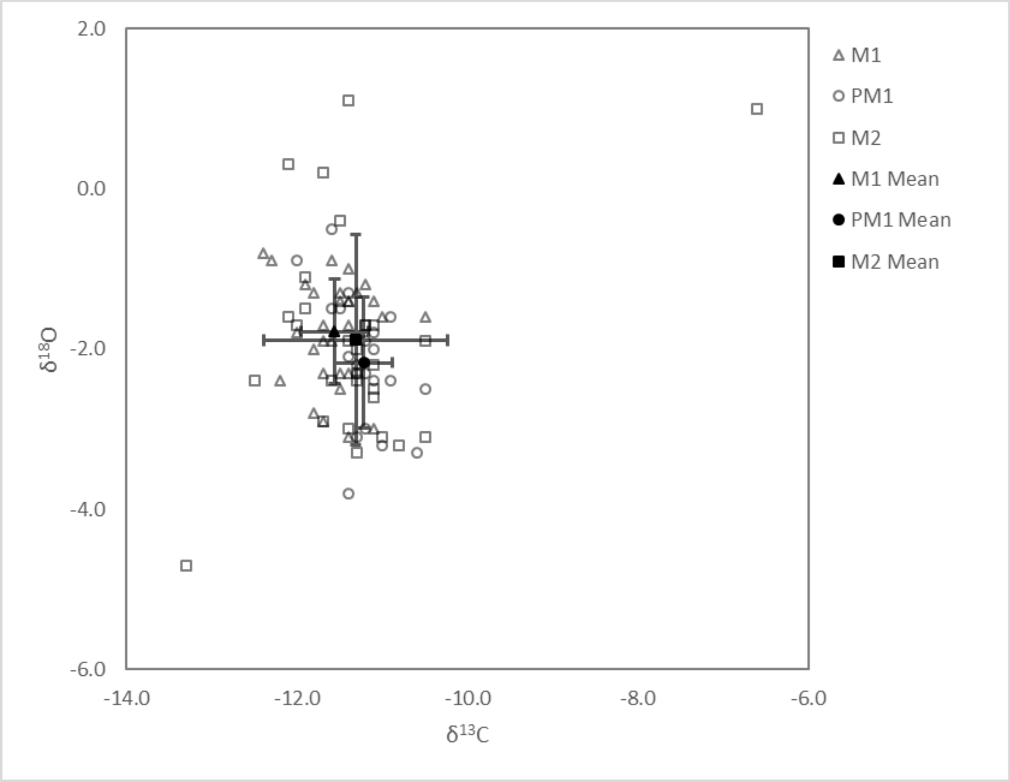

Figure 2. $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values labeled by tooth class showing mean and standard deviation.

The $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values for the eleven individuals with multiple teeth sampled are reported in Table 2. The $\delta^{13}C_{ap}$ values were expected to increase between first molars and first premolars as supplementary food became a larger component of the diet than breastmilk, to decrease between first premolars and second molars as weaning was completed and an adult diet was adopted, and to decrease between first molars and second molars since the latter should represent an adult diet (Dupras and Tocheri 2007; Wright and Schwarcz 1998). Eight individuals (B.5:34 Skull #13, B.6:27 Layer 2 #30, B.6:44, B.7:27, B.7:31 skull E. end, B.7:31 cranium/mandible W end, B.7:34 mand#3, and F.1:28.97) follow these expected patterns (Figure 3). The three remaining individuals either had an increase in values from the first molar to the second molar (B.5:34 max #20 and F.1:28.115) or an increase between the first premolar and the second molar (B.6:42). The only outlier in the values of the individuals was the first molar of F.1:28.115.35.f (MEP82) at a $\delta^{13}C_{ap}$ value of -12.3‰. This value is lower than the 95% confidence level for the first molar (95% CI [-11.7, -11.4]) which could be caused by the mother's diet consisting of more C_3 food sources than that of other individuals.

The $\delta^{18}O_{ap}$ values for the individuals were expected to gradually decrease between first molars and first premolars, between first premolars and second molars, and between first molars and second molars as individuals were weaned off enriched breastmilk. Seven individuals (B.5:34 max #20, B.6:27 Layer 2 #30, B.6:42, B.7:27, B.7:31 skull E. end, B.7:34 mand#3, and F.1:28.97) followed the expected pattern (Figure 4). The four individuals that did not follow the expected pattern either had an increase in values from the first molar to the second molar (B.6:44 and F.1:28.115) or from the first premolar to the second molar (B.5:34 Skull #13 and B.7:31 cranium/mandible W end).

Table 2. Results of the $\delta^{13}C$ and $\delta^{18}O$ isotope analysis of enamel apatite of eleven individuals with multiple teeth.

Individual	Age	Sex	Cementum Age	Sample ID	Context	Database Accession	Sample Type	$\delta^{13}C_{ap}$ (‰)	$\delta^{18}O_{ap}$ (‰)
1	Adult	--	--	MEP12	B.5:34 Skull #13	B.5:34.55.a	RPM^1	-10.9	-2.4
				MEP26	B.5:35 Skull 13	B.5:35.55.a	LM^2	-11.1	-2.2
2	Adult	--	--	MEP16	B.5:34 max #20	B.5:34.j	RM^1	-11.9	-1.2
				MEP18	B.5:34 max #20	B.5:34.53.a	LM^2	-11.3	-2.0
3	Adult	--	--	MEP30	B.6:27 Layer 2 #30	B.6:27.d	RM$_1$	-11.0	-1.6
				MEP29	B.6:27 Layer 2 #30	B.6:27.b	LPM$_1$	-10.9	-1.6
				MEP31	B.6:27 Layer 2 #30	B.6:27.30.a	RM^2	-12.0	-1.7
4	Adult	--	--	MEP41	B.6:42	B.6:42.418.a	LPM$_1$	-11.2	-2.3
				MEP43	B.6:42	B.6:42.418.b	LM$_2$	-11.1	-2.6
5	Adult	--	--	MEP49	B.6:44	B.6:44.446.b	LM^1	-11.7	-2.3
				MEP50	B.6:44	B.6:44.446.a	LM^2	-11.9	-1.5
6	Adult	--	--	MEP58	B.7:27	B.7:27.a	RPM^1	-11.2	-3.0
				MEP60	B.7:27.76	B.7:27.76.a	RM^2	-11.3	-3.3
7	Adult	Female	--	MEP63	B.7:31 skull E. end	B.7:31.l	RM^1	-11.5	-1.3
				MEP61	B.7:31 cranium E. end	B.7:31.a	RPM^1	-11.4	-2.1
8	Adult	Male	52 years	MEP62	B.7:31 cranium/mandible W end	B.7:31.c	LPM$_1$	-10.6	-3.3
				MEP65	B.7:31 skull W. end	B.7:31.m	RM$_2$	-11.0	-3.1
9	Adult	Female	--	MEP70	B.7:34 mand#3	B.7:34.e	M$_1$	-11.7	-1.9
				MEP68	B.7:34 mandible 3?	B.7:34.b	LPM$_1$	-11.3	-2.3
				MEP69	B.7:34 mand#3	B.7:34.a	PM$_1$	-11.3	-2.2
10	Adult	--	37 years	MEP78	F.1:28.97	F.1:28.97.69.a	LM^1	-11.3	-1.3
				MEP79	F.1:28.97	F.1:28.97.69.a	LM^2	-11.4	-3.0
11	Adult	--	--	MEP82	F.1:28.115	F.1:28.115.35.f	RM^1	-12.3	-0.9
				MEP83	F.1:28.115	F.1:28.115.35.f	RM^2	-11.5	-0.4

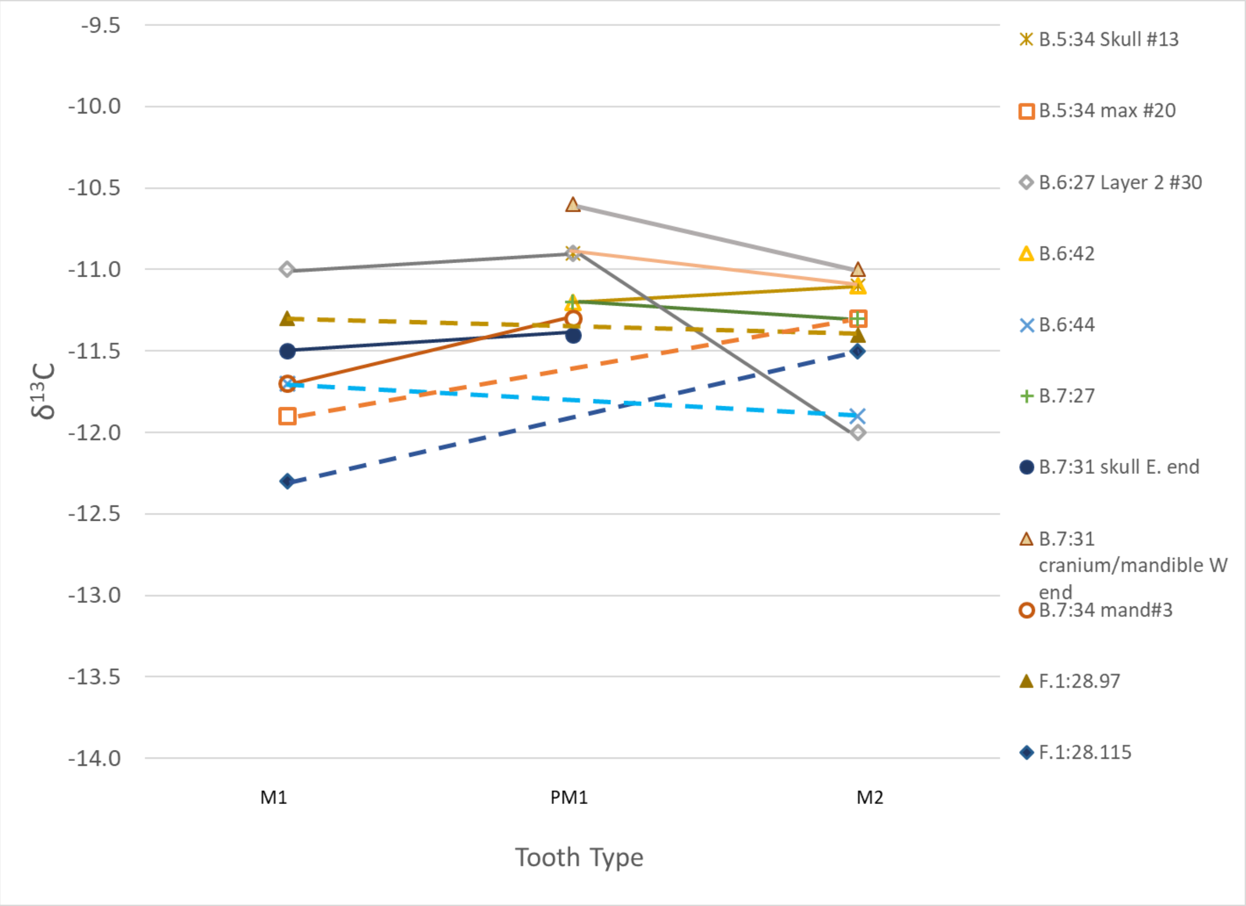

Figure 3. Within-individual variation in $\delta^{13}C_{ap}$ values of the eleven individuals with at least two teeth from the Petra North Ridge tombs. The dashed lines indicate changes between the M1and M2 only.

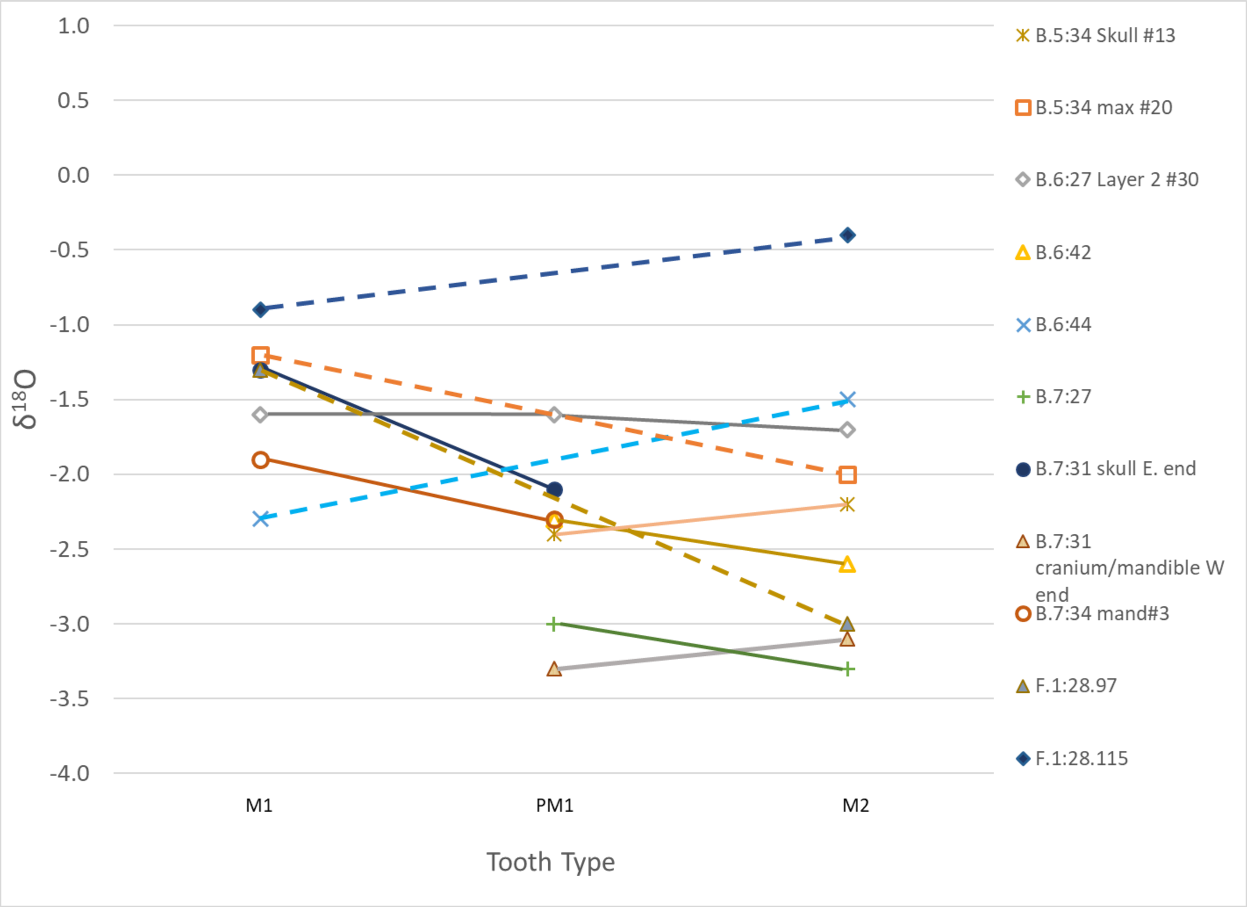

Figure 4. Within-individual variation in $\delta^{18}O_{ap}$ values of the eleven individuals with at least two teeth from the Petra North Ridge tombs. The dashed lines indicate shifts between the M1 and M2 only.

Table 3. Statistical comparisons of $\delta^{13}C_{ap}$ values of the dental and bone apatite by general age of tissue formation from the Petra North Ridge tombs.

	N	Mean	SD	Mann-Whitney U*
0.0-2.5	33	-11.6	0.4	**0.0-2.5 vs 2.6-4.5: Z=-2.949, p=0.003**
2.6-4.5	21	-11.3	0.5	0.0-2.5 vs 4.6-7.0: Z=-1.713, p=0.087
4.6-7.0	29	-11.3	1.1	**0.0-2.5 vs 7.1-19.9: Z=-3.192, p<0.001**
7.1-19.9	4	-12.9	0.4	2.6-4.5 vs 4.6-7.0: Z=-0.996, p=0.319
20.0+	27	-12.9	1.0	**2.6-4.5 vs 7.1-19.9: Z=-3.050, p<0.001**
				4.6-7.0 vs 7.1-19.9: Z=-3.041, p<0.001
				0.0-2.5 vs 20.0+: Z=-4.746p<0.001
				2.6-4.5 vs 20.0+: Z=-4.433p<0.001
				4.6-7.0 vs 20.0+: Z=-4.614p<0.001
				7.1-19.9 vs 20.0+: Z=-1.035, p=0.316

*Bolded items indicate significant results at the Bonferroni-corrected alpha of 0.01.

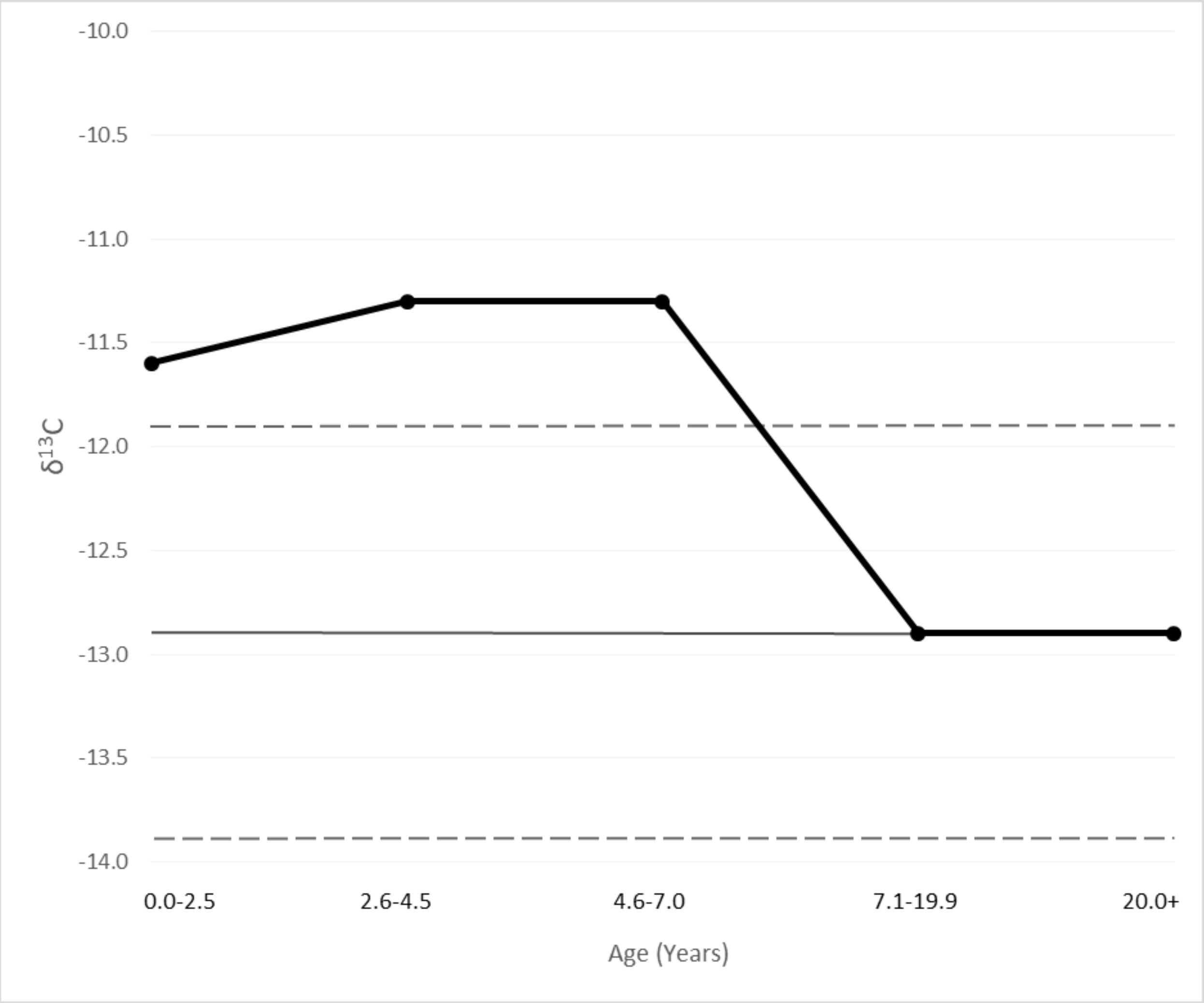

Figure 5. Mean δ13C_{ap} values from dental and bone apatite by age in the Petra North Ridge tombs sample. The solid horizontal line represents the mean for the adult values and the dashed lines represent the standard deviation.

Table 4. Statistical comparisons of $\delta^{18}O_{ap}$ values of the dental enamel by age of crown formation (M1, PM1, M2) from the Petra North Ridge sample.

	N	Mean	SD	Mann-Whitney U*
0.0-3.0	30	-1.8	0.7	0.0-3.0 vs 2.0-4.5: Z=-1.936, p=0.053
2.0-5.0	21	-2.2	0.8	0.0-3.0 vs 3.0-7.0: Z=-1.397, p=0.162
3.0-7.0	29	-1.9	1.3	2.0-4.5 vs 3.0-7.0: Z=-0.453, p=0.651

*Significance based on Bonferroni-corrected alpha of 0.0167.

The communal tombs at Petra are hypothesized to represent different lineages in the city (Perry 2017). In order to determine if variation in isotope shifts by age was linked to lineagespecific dietary practices that could have resulted in intra-tomb differences in shift patterns, the age-at-tissue formation of bone and enamel $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ results were broken down by tomb. The mean values of $\delta^{13}C_{ap}$ in Tombs B.5, B.6 and F.1 increase between the age ranges 0.0–2.5 and 2.6–4.5 and decrease from 2.6–4.5 to 4.6–7, which follows the expected pattern, while the mean values in Tombs B.4 and B.7 either remain unchanged or increase randomly across the three youngest age categories (Figure 6). Tombs B.4 and B.5, the two tombs with bone apatite data representing diets between 7.1 and 19.9 years, show a decline from the previous age category. The final shift from older childhood to adult diet presents as a mean $\delta^{13}C_{ap}$ decline for Tomb F.1 but a decrease for Tomb 2 and B.5.

The mean $\delta^{18}O_{ap}$ values in all tombs decreases as expected from the youngest to the middle childhood age category, but variability exists in whether the values increase or decrease between the second and third age category (Figure 7).

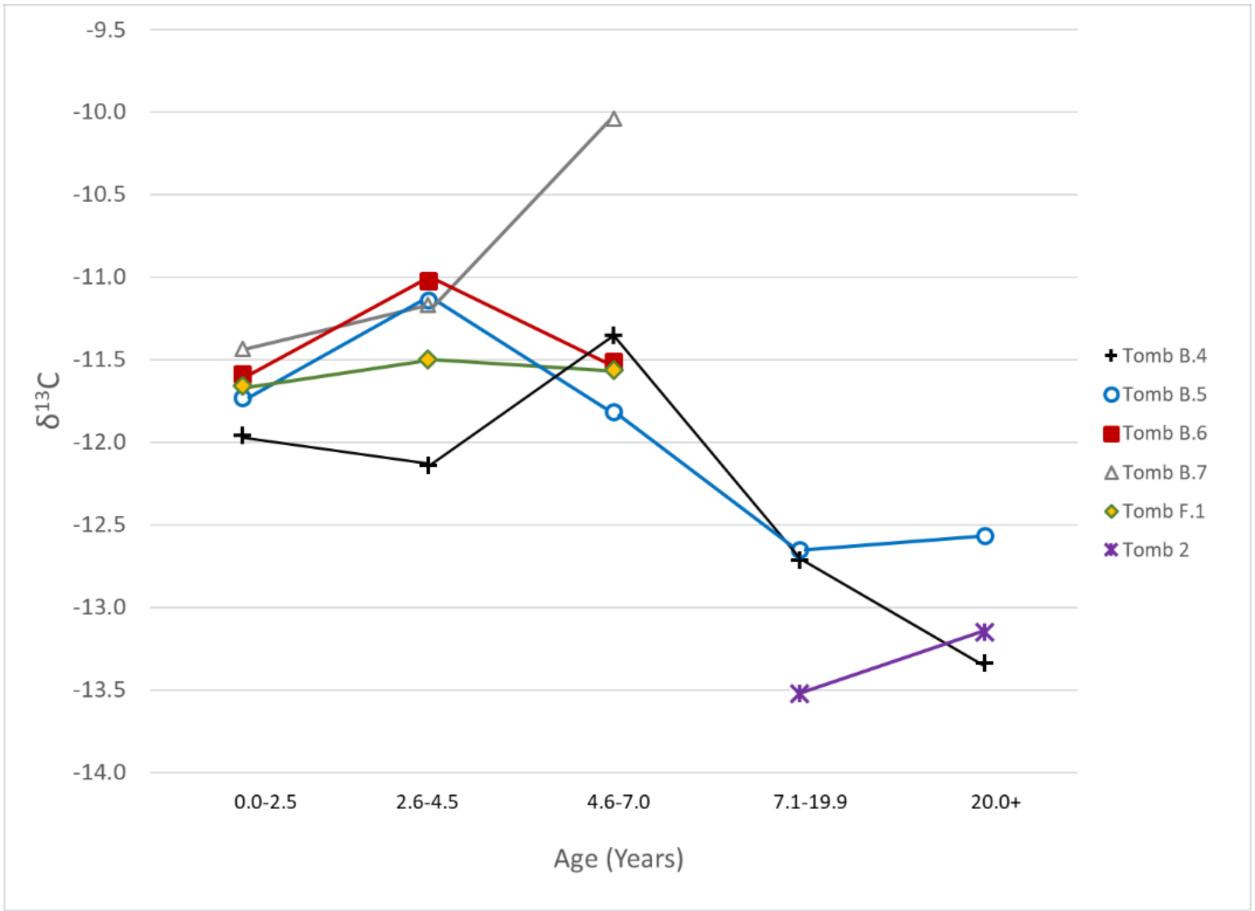

Figure 6. Intra-tomb variation in $\delta^{13}C_{ap}$ values of the dental and bone apatite from the Petra North Ridge tombs.

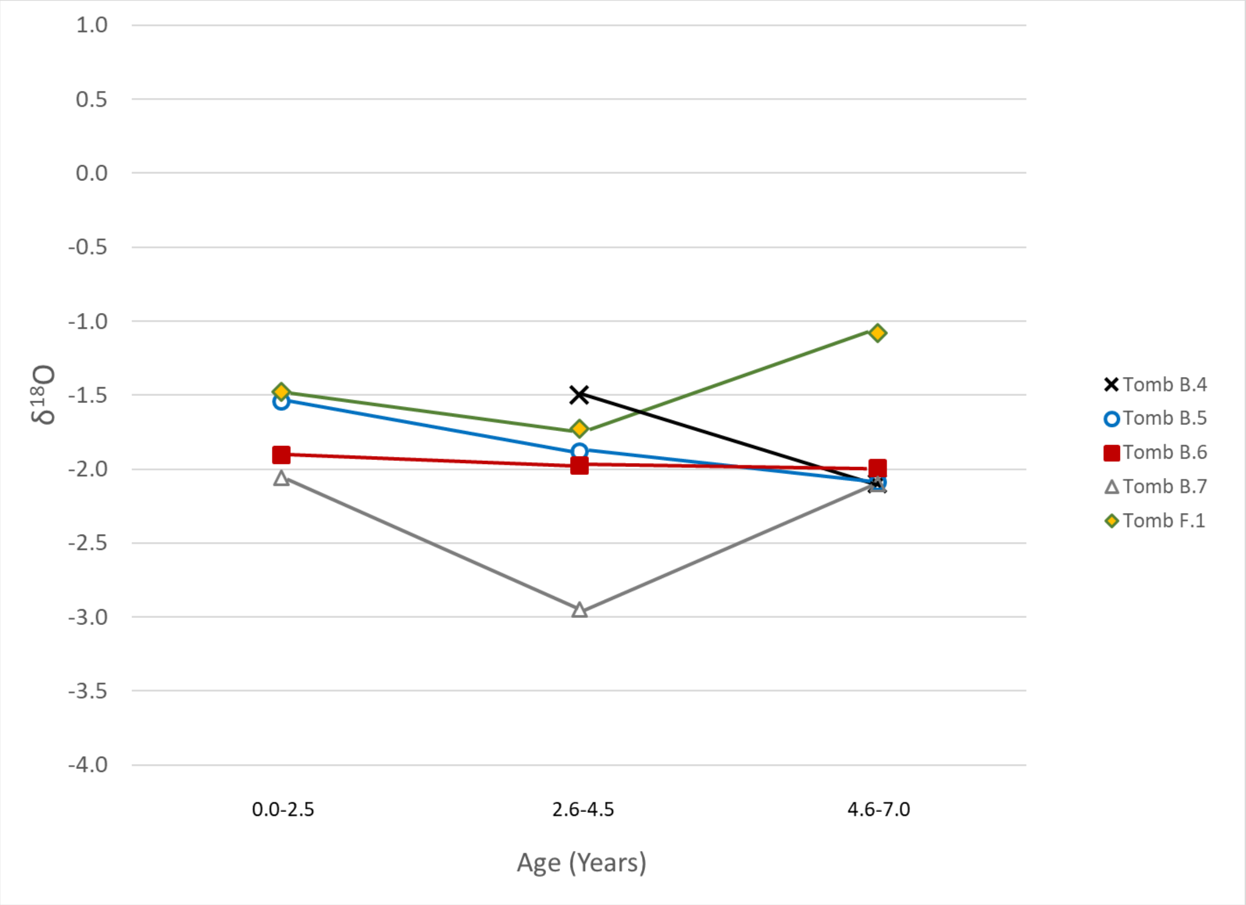

Figure 7. Intra-tomb variation in $\delta^{18}O_{ap}$ values of dental apatite from the Petra North Ridge tombs.

Finally, isotope values were compared between tissues of those who died in childhood versus those who lived past 20+ years to identify potential differences in childhood diets or water sources which could have resulted in frailty. The $\delta^{18}O_{ap}$ and $\delta^{13}C_{ap}$ in the bone or enamel tissues forming between birth and 2.5 years represent the diet and water sources during this period. The first molars of nine individuals who died before the age of 20 years along with the bones of two infants who died during this age range reflect a "non-survival" diet. This was compared to the first molar dental enamel values of the 22 individuals who lived past 20 years of age. No differences were identified between the $\delta^{13}C_{ap}$ or $\delta^{18}O_{ap}$ values of those who died during childhood versus those living past 20 years of age using a Mann-Whitney U test (Table 5 and 6).

Therefore, diet and water source during the first 2.5 years of life does not appear to impact the likelihood of an individual surviving past 20 years of age at Petra.

Table 5. Analysis of $\delta^{13}C_{ap}$ values of skeletal tissues forming between 0–2.5 years of individuals dying before 20 years and those dying after 20 years of age.

	N	Mean	SD	Mann-Whitney U
Non-Adult (<20 years)	10	-11.6	0.1	Z=-0.777, p=0.437
Adult (>20 years)	22	-11.6	0.1	

Table 6. Analysis of $\delta^{18}O_{ap}$ values of skeletal tissues forming between 0–2.5 years of individuals dying before 20 years and those dying after 20 years of age.

	N	Mean	SD	Mann-Whitney U
Non-Adult (<20 years)	8	-1.9	0.2	Z=-0.823, p=0.411
Adult (>20 years)	22	-1.7	0.1	

The overall analyses show that major shifts in $\delta^{13}C_{ap}$ values toward the adult dietary values did not occur until after the second molar crown had formed. Thus weaning likely had started by the time the first premolar crown began forming at 3 years, represented by the slight increase in $\delta^{13}C_{ap}$ values compared to the earlier age range, but perhaps had not completed past three years of age when the second molar began forming. However, the individual-level analysis indicated that dietary shifts in childhood represented by the first molar, first premolar, and second molar $\delta^{13}C_{ap}$ values were quite varied, perhaps indicating some element of personal choice or circumstance in the age that weaning occurred and the foods introduced during weaning. In addition, there was also variability among individuals in whether the shift was an increase or decrease in value, masking any notable shift from the first premolar to the second molar in the overall sample. The intra-individual analysis of isotope values identify that some individuals might have begun a shift to an adult diet quite late in formation of the first premolar and early in the formation of the second molar.

Shifts in the $\delta^{18}O_{ap}$ by tooth type were expected to gradually decline as the infant slowly transitioned from one trophic level to another during weaning. The values decrease as hypothesized from the age of first molar formation to the age of first premolar formation, but unexpectedly increased between first premolar and second molar formation. However, none of these shifts were statistically significant. Similar to the intra-individual analysis of $\delta^{13}C_{ap}$ across tooth types, whether the $\delta^{18}O_{ap}$ values increased or decreased with age, and the extent of the change, varied by individual. In general, a decrease in $\delta^{18}O_{ap}$ between 0.0–3.0 years of age and 2.0–5.0 years of age in an individual may not indicate that weaning was occurring, but that the water sources between 2.0–5.0 years and 3.0–7.0 years may have changed drastically for that individual, possibly due to migration, resulting in an unexpected increase.

Finally, the diet or water source a child was exposed to from birth to 2.5 years, based on bone and dental enamel $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values that formed during those years, had no impact on whether or not the individual died before reaching 20 years of age.

CHAPTER 5: Discussion

The stable isotope data presented in the previous section is interpreted through several lenses in this chapter. The $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values is used to study the general timeline of weaning, supplemental food used during weaning, intra-individual trends by age, and implications of early nutrition on health and mortality rates. In addition, these results are compared to other sites that fall within the Greco-Roman cultural sphere to identify any differences in weaning patterns from ancient medical norms.

Weaning Estimations

Previous studies have determined that $\delta^{13}C$ values may be reliable indicators for the timing and overall length of the weaning process (Fuller et al. 2006a; Wright and Schwarcz 1998). $\delta^{13}C$ values from collagen have an enrichment factor of ~1‰ during exclusive breastfeeding (Bourbou et al. 2013; Fuller et al. 2006a), which should begin to decrease to maternal levels upon the introduction of solid foods (Fuller et al. 2006a). While $\delta^{13}C$ values from enamel apatite do not experience fractionation between solely breastfeeding and the incorporation of solid food, they do reflect the macronutrients of solid food introduced to the infant during weaning. Thus, $\delta^{13}C_{ap}$ would show evidence of weaning through a value shift, the direction of which depends on the C_3 or C_4 composition of the foods used for weaning supplementation as opposed to the adult/maternal diet. After weaning is completed, $\delta^{13}C$ values from both collagen and apatite should be representative of an adult diet.

The exact age at which supplemental foods were introduced into an infant's diet in the sampled population is difficult to discern due to the wide age range for each value. However, the shift of $\delta^{13}C$ values from the first molar to the first premolar can give some indication. First molars can be presumed to have values that reflect the $\delta^{13}C$ of breastmilk in conjunction with solid foods that provide some enrichment (Lee-Thorp et al. 1989; Wright and Schwarcz 1998). Since the development of first molars and first premolars overlap from two to three years of age, a large difference in values between those two tooth types would suggest that breastmilk was no longer a substantial part of the diet by the time first premolars began to form (Wright and Schwarcz 1998), thus suggesting, supplementary foods would have already been introduced by the age of two. Wright and Schwarz (1998) were able to determine that the Kaminaljuyú likely began weaning at one year of age based on the significance of this shift along with ethnographic data from the modern population. Since the $\delta^{13}C_{ap}$ values at Petra have a significant difference between the 0.0–2.5 years and the 2.6–4.5 age groups, it is probable that solid foods were introduced before the age of two years.

As far as estimating the termination of the weaning process at Petra using $\delta^{13}C$ values, the most informative shift is between the children 4.6–7.0 years-old and those 7.1 years of age and older. There are two possible explanations for the sizable difference in values between these two age categories. The first option is that the completion of the weaning process did not occur until after 4.6 years of age, which would cause second molars and bone forming after that age but before 7 years to retain weaning-diet signatures. Another possibility is that there was a specialized diet for children distinct from that of an adult that was introduced during weaning and lasted until around seven years of age. The stable isotope analysis conducted by Appleton (2015) determined that the Petraean adults largely relied on C_3 food sources, such as barley, wheat, legumes, nuts, fruits, vegetables, and animals that subsisted on C_3 vegetation. It was expected that childhood diet before, during, and after weaning would show a similar consistency in primarily C_3 signatures, assuming the foods used to supplement breastfeeding were the same as the adults in terms of the C_3/C_4 balance. Enamel carbonate values indicating C_3 based diets are between -22 to -11‰, while values for C_4 diets are between -5 to 2‰ (Krueger and Sullivan 1984; Smith and Epstein 1971). In general, the enamel $\delta^{13}C_{ap}$ values, which range from -13.3‰ and -6.6‰ with a mean of -11.4 ± 0.7‰ indicate that the children had a C_3-dominant diet. However, the comparisons by age of tissue formation show a significant enrichment in $\delta^{13}C_{ap}$ values from children under 2.5 years versus those 2.6–4.5 years, indicating that C_4 food sources probably supplemented breastmilk during the weaning process. Once children reached 7.0 years of age, however, C_3 plants were consumed essentially at the same level as adults.

According to paleobotanical studies at Petra and the surrounding hinterlands, the most likely source would be from millet as few, if any, other C_4 plants have been found during archaeological investigations (Bedal et al. 2007; Ramsay and Bedal 2015; Ramsay and Smith 2013). Sorghum is another C_4 plant indigenous to the area which could have been implemented during weaning, but it is not preserved in the archaeological record of this area.

The $\delta^{18}O$ values are useful in detecting shifts during the weaning process because breastmilk is more enriched in $\delta^{18}O$ than drinking water (Dupras and Tocheri 2007). Therefore, as breastfeeding lessens and water from other sources is used, the $\delta^{18}O$ values are expected to decrease with age. Since there are no data available on adult levels of $\delta^{18}O$ for Petra, this study could not use the analysis of $\delta^{18}O$ to determine a relative age at which children had stopped drinking breastmilk and were completely weaned. Instead, $\delta^{18}O$ values were assessed for indications of shifts among the three tooth types during the weaning process. None of the tooth types had significant differences from first molars ($\bar{x}$ = -1.8‰ ± 0.7) to first premolars ($\bar{x}$ = -2.2‰ ± 0.8), or from first premolars to second molars ($\bar{x}$ = -1.9‰ ± 1.3). There are three possible explanations that $\delta^{18}O$ did not decrease by age as expected, which can be clarified by the intra-individual analysis: 1) immigration during early childhood, 2) non-human breastmilk being used as supplementary food during weaning, or 3) early introduction of environmental water.

Within Individual Variation

The intra-individual analysis of dental enamel $\delta^{13}C$ and $\delta^{18}O$ values across tooth classes demonstrates that Petraeans varied in terms of when weaning occurred, the types of foods they introduced during weaning, whether other liquids replaced human breastmilk during weaning, and their levels of childhood mobility. For instance, the individuals from B.6:44 and B.6:27 Layer 2 #30 have lower second molar $\delta^{13}C_{ap}$ values, indicating that they might have finished weaning earlier than the other individuals. The only individual with $\delta^{13}C_{ap}$ values that increase from the first premolar to the second molar is from context B.6:42. This could be due to extended weaning, but the $\delta^{18}O_{ap}$ values for that individual shift downward as expected with decreased breastmilk consumption. Another explanation is that the individual consumed a larger amount of C_4 based foods after weaning was completed than during the process, whether due to individual preference or food availability. These variabilities in $\delta^{13}C_{ap}$ changes by age suggest that weaning diets and timing varied at Petra.

There is heterogeneity in the within-individual $\delta^{18}O_{ap}$ values as well. Four of the individuals (B.5:34 Skull #13, B.6:44, B.7:31 Cranium/Mandible W End, and F.1:28.115) have $\delta^{18}O_{ap}$ values that increase over time instead of the expected decrease, suggesting that they might have immigrated during childhood and been exposed to different water sources for the first years of life. Studying oxygen isotopes can assist in determining migration as oxygen isotopes are affected by elevation, rainfall levels, humidity, and distance from large bodies of water, Jordan's varying topography causes there to be a range of groundwater values and makes it an optimal region for oxygen isotope analysis (Perry et al. 2009).

Samples underwent $^{87}Sr/^{86}Sr$ isotopic analysis as well as $\delta^{13}C$ and $\delta^{18}O$ analyses can shed more light on whether childhood immigration to Petra resulted in the increase in $\delta^{18}O_{ap}$ with age. Only one sample that had an outlying $\delta^{18}O_{ap}$ value had also been analyzed for $^{87}Sr/^{86}Sr$: sample MEP19/Petra038 from context B.5:34. Despite the outlying $\delta^{18}O_{ap}$ value, the $^{87}Sr/^{86}Sr$ data was within the normal range based on faunal samples from Petra ($^{87}Sr/^{86}Sr$ $\bar{x}$=0.7080 ± 0.0003, Petra038=0.7080). Although the first molar from context B.6:27 Layer 2 #30 (MEP30/Petra006) did not have an outlying $\delta^{18}O_{ap}$ value, its $^{87}Sr/^{86}Sr$ value was the most divergent in the entire Petra sample (Petra006=0.706391). This was also the individual that had close to no change in $\delta^{18}O_{ap}$ values across teeth classes (first molar: MEP30= -1.6, first premolar: MEP29= -1.6, second molar: MEP31= -1.7). This indicates that the individual from context B.6:27 Layer 2 #30 had non-local origins during the formation of the first molar and migrated shortly before or at the start of the first premolar forming.

Another cause for an increase in $\delta^{18}O_{ap}$ values through childhood could be consuming other liquids such as non-human breastmilk or environmental water either pre-weaning or during weaning period. The higher $\delta^{18}O$ values of milk from grazing animals can offset any weaning trophic level shift or potentially cause an increase over time (Daux et al. 2008). Grazing animals might also have access to a more varied range of water sources. In addition, introduction of environmental water early in infancy would reduce any trophic level shift expected with a reduction in breastmilk consumption (Williams et al. 2005). Another consideration is the seasonal climatic fluctuations that affect $\delta^{18}O$. During Wright's (2013) study at Kaminaljuyu, it was determined that $\delta^{18}O_{ap}$ values in a single tooth can vary 2‰ and the rainwater in that region of Guatemala could vary 7‰ due to seasonal changes.

The shift in $\delta^{18}O$ by tooth class in the overall sample does not reliably indicate the general age that weaning occurred in Petra. However, the intra-individual findings suggest that the two individuals for whom we have both a first molar and a first premolar see a decline of 0.6‰ on average between these two age ranges, indicating a decline in breastmilk consumption before formation of the first premolar. Greater variation exists in the change of $\delta^{18}O$ values from the first premolar to the second molar. Two individuals have an average +0.25‰ shift between the age ranges represented by these two teeth, while the others with these two teeth average a -0.35‰ change. To this, four other individuals with only first molars and second molars could be added, two showing a decline averaging -1.25‰ and two an increase averaging +0.65‰ between the ages of crown formation. The value of the first premolar in these four cases can be assumed to fall somewhere in between the M1 and M2 values, marking a linear, gradual change over time. Overall, the $\delta^{18}O_{ap}$ patterns at Petra demonstrate that some individuals may have been weaned gradually across all ages represented by the first molar, first premolar, and second molar, starting before formation of the first premolar. Others could have had a supplementation of breastmilk by non-human milk during formation of the first molar, and increasing proportions of non-human milk enriched in $\delta^{18}O$ drove child values higher with age. Finally, although immigration could have been a confounding factor, it is unlikely to be the case for any individual besides B:6.27 Layer 2 #30.

Petra Weaning Practices in a Greco-Roman Context

Comparing these data to contemporary populations in the circum-Mediterranean and European region can illuminate whether or not weaning practices at Petra were similar to other groups in the Greco-Roman realm. Three such sites have studied childhood diet through $\delta^{13}C$ and/or $\delta^{18}O$ isotopic analysis: Kellis 2 cemetery in Egypt, Isola Sacra necropolis in Italy, and Queenford Farm cemetery in England. The $\delta^{13}C$ values from these sites were converted to general age at tissue formation for comparison with the Petra $\delta^{13}C$ results (Table 7).

The only comparative site that utilized isotope analysis of dental enamel apatite was the Kellis 2 cemetery in Dakhleh Oasis, Egypt. This cemetery was used by the inhabitants of the Roman period town of Kellis from 100 A.D. to 450 A.D. Dupras and Tocheri (2007) analyzed $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ from enamel apatite and $\delta^{15}N$ and $\delta^{13}C$ from dentin collagen in all permanent and deciduous teeth except third molars (297 teeth from 102 juveniles and adults). The authors concluded that exclusive breastfeeding occurred for the first six months and then infants were slowly weaned until 3 years of age. The Dakhleh $\delta^{13}C_{ap}$ values show an increase between formation of the permanent anterior dentition and the first and second premolars that they interpret as supplementing breastmilk with foods such as millet gruel and milk from cows and goats who consumed millet, which would have enriched $\delta^{13}C$ values in children during weaning. This pattern is similar to the overall Petra sample (Figure 7), with an increase in $\delta^{13}C_{ap}$ between the two earliest age categories, a few years of stasis, followed by a sharp decline in later childhood. The Dakhleh values fall lower than those at Petra, which may indicate that the individuals at Petra consumed more C_4 resources throughout their lifespan. The $\delta^{18}O_{ap}$ at Dakhleh showed a decrease between dentition forming *in utero* and during breastfeeding and those forming during weaning. The expected pattern at Petra appears to be confounded by other factors not as prevalent at Dakhleh, such as seasonal fluctuations, more substantial supplementation of human by non-human milk, or population mobility. This could be explored further through analysis of the intra-individual data at Dakhleh.

While the other Roman sites only studied weaning through collagen in bone, the fact that the Dakhleh study implemented both dentin collagen and enamel apatite can provide some indication for what the $\delta^{13}C$ collagen pattern would look like at Petra based on the sites' similarities in the $\delta^{13}C_{ap}$ data by age. The Isola Sacra necropolis in Portus, Italy was in use during the Roman Period from the 1st to 3rd centuries A.D. (Prowse et al. 2008). Bone collagen from 37 rib samples from subadults and adults were analyzed for $\delta^{15}N_{co}$ and $\delta^{13}C_{co}$. According to this study, weaning process at Portus began by the end of the first year and finished between two and 2.5 years of age, a slightly shorter period than at Dakhleh and, apparently, at Petra. The $\delta^{13}C_{co}$ values for fully weaned children were still lower than those of adults, likely due to a specialized childhood diet past weaning. The lower $\delta^{13}C_{co}$ values also indicate that this specialized childhood diet was mostly terrestrial-based (C_3 plants).

Queenford Farm cemetery, in Dorchester-on-Thames, Oxfordshire, UK, was in use during the Late/Sub-Roman period between the 4th and mid-6th centuries A.D. (Fuller et al. 2006b). There are an estimated 2,000 individuals in the cemetery, of which 164 individuals were excavated. Bone collagen from ribs and femora of 87 subadult and adult individuals were analyzed for $\delta^{15}N_{co}$ and $\delta^{13}C_{co}$. The lack of individuals between birth and 1.5 years old made it impossible for this study to determine when weaning began, but researchers discovered that supplementary food was introduced by the age of 1.5 years and the weaning process continued until two to four years of age. The lower $\delta^{13}C_{co}$ values during weaning indicate that the weaning diet likely consisted of more cereals than in the adult diet.

The pattern of values for Isola Sacra and Queenford Farm (shown in Figure 7) are very similar. The mean $\delta^{13}C_{co}$ values decrease from 0.0–2.5 to 2.6–4.5, followed by a gradual increase starting at 4.6–7.0 and extending into the adult age groups. Whereas Dakhleh's $\delta^{13}C_{co}$ values decrease between 0.0–2.5 and 2.6–4.5, then increase from 4.6–7.0 only to be followed by a decrease to the adult values. Although Isola Sacra and Queenford Farm do have different patterns from Dakhleh, Dakhleh's $\delta^{13}C_{co}$ pattern is much more similar compared to the site's $\delta^{13}C_{ap}$ pattern. This indicates that differences in age-related shifts seen at Petra versus Isola Sacra and Queenford Farm could result from comparing values derived from apatite and those from collagen, not necessarily from actual differences in foods consumed or shifts related to weaning.

Enamel apatite and bone collagen provide information on slightly different aspects of diet: bone collagen contains carbon from dietary protein, while enamel apatite represents the carbon from the whole diet (protein, carbohydrates, and lipids) (Ambrose and Norr 1993). This leads to a difference in isotopic values, called the apatite-collagen spacing, between the two tissue types, with collagen tending to have lower $\delta^{13}C$ values than apatite (Lee-Thorp et al. 1989). There is no standard difference between the two as it depends on the type of diet and reliance on certain macronutrients, but the intra-individual apatite-collagen spacing tends to remain stable (Loftus and Sealy 2012) and dietary values can be calculated using an enrichment factor (Ambrose and Norr 1993). In this study, dietary values were not used since the purpose of comparing these sites was to determine overall patterns of change. Although analysis of the Kellis 2 skeletons included collagen and apatite analyses on all deciduous and permanent dentition, only the permanent first molars, first premolars, and second molars were used for comparison to ensure that the age ranges matched the ones in this study. The results (shown in

Table 7) of each site’s $\delta^{13}C$ analysis were grouped into the age ranges established earlier in this chapter.

Table 7. Mean and standard deviation of $\delta^{13}C$ values across age ranges for Petra, Dakhleh, Isola Sacra, and Queenford Farm. The data from Dakhleh did not include apatite values reflecting adult diet.

Site	Tissue Type	0-2.5			2.6-4.5			4.6-7.0			Adult		
		N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Petra	apatite	32	-11.6	0.4	22	-11.2	0.5	29	-11.3	1.07	27	-12.9	1.0
Dakhleh[1]	apatite	18	-12.6	0.6	20	-11.7	0.5	11	-11.9	0.5	--	--	--
	collagen	58	-18.7	0.4	11	-18.9	0.6	56	-18.7	0.2	35	-19.1	--
Isola Sacra[2]	collagen	23	-18.5	0.5	8	-19.1	0.3	0	--	--	32	-18.9	0.3
Queenford Farm[3]	collagen	14	-20.0	0.5	16	-20.1	0.3	7	-20.0	0.2	33	-19.7	0.3

[1] Adult female mean used from Dupras et al. (2001). No standard deviation given.
[2] Adult female mean used from Prowse et al. (2004).
[3] Adult female mean used.

Table 8. Mean and standard deviation of $\delta^{18}O$ values across tooth types for Petra (reported as VPDB) and Dakhleh (reported as SMOW).

Site	M1			PM1			M2		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Petra	30	-1.8	0.7	21	-2.2	0.8	29	-1.9	1.3
Dakhleh	18	25.7	0.5	20	25.9	1.1	11	25.4	0.8

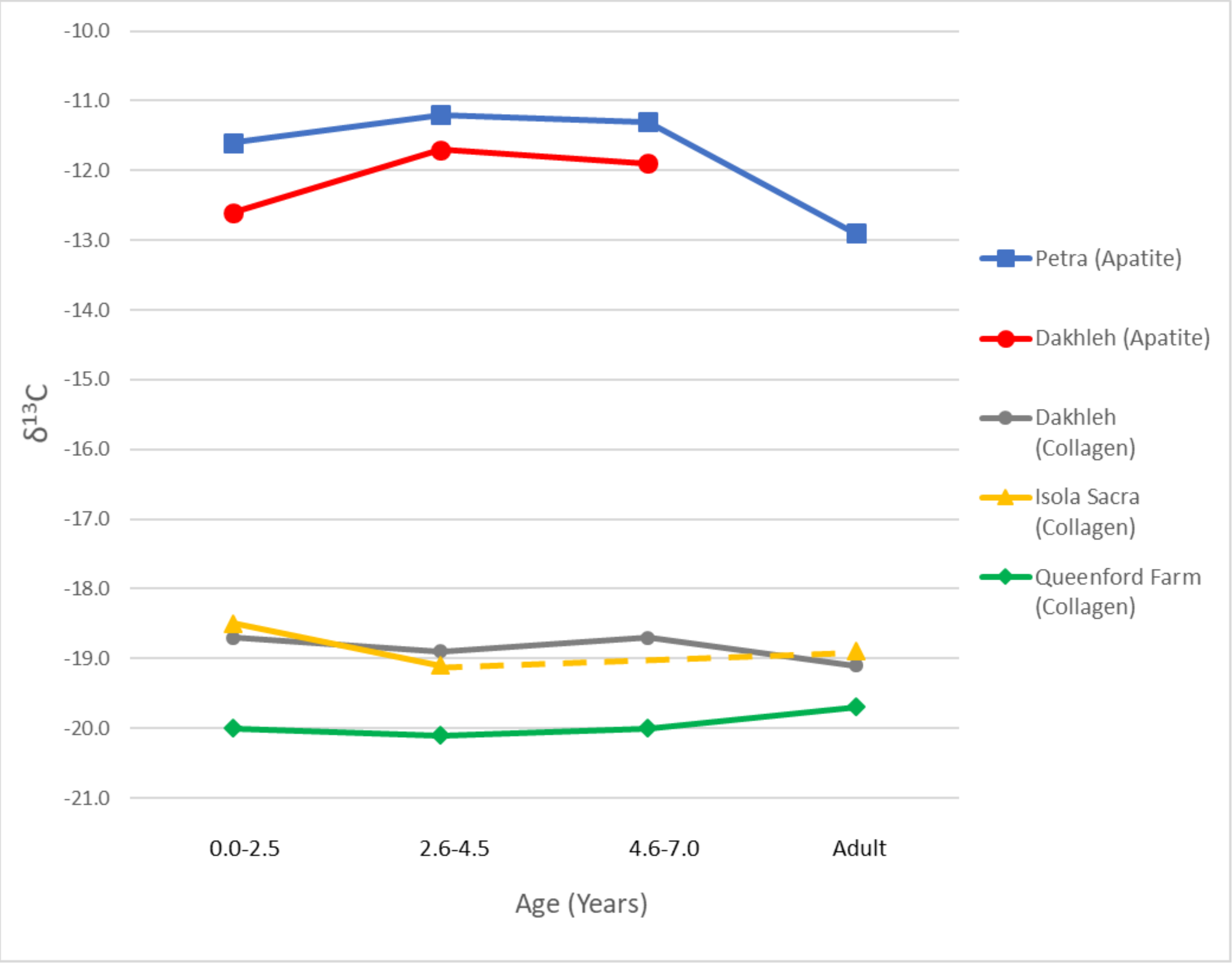

Figure 8. Change in δ^{13}C values by age for Petra, Dakhleh, Isola Sacra, and Queenford Farm.

Despite there being differences between the δ^{13}C values, there does appear to be a similar pattern among the sites (Figure 8) with either an upward or downward shift with age until 2.6–4.5 years, after which the isotope values migrate toward that seen in adults at each site. The patterns at Dakhleh and Queenford Farm were interpreted as cessation of weaning sometime between two to four years of age (Dupras and Tocheri 2007; Fuller et al. 2006b), while at Isola Sacra it was interpreted as cessation of weaning between 2 and 2.5 years old (Prowse et al. 2008). The data from Petra demonstrate a more drastic difference between 4.6 to 7 years and adult $\delta^{13}C_{ap}$ values than these other sites, suggesting that weaning extended past four years of age.

Dakhleh was the only comparative site that also conducted a $\delta^{18}O$ analysis. The results from Dakhleh and Petra's analysis are shown in Table 8. Neither site showed significant differences between permanent tooth classes (Dupras and Tocheri 2007). However, Dupras and Tocheri (2007) found a significant difference between the mean $\delta^{18}O_{ap}$ values of deciduous and permanent dentition. Since deciduous dentition forms during fetal development and the first year of life, it should not be affected by external water sources. Whereas the formation of permanent dentition includes signatures from both breastmilk and external water sources as they form from birth until at least three years of age. This could indicate that the shift from breastmilk to external water sources cannot be fully captured by permanent dentition alone.

Implications for Health at Petra

The overall health and nutrition of children can have long-term effects on adult health. The length of exclusive breastfeeding and appropriate supplementary food during weaning are important determinants of health. Infants who are given solid foods and not being exclusively breastfed before six months are more likely to develop nutritional deficiencies that are highly associated with infant mortality in the first year (Katzenberg et al. 1996; Schurr 1997). Exclusive breastfeeding for at least six months is important as it provides support for an infant's immune system development; it contains immunoglobins, antistaphylococcal factor, and other elements of the immune system to protect infants from pathogens (Katzenberg et al. 1996). Reducing the length of breastfeeding leaves the infant with less immunity and introducing solid foods into an infant's diet introduces new environmental pathogens; this combination can result in diseases and chronic illnesses such as iron-deficiency anemia (Bourbou and Garvie-Lok 2009). Megaloblastic anemia in infants is often caused by consuming goat's milk, which is lower in folate necessary for proper iron absorption, in place of human breastmilk (Dupras et al. 2001). Cribra orbitalia and porotic hyperostosis are common skeletal manifestations of anemia and have a high prevalence in some regions of the Greco-Roman world (Beaumont et al. 2015). Linear enamel hypoplasias (LEH) and Wilson bands, which form in tooth enamel, are also good indicators of potentially weaning-related nutritional stress during childhood (Geber 2014; Prowse et al. 2008).

Since Petra has very few subadult remains, the prevalence of cribra orbitalia and porotic hyperostosis in children potentially related to weaning is unknown. One sample of the predominantly adult sample interred in Tombs B.4 and B.5 (based on 670 frontal and parietal fragments) showed no evidence of these anemia-related conditions (Canipe 2014). The primarily adult sample at Petra also had a relatively low frequency of LEHs (12.4%, based on 621 teeth); most of the ones that were present formed between 2.5 and 4 years of age (Lieurance 2018). This is contemporary with the period that most children at Petra were experiencing a dietary shift based on $\delta^{13}C$ results. As mentioned above, the Petra sample may suffer from mortality bias because it mostly includes adults who survived childhood, and thus were more likely to have had better nutrition. However, lack of a significant difference between the $\delta^{13}C$ values during the critical birth to 2.5 years of age of those who survived into adulthood and those who died in childhood does not support that theory.

The low prevalence of skeletal indicators of non-specific stress within Petra adults may be linked to childhood dietary practices. Extended breastfeeding until two years or more has been shown to lead to less incidents of illness during childhood as breastmilk continues to provide immunological support and nutrients that might not be available from supplementary foods alone throughout the second year (Heinig 2001; Mortensen and Tawia 2013). Lactoferrin, lysozyme, and secretory IgA, the immune components of breastmilk, continue to be produced at the same level throughout breastfeeding (Goldman et al. 1983). The change over time in $\delta^{13}C$ at each site in Figure 7 may indicate that the weaning period extended slightly longer at Petra than was typical for the Mediterranean during the Greco-Roman era. This allowed infants and young children the benefit of their mother's immune responses through extended breastfeeding.

Petraeans might have avoided practices that contributed to the high infant mortality rate found in the other sites. For instance, Soranus and Galen recommended giving newborns a mixture of honey and water for the first few days after birth as they thought colostrum was harmful (Bourbou and Garvie-Lok 2009). Colostrum is enriched breastmilk that the mother produces for a few days after giving birth and has a high concentration of immunoglobins to boost an infant's immune system (Katzenberg et al. 1996). Not only does replacing breastmilk with honey and water take away the immune benefits, but it also introduces contaminants as honey frequently contains *Clostridium botulinum* that can lead to botulism (Dupras et al. 2001; Fairgrieve and Molto 2000). While some Petraeans may have supplemented breastmilk with non-human milk during early childhood, evidence for this does not exist in their skeletal remains (Canipe 2014). It is always possible that, despite the lack of evidence for an infant mortality bias in the sample, children with these conditions perished and are not represented in the sample studied here.

Summary

The general patterns of the $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ values suggest that Petraean weaning practices followed that of contemporaneous Roman populations, suggesting that it, too, had a general weaning period of 6 months to 2–4 years (Dupras and Tocheri 2007). However, breastmilk likely remained a major component for a longer period of time than at the other sites, with $\delta^{13}C_{ap}$ remaining essentially the same through 2.6–4.5 years and 4.6–7.0 years. This indicates that the instructions of Galen and Soranus for weaning to be completed by three years of age (Galen, *A Translation of Galen's Hygiene: De Sanitate Tuenda* 1:9; Soranus, *Soranus' Gynecology* 2:21) was not necessarily followed at Petra. The weaning diet of children at Petra consisted of more C_4 food sources than the diet of the adults, and it may be that the post-weaning diet continued to have a reliance on C_4 consumables. One part of this may be the replacement of breastmilk during weaning by milk from C_4-consuming animals. The absence of significant shifts between any of the tooth types for $\delta^{18}O_{ap}$ values may provide support for non-human milk being implemented as a supplementary food. Although as noted above, in one individual this lack clearly results from childhood mobility; in others it could reflect seasonal variation in drinking water $\delta^{18}O$.

CHAPTER 6: Conclusions

The $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ analyses of the non-elite inhabitants of Petra illuminated overall childhood diet from birth to seven years of age and general weaning patterns. $\delta^{13}C_{ap}$ data indicate a potential for an extended weaning process that was not completed until at least after three years of age as the values of the second molar (which form between three and seven years) are not at the same level of an adult diet. The food sources that supplemented weaning had a higher C_4 input than seen in adults, and it is possible that a specialized childhood diet was implemented for a period after children were weaned and before fully transitioning to an adult diet. The overall $\delta^{13}C_{ap}$ pattern at Petra does show some similarities to the Greco-Roman comparative sites, Dakhleh, Isola Sacra, and Queenford Farms, but Petra's weaning period seems to extend longer than at the other locales. $\delta^{18}O_{ap}$ values were not as useful in estimating the length of weaning in this case as they appeared to be confounded by other factors such as childhood mobility, the use of non-human milk, early implementation of environmental water as a drinking source, or seasonal variability in drinking water $\delta^{18}O$ enrichment.

While the childhood diet has a greater component of C_4 foods than the one consumed by adults, it still appears to be largely based on C_3 food sources with the $\delta^{13}C_{ap}$ values on the higher end of the C_3 spectrum. Millet, a C_4 plant, found at sites in Petra and the surrounding hinterlands (Ramsay and Smith 2013) provided a likely weaning food, similar to the conclusions made about the weaning diet at Dakhleh Oasis (Dupras and Tocheri 2007). Non-human milk being implemented could also explain some of the variation in values as grazing animals could have consumed some of the C_4 vegetation found in Petra and there is evidence of millet being used as fodder at other sites (Dupras and Tocheri 2007).

The eleven individuals with more than one tooth sampled were important in highlighting the variation present in the population. Using only the overall site pattern to interpret the data could blur the finer details that indicate differences in weaning ages and supplementary foods. There is within-individual variability present in both $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ data, suggesting significant heterogeneity in the age that solid foods were introduced, the type of supplementary food used, whether or not non-human milk was also used to supplement breastmilk, and the source of the main drinking water (either within Petra or, in the case of at least one individual, from another region entirely).

The isotopic analyses conducted in this study along with Canipe’s (2014) paleopathological findings can aid in the understanding of childhood, and the overall population’s health. In both studies, there do not appear to be any indications of common periods of nutritional stress across the population, particularly those related to weaning. The lack of cribra orbitalia, the moderately low prevalence of enamel hypoplasias, and little to no isotopic support for nutritional deficiency during childhood all point to a relatively healthy population that might not have had as high an infant and child mortality rate as the contemporaneous Roman sites. It does need to be noted, however, that a lack of skeletal manifestations of pathologies does not necessarily equate to good health as the individual could have died before a bony reaction occurred.

Overall, this study was a step forward in gaining an understanding the lives of the non-elite Petraeans. Although the results were not conclusive on certain aspects of weaning practices or how cultural interactions impacted said practices, it did provide information not previously available on childhood diet.

Future Research and Considerations

One of the main setbacks of this study is the lack of specificity in terms of age associated with each value. This has led to the inability to determine a more exact age for the start and completion of weaning. Sequential micro-sampling of enamel could address this issue. Micro-sampling consists of sectioning enamel longitudinally at 1 mm thickness and then removing samples from multiple locations that represent different ages during the formation of that tooth enamel (Wright 2013). In addition to the ability to have smaller age range per sample, this method is beneficial in that each tooth represents an individual that has multiple isotopic values over time. As the burials in Petra are largely commingled, finding teeth from the same individual for within-individual analyses was difficult; micro-sampling would circumvent that complication. Wright (2013) used this method to determine weaning practices and residential mobility. Fuller (2003) was successful in using microsampling on dentine collagen to investigate breastfeeding and weaning patterns, as was Burt and Garvie-Lok (2013) and Beaumont et al. (2015).

Laser ablation is a technique similar to micro-sampling in terms of being able to take multiple samples representing different ages from the same tooth. The difference between the two is the method in which the sample is removed. Laser ablation implements a focused laser beam to heat up a 0.5 mm^2 area on the enamel surface, which then gives off CO_2 gas (Ambrose 2006; Cerling and Sharp 1996). Carbon and oxygen can then be analyzed from the expelled gas. This has been a popular method for specimens that are rare or small in nature as the laser just barely penetrates the enamel, meaning almost no destruction to the sample. Ambrose (2006) used this method on robust-australopithecine teeth to determine seasonal variations in diet and was able to avoid causing noticeable damage to the specimen. Passey and Cerling (2006) found this to be a successful method when conducting isotopic analyses on smaller mammals as their teeth have less surface area from which to sample.

Another difficulty in determining weaning practices was the lack of $\delta^{18}O_{ap}$ values for adult Petraeans. Since the $\delta^{18}O_{ap}$ values for the first molars, first premolars, and second molars did not follow the expected decrease over age that is consistent with decreasing levels of breastmilk, it could have been useful to have adult values for comparison. This wold enable me to determine which age range represented the largest variation to the pattern.

It would be beneficial if future studies incorporated other isotopic ratios, such as nitrogen ($\delta^{15}N$) and calcium ($\delta^{44/42}Ca$). Adding data from $\delta^{15}N$ analysis to those of $\delta^{13}C_{ap}$ would flesh out the importance of protein in the childhood diet over other macronutrients. It would also make it possible for more complete inter-site comparisons as many of the childhood diet studies implement $\delta^{15}N$ instead of $\delta^{18}O$. However, the poor collagen preservation at Petra (Appleton 2015) could limit results. $\delta^{44/42}Ca$ analysis can provide insight into weaning through enamel apatite as $\delta^{44/42}Ca$ values in mammal milk are significantly different than dietary values and this isotope is not easily affected by diagenesis (Tacail et al. 2017).

The teeth used in this analysis still have enough remaining enamel that further isotopic analyses could be conducted. Having future studies use at least some of the same samples would aid in the interpretations as well as continue to expand on the understanding of non-elite Petraean lives.

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