BREASTFEEDING AND WEANING HISTORIES IN A 19TH CENTURY SPANISH SAMPLE

INDIVIDUAL BREASTFEEDING AND WEANING HISTORIES IN A 19TH CENTURY SPANISH SAMPLE USING STABLE ISOTOPE ANALYSIS OF INCREMENTAL DENTINE SECTIONS

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Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Master of Arts

MASTER OF ARTS (2018) McMaster University
(Anthropology) Hamilton, Ontario

TITLE: Individual breastfeeding and weaning histories in a 19th century Spanish sample using stable isotope analysis of incremental dentine sections

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NUMBER OF PAGES: xi - 159

Abstract

Through stable isotope analysis of human tooth dentine, this thesis investigates breastfeeding and weaning patterns in relation to rickets in a sample of sub-adults (*n*=12) interred in the nineteenth century sub-adult cemetery located at the Church of the Trinitarias in Madrid, Spain. The main objectives of this research are to create early life feeding histories for each individual using dentine serial sectioning techniques and apply these histories to investigate the relationship between breastfeeding, weaning, and vitamin D deficiency. The early life feeding histories allows for the determination of the onset and complete age of weaning at an individual level. These stable isotope data are then used to estimate general trends in breastfeeding and weaning practices in nineteenth century Spain. The results of this study indicate that the onset age of weaning for most of the individuals in this sample was between 10 and 14 months of age. The age at which breastmilk consumption stopped entirely was more difficult to estimate, however, in individuals that did show evidence of completed weaning, it was estimated to occur around the age of three. These ages are slightly higher than those discussed in historical sources for nineteenth century Spain. When these results are compared to contemporaneous, more industrialized, European countries the comparison shows that the initialization of weaning occurred slightly later and was a more gradual process in this Spanish sample. The results also demonstrate that there is no direct relationship between weaning patterns and the occurrence of skeletal vitamin D deficiency. It may be that the high prevalence of vitamin D deficiency is linked with the nuances of breastfeeding and other biocultural variables, such as a lack of sun exposure, an inadequate weaning diet, or childcare practices.

Acknowledgements

First and foremost, I cannot express how grateful I am for the support and guidance of my supervisor, Dr. Tracy Prowse. Stable isotope analysis was a whole new world to me, without her training, patience, and assistance throughout the learning, research, and writing processes, this thesis would not have been possible. As well, I would like to thank my committee members. First, Dr. Megan Brickley for granting me access to the samples used in this thesis and for her guidance on the topic of vitamin D deficiency. Second, Dr. Tina Moffat, whose door was always open when I needed guidance in the area of infant feeding research. I would also like to thank Dr. Sang-Tae Kim for allowing me to use his stable isotope prep lab, and Martin Knyf for his training and banter that was indispensable in helping me understand stable isotope geochemistry. My appreciation also extends to the entire team at the G. G. Hatch lab in Ottawa where these samples were analyzed. Additionally, I would like to thank the Social Sciences and Humanities Research Council of Canada (SSHRC) for supporting this research.

Grad school, unsurprisingly, has not been an easy process, however, thanks to the support of my colleagues and friends in the anthropology department at McMaster, it has been a manageable adventure. Special thanks go out to my friends, Aaron Cathers, Beatrice Fletcher, and Emil Karpinski, whose motivation and assistance throughout the writing process has been invaluable. I also would like to thank my family, specifically my grandma, Joan, and my father, Victor, for their continued support throughout my academic career, and for putting up with my stable isotope rambling that must sound like a foreign language.

Table of Contents

List of Tables

List of Figures

List of Equations

Chapter 1: Introduction

1.1 Research overview

The stable isotope analysis of human bones and teeth has contributed greatly to how we understand the past. Established isotopic methods typically employ bulk analysis of skeletal and dental tissue to provide broad estimates of diet; however, the development of incremental dentine sampling methods has led to more chronologically-refined estimates of dietary change. Based on the stable carbon and nitrogen isotope analysis of tooth dentine in both permanent and deciduous teeth, these methods enable the study of variation in individual feeding histories. This thesis is concerned with the analysis of individual breastfeeding and weaning histories using this method. Understanding infant and young child feeding practices are crucial to understanding children's health in the past. Adequate nutrition and health during the first few months of an individual's life is essential for growth and ultimately survival. Breastfeeding is a common means of providing infants and young children with the nutritional support they require as well as providing them with passive immunity from their mother.

This thesis presents a detailed investigation of the infant and young child feeding practices in a sample from a nineteenth century infantile cemetery located at the Church of the Trinitarias in Madrid, Spain. Initial bioarchaeological analysis of the infants and children buried in the Trinitarias cemetery found a high prevalence of vitamin D deficiency (~60%) (Ríos et al., 2016), making this a unique sample to investigate children's health in the nineteenth century.

The primary objective of this thesis is to investigate the infant and young child feeding practices of individuals buried at the Trinitarias cemetery through stable isotope analysis of tooth dentine. Using the stable carbon and nitrogen values found within the tooth dentine of deciduous first molars and deciduous central incisors, this thesis presents individual histories of the onset age of weaning and the age at which breastmilk consumption stopped. This is possible due to the predictable shift in $\delta^{15}N$ (~3‰) and $\delta^{13}C$ (~1‰) values associated with the consumption of breastmilk and the weaning period (Fogel et al., 1989; Fuller et al., 2006).

The second objective of this thesis is to determine what influence breastfeeding has on vitamin D deficiency in this sample of infants and children from Madrid. While vitamin D deficiency is generally caused by insufficient exposure to the ultraviolet B (UVB) radiation found in sunlight, the amount of dietary vitamin D can play an important role in maintaining sufficient levels, albeit to a lesser extent (Chaplin and Jablonski, 2009, 2013). Data on vitamin D deficiency in the infants and children excavated from the Trinitarias cemetery were collected by the initial archaeological team in charge of the excavation. This thesis compares the findings from the stable carbon and nitrogen isotope analysis with these existing data on vitamin D deficiency, in order to understand the relationship between breastfeeding and weaning practices and vitamin D deficiency. This research tests the hypothesis that there may be a relationship between the variation seen in breastfeeding and weaning patterns, and prevalence of vitamin D deficiency.

This is the first study to apply stable isotope analysis to a nineteenth century Spanish sample of sub-adults, both with and without rickets. Historians have reported on

childrearing practices during this period; however, there is still little information published on the breastfeeding and weaning practices in English-language literature. This stable isotope investigation provides a point of comparison to historical sources and other bioarchaeological studies of weaning from nineteenth century Europe.

1.2 Thesis structure

Chapter two presents the historical context for nineteenth century Spain, before moving to a discussion of the socio-cultural views on women and children during this period. The beliefs and views on breastfeeding, weaning, and childhood diet are also discussed here. Due to interconnected nature of nineteenth century Europe, this chapter looks at trends in breastfeeding and weaning throughout Europe as studied through historical and bioarchaeological analyses.

Chapter three provides a detailed review of the bioarchaeology of breastfeeding and weaning, as well as discussing stable isotope geochemistry and its application to bioarchaeology. The chapter opens with a discussion on the importance of studying breastfeeding and weaning in the past. Then a review of the bioarchaeological and stable isotope methods of studying weaning through the analysis of bone and teeth is provided.

Chapter four is the final background chapter. It is a detailed discussion on vitamin D deficiency, both through clinical and bioarchaeological literature. The chapter includes an overview of the relationship between vitamin D and breastfeeding, as well as a review of the methods and criteria used to diagnose vitamin D deficiency in a bioarchaeological context.

Chapter five outlines the methods used in this thesis, including the microsampling of tooth dentine and the extraction of dental collagen. This chapter also provides the details on the Trinitarias cemetery and the sample of individuals studied in this thesis. Chapter six presents the stable isotope results from the dentine microsamples. Included in this chapter are also the individual isotope profiles for each of the individuals sampled. Finally, chapter seven discusses and interprets the stable isotope data presented in the previous chapter. The breastfeeding histories of the individuals are discussed alongside the historical information on nineteenth century European/Spanish customs. As well, this chapter presents the integration of stable isotope data with information on vitamin D deficiency in this sample and interprets the evidence within the biocultural context of nineteenth century Spain.

Chapter 2: Social and Historical Context

2.1 Introduction

In order to contextualize and begin to understand the process of weaning in nineteenth century Spain, stable isotope results must be considered alongside historically-informed social and cultural understandings of breastfeeding and weaning. While lactation and weaning are undoubtedly biological processes, both practices are also cultural (Stuart-Macadam, 1995). The views on, and practices of, breastfeeding vary over time and in different socio-cultural contexts. This chapter will provide the social and historical context in which the isotopic results will later be interpreted. A brief history of nineteenth century Spain is presented to understand the political context of the time period and region. The remainder of the chapter is dedicated to examining the social and cultural views on breastfeeding and weaning in western Europe and Spain specifically. The role of women and children in nineteenth century Spain will also be discussed as it is an important factor when studying breastfeeding and weaning.

While nineteenth century Spain has been subject to numerous historical studies, there has been little archaeological research. Historians have been able to create a rich historical account of nineteenth century Spain due to the abundance of writing from the time period. However, because Spain was in such a dynamic state during this time, the written sources from the nineteenth century tend to focus on political conflicts, specifics of war, and changes in legislature. Currently, there is little writing on day-to-day life, rather, the social changes that are documented in historical sources are ideals shown through

constitutional changes. Through bioarchaeological and archaeological study, the nuances of daily life can begin to be explored.

Year	Important Event	Source
1808	King Ferdinand is forced out of Spain by Napoleon, Joseph Bonaparte assumes the throne.	Cowans, 2003
1810	Cadiz *Cortes* is formed.	Cowans, 2003
1812	The Cadiz *Cortes* drafts a constitution.	Cowans, 2003
1814	King Ferdinand returns and abolishes the constitution formed by the Cadiz *Cortes.*	Esdaile, 2000
1820	King Ferdinand returns constitutional power.	Esdaile, 2000
1823	Under King Louis XVIII, Ferdinand is returned to absolute power.	Herr, 2000
1830	King Ferdinand's daughter, Isabel II, is born. A female heir to the throne.	Herr, 2000
1833	King Ferdinand passes away, his wife assumes the throne until Isabel II is of age. The Carlist movement begins.	Cowans, 2003
1841	Maria Cristina, the Queen, is forced into exile. Espartero is appointed to the throne.	Carr, 2000
1843	Isabella II turns 13. Espartero leaves Spain. Isabella II is appointed Queen.	Carr, 2000
1845	The constitution of 1845 is introduced by the Moderates under Narvaes.	Vilar, 1977
1854	Progressive revolution led by O'Donnell. A new constitution is drafted but never implemented.	Carr, 2000
1856	Moderates are effectively back in power.	Esdaile, 2000
1868	The Glorious Revolution. A military uprising under Generals Prim and Serrano. The Queen is forced to flee.	Cowans, 2003
1870	Amadeo of Italy accepts the throne. The same day, Prim is murdered.	Vilar, 1977
1873	Amadeo flees Spain, in response, the first Spanish Republic begins.	Cowans, 2003
1874	With aid from the military, the Republic is overthrown. Alfonso XII becomes King.	Carr, 2000
1876	The constitution of 1876 is written and introduced.	Carr, 2000
1876	The political system, soon known as the *turno pacifico* is introduced.	Esdaile, 2000
1885	Alfonso dies, his wife becomes Queen while expecting a son.	Vilar, 1977
1892	Universal male sufferage is introduced.	Carr, 2000

Table 2.1: A detailed time-line of key events that occurred in Spain during the nineteenth century.

2.2 The shifting political landscape of nineteenth century Spain

Spain experienced a great deal of political and social dynamism throughout the nineteenth century. Over the course of this century, Spain' political system shifted from an absolute monarchy to a constitutional monarchy, providing the framework for the parliamentary monarchy that Spain follows today. This section of the chapter will briefly highlight crucial events that shaped nineteenth century Spain. Table 1 provides a timeline of key events that occurred throughout the century.

During the eighteenth, and into the nineteenth century, the Bourbon family ruled over Spain (Esdaile, 2000). While the nineteenth century began as a prosperous period for Spain, matters quickly changed. Spain suffered a great loss in 1805 at the Battle of Trafalgar, a navy battle between combined French and Spanish forces and the British Navy (Esdaile, 2000). Shortly after this loss, Napoleon Bonaparte, the French Emperor, invaded Spain and the country came under French rule in 1808 (Cowans, 2003). His brother, Joseph Bonaparte, was then appointed to the throne, but the people of Spain did not recognize Bonaparte as king (Cowans, 2003); their reaction sparked the myriad of political changes that occurred during the remainder of the century.

The French rule of Spain lead to revolts by the Spanish citizens, resulting in the formation of local *Juntas*, small committees dedicated to the ousted king of Spain (King Ferdinand) (Cowans, 2003). According to Esdaile (2010), areas of Spain unoccupied by the French then came together forming a central *Junta* and, by 1810, the Cadiz *Cortes*. The Cadiz *Cortes* was a parliament formed in the city of Cadiz to govern sovereign Spain, marking the beginning of the liberal movement (Herr, 2000). By 1812 the *Cortes* wrote a

constitution that stated the returning king would have limited power, and the elected *Cortes* officials would ultimately have control (Cowans, 2003). After a defeat by the British, Napoleon allowed King Ferdinand to return to the throne in 1814 (Esdaile, 2000). Cowans (2003) outlines that once the king returned he immediately abolished all the work of the *Cortes*, re-established the absolute monarchy, which threw Spain into six years of economic and social stagnation.

In 1820 Spain experienced the first *pronunciamientos*, or military-driven coups, setting the precedent for the remainder of the century; the goal of which was to ensure the return of constitutional order, in hopes of bringing back a more liberal Spain (Cowans, 2003). Spain then returned to following the constitution of 1812, however, this was short lived. Aided by the French military, King Ferdinand assumed absolute power once again in 1823 and after a bleak decade of absolute rule he died in 1833, leaving his daughter as the rightful heir to the throne (Esdaile, 2000). As his daughter was still young, Ferdinand's widow, Maria Cristina, was appointed queen until his daughter came of age. Soon after coming to power, Queen Maria introduced the Royal Statute that created a new form of legislature for Spain (Burdiel, 1998). Carr (2000) notes that following this, three political parties were established, the Progressives, the Moderates, and the Liberal Union. There were a number of *pronunciamientos* between these three parties over the next 35 years, but none of these resulted in sweeping social or economic change (Carr, 2000; Vilar, 1977).

The country remained under the parliamentary rule of these parties, with Queen Isabella II (Ferdinand's daughter) on the throne until 1868, the year of the 'Glorious Revolution' (Carr, 2000). Following the Glorious Revolution and the short-lived rule of

Amadeo of Savoy as the newly appointed king, Spain entered a short-lived Republic period after the new king fled (Carr, 2000). After one year, the republic fell, making way for the Restoration Monarchy. Alfonso XII (the son of Isabella) was appointed the new ruler of Spain and the *turno pacifico* was introduced, a two-party system that worked by alternating periods of power of the two main political parties, the Liberals and the Conservatives, ensuring a peaceful transition after each period in office (Carr, 2000). The *turno pacifico* system stayed in use until the early twentieth century.

2.3 Women and children in nineteenth century Europe

Having presented the political landscape of nineteenth century Spain, the roles of women and children will now be discussed. Traditionally, historical writings have provided an androcentric view of the past, focusing on the history of men; while there has been a recent increase in feminist historians and a focus on the history of women, there is still little written on women in nineteenth century Spain (Lerner, 1979). To provide enough context to situate this study, women and children will be looked at in Western Europe as a whole before the context of Spain is discussed.

Throughout nineteenth century Europe the divide between the roles of men and women became increasingly distinct; men were defined as belonging in the public sphere as the breadwinners, while the women's sphere of activity was expected to be the household (Clark, 2008). There are two key events that can be attributed to the marked divide of gender roles: the Industrial Revolution (ca. 1760 – 1840), and the French Revolution (1789 – 1799). While the Industrial Revolution began in Britain at the end of the eighteenth century, by the mid-nineteenth century much of western Europe (Belgium, France, and

Germany) was moving towards industrialization. While industrialization did occur in Spain, the extent of it was minimal in comparison to other European countries (Stearns, 2007). Home and work, while previously synonymous, were now growing further apart as production moved from the home to the factory (Fuchs, 2005). Men dominated the industrial labor force, while women were expected to act as homemakers (De Vries, 1994). Young single women were an exception. While it was less common for married women to engage in factory work, Scott and Tilly (1975) report that unwed women often worked in textile manufacturing and domestic service. However, by the mid-nineteenth century, it was expected that men were to work in the factories, and married women were to stay and care for the home (De Vries, 1994). Fuchs (2005) explains that despite the beliefs held at the time, the delineation of homework and factory work for nineteenth century European women was not so clear. With industrialization, women engaged more in low-paying factory work, alongside work at home, to support their families when the household incomes were not enough. Despite the involvement of married women in factory work, men were still perceived as the bread-winners and women as the homemakers due to a large pay gap between men and women.

The French Revolution also sparked social and political change across Europe. In the first year of the Revolution, the *Declaration of the Rights of Man and of the Citizen* was passed and spread throughout Europe, proclaiming the equality and universal rights of all men (Clark, 2008). Women, as 'passive citizens', were excluded from universal rights, suffrage, and citizenship in this declaration, which caused a surge of female-led protests throughout the Revolution (Fuchs, 2005; Dalton, 2001; Desan 1989).

In post-revolutionary Europe, the extreme delineation of gender roles was visible in both social beliefs and in the legal codes. In both instances, women were assumed to reside in the private sphere, while men occupied the public sphere. Such ideas were backed by the medical and scientific communities, arguing that such a divide was ingrained in the 'nature' of men and women (Clark, 2008). This resulted in women being excluded from politics and other public matters and limited to domestic work. Such divides manifest in the drive for marriage in the nineteenth century. Marriage was considered first and foremost an economic union, and it was viewed as the one way a woman could ensure economic stability (Fuchs, 2005). Marriage, however, also stripped a woman of her rights; single women had more rights and liberty than married women, but they risked a life of poverty due to minimal opportunities for work (Fuchs, 2005). These social beliefs were not solely propagated through the scientific and medical communities; the church also played a crucial role due to its power throughout Europe (Scott and Tilly, 1975).

During the nineteenth century views on children were shaped by the patriarchal ideals of the century. According to Clark (2008) male children were viewed as future citizens, workers, and soldiers, and the education they received reflected this. Schools were set up to teach young boys to become military leaders, while it was not until the late nineteenth century that schools for female children started to become more common (Clark, 2008). Regardless of the views on education, at the end of the eighteenth and beginning of the nineteenth century, children were often an essential part of the labor force. During much of the Industrial Revolution, children worked in factories and mines to help support their families (Stearns, 2007). Prior to this, although children worked in the domestic

sphere from a young age, the Industrial Revolution brought forth the exploitation of child labour (Horrell and Humphries, 1995; Stearns, 2007). Within the first few decades of the nineteenth century, stemming from both an advance in technology and ethical concerns, child labour laws begin to arise and child labour itself began to decline (Stearns, 2007).

2.3.1 Women and children in Spain

Similar to the rest of Europe, there were clear expected gender roles in nineteenth century Spain, segregating women to the domestic sphere and men into the public sphere (Beltrán Tapia and Gallego-Martínez, 2017). In this strongly male-oriented society, women were socially and legally subordinate to men; they were unable to participate in politics and would face jail-time for disobeying their husbands (Shubert, 1990). This inequality was expressed in the daily lives of men and women. Borderias and colleagues (2010) note that men were viewed as harder working and thus received more food, and food of a higher nutritional and caloric quality than women. It was argued that the work of women was not labor intensive, thus they were able to survive on the remaining food scraps (Borderias et al., 2010).

Single women in Spain, as in much of Europe, had different rights and privileges than married women. A single woman of legal age was able to carry out her own business, sign contracts, and own property; all of which was taken away upon marriage (Shubert, 1990). However, women who were able to engage in the workforce made significantly less money than men, thus marriage was often an economic necessity (Borderias et al., 2010). According to Shubert (1990), the percent of unmarried individuals in Spain dropped significantly over the nineteenth century; the amount of unwed men throughout Spain

dropped from 12% to 6% from 1787 to 1900. This increase in marriage rates throughout nineteenth century Spain was due to the economic incentives they offered, thus there were few benefits of remaining single.

The situation for children was hardly different from adults. The education system trained female children in "domestic competence" (Shubert, 1990: 36), in contrast to literacy skills and preparation for higher education for male children. Female children were also limited in their overall access to education. While there was a sufficient number of schools in place throughout Spain, the majority of them were strictly boys' schools; girls' schools were significantly less common (Sarasua, 2002). In rural areas, schooling would often not be available where it was more common for female children to engage in work to assist the family.

2.4 Breastfeeding and weaning in nineteenth century Europe

While the biological basis for breastfeeding and weaning will be discussed in more detail in Chapter Three, the remainder of this chapter will examine the social context of breastfeeding and weaning in nineteenth century Europe. The different nineteenth century social beliefs that were propagated through the medical, scientific, and political communities provide an important context for the isotopic investigation of breastfeeding, weaning, and childhood diet.

Like all mammals, human females are physiologically adapted to lactate and breastfeed their young. Breastmilk is able to provide newborn infants with the nutrients they require along with immunity support and the bacteria required to develop the infant gut microbiome. The duration of breastfeeding is greatly variable between species; this can

be attributed to a number of variables, including body size and sociality (Sellen, 2007). While breastfeeding is a biological necessity, in the case of humans, culture also plays a significant role in the practice of breastfeeding, influencing the duration, the use of non-breastmilk foods, and whether children are breastfed or not. To demonstrate the unique influence of culture on breastfeeding practices, studies have attempted to estimate the biological age at which humans should be weaned. Humans, when compared to other primates, begin weaning at a young age, especially when considering birth weight and gestation length. Compiling data from a number of studies, Dettwyler (1995) demonstrates that the 'natural' (eschewing the influence of culture) age in which humans would wean their children, is between 2.5 and 7 years of age. This is based on the application of certain 'rules' used when examining age of weaning in non-human primates, including eruption of the first molar, quadrupling of birth weight, and adult body size. While still possessing considerable variation, children are often weaned younger than this, between 1 and 4 years of age (Dettwyler, 1995). The remainder of this section will look at the variation in breastfeeding practices throughout nineteenth century Europe.

After the Industrial Revolution, there was a good deal of variation in breastfeeding practices in nineteenth century Europe. Wet nursing, the practice of having a woman breastfeed another's child, had peaked in Europe in the century prior; however, by the nineteenth century it was likely uncommon but still practiced (Stevens et al., 2009). In some northern areas of Europe, such as parts of Germany, Finland, and Sweden, it was common for children to be fed cow's and sheep's milk instead of breastmilk altogether (Fildes, 1995). Throughout other areas of Europe there is no universal breastfeeding model,

rather, historical accounts show that children were fed maternal breastmilk, by a wet nurse, or through artificial feeding (Fildes, 1995). These, however, are not mutually exclusive, children would likely be fed by a combination of these methods early in life. There are two variables that had an influential effect on the feeding practices of children: socio-economic status and community setting (rural/urban). Both variables influenced the kind of work mothers engaged in, which likely had the greatest influence over infant and childhood feeding practices (Fildes, 1995).

During and after the Industrial Revolution, it became more common for women living in urban areas to engage in work outside the home. While this went against the cultural ideal of women as the homemaker, at times it was necessary if the husband's income was insufficient. In these settings, breastfeeding rates were lower as mothers had to be in the factories or mills for long periods, limiting their ability to breastfeed. It is suggested that by the end of the nineteenth century, the percentage of children being breastfed in such areas was as low as 50% (Fildes, 1995:107). In areas where breastfeeding was not possible due to work, mothers turned to artificial feeding of animal milk or cooked cereals, however, mortality rates were significantly increased (Nitsch et al., 2011). Some workplaces, however, did introduce nurseries at the workplace which allowed women the opportunity to breastfeed their children during the work day (Fildes, 1995). In rural areas, it was much more common for children to be exclusively breastfed within the first year of their life (Fildes, 1995). Again, these feeding practices were not mutually exclusive, as mixed feeding was often practiced.

Throughout Europe the duration of breastfeeding was highly variable, based on geography and individual situation. Fildes (1995) estimates that throughout nineteenth century Europe, children were gradually weaned off breastmilk between one and two years of age (Fildes, 1995). In a bioarchaeological study of weaning age and enamel hypoplasia in a nineteenth century Italian sample, the onset age of weaning was estimated to be around one and a half years, consistent with historical data (Moggi-Cecchi et al., 1994). In England and other highly industrialized urban areas, the age of weaning would often be earlier to accommodate the need to work (Newman and Gowland, 2017; Fildes, 1995). Henderson and colleagues (2014) present a stable isotope investigation into the age of weaning in eighteenth and nineteenth century London, yielding results in-line with these general patterns, concluding that children were beginning to be weaned off breastmilk by around six months of age. Analysis of historical literature shows that even breastfeeding children could not escape the gender inequality of the nineteenth century. In some cases, it was recommended by medical practitioners that male children be breastfed longer than females – resulting in increased female infant mortality (Borderias et al., 2010; Fildes, 1995).

2.4.1 Breastfeeding and Weaning in Spain

While there is some information about general patterns of breastfeeding and weaning in the whole of Europe, there is comparatively little discussion about the specifics of Spain. While in some regions of Europe artificial feeding was common, in Spain, breastfeeding was widespread and most prevalent (Reher and Gonzalez-Quifiones, 2003; Reher et al., 1997). Data on infant mortality throughout the nineteenth century in Spain show reduced mortality in the first year of life, which points to both the widespread

practice of breastfeeding and a potential introduction of complementary foods at around one year (Ramiro Farinas and Sanz Gimeno, 2000). The Industrial Revolution had less of an impact on nineteenth century Spain which resulted in a lower female workforce, more mothers at home, and a reduced divide between rural and urban infant and child feeding practices (Martínez-Carrión, 2016). Since Spain's female labour participation was considerably lower than countries such as England, Spanish mothers were likely afforded the ability to breastfeed their own children (Reher, 2001).

There is no historical information on precisely what age the weaning process began throughout nineteenth century Spain. Again, due to the less industrialized nature of Spain, the initiation of weaning would likely have been slightly later than other European countries (Reher and Gonzalez-Quifiones, 2003). There is also the possibility of gender differences in weaning practices, as it was common for males to be breastfed more often, and for longer, than females following the gender divide present throughout the country (Borderias et al., 2010). Reher and colleagues (1997) note that by the latter half of the nineteenth century it was standard to breastfeed an infant until six to 11 months, after which supplemental foods were introduced; however, no information is provided on the age at which weaning was complete.

Near the end of the nineteenth century, Spain saw an increase in artificial feeding following the introduction of infant formula. While introduced to combat the high infant mortality of the 1800s, formula did not catch on and become widespread until the early twentieth century (Ramón and Enrique, 2008). When women chose not to breastfeed, or due to circumstances, could not breastfeed, wet nursing was a solution. Similar to patterns

seen in other parts of Europe, the hiring of a wet nurse was a sign of high socio-economic status, the practice even being promoted by doctors at the time (Ragan, 2004; Bergmann, 2000). Most wet nurses came from rural areas or the lower working class as they were able to receive fair pay for their work (Ragan, 2004). There was, however, an active campaign against wet nursing in nineteenth century Spain, creating a stigma around wet nurses as immoral and unhygienic (Regan, 2004; Bergmann, 2000; Sherwood, 1991). This stigma was most prevalent concerning the wet nurses hired to breastfeed abandoned children as they were paid less than the wet nurses hired by the upper-class families (Bergmann, 2000; Sherwood, 1991). This stigma is more a reflection of the blatant classism present in the nineteenth century in contrast to a true critique of wet nursing. By the end of the nineteenth century, while wet nursing was still practiced, medical campaigns targeted at reducing infant mortality promoted that only maternal breastmilk could ensure a child's health (Ramón and Enrique, 2008).

2.5 Conclusions

The nineteenth century in Spain, and more broadly in Europe, was a truly dynamic period. The century prior saw the birth of industrialization and the French Revolution, which would change the face of the continent. While Spain was not affected by the Industrial Revolution to the same extent as other European countries, it was still present. The gender divide grew as men were expected to fill the role of bread-winners and married women were expected to remain in the domestic sphere. While married women did engage in factory work, their rates of participation in the labour force were relatively low, compared to men and young single women.

While the topic of breastfeeding in the greater context of Europe has received study from historians, demographers, and archaeologists, Spain has not received as much attention in the English-language literature. We do know that breastfeeding was more widespread in Spain due to the less industrialized nature of the cities, so complementary foods were likely introduced slightly later than in the rest of Europe. Following the gender inequalities present in nineteenth century Spain, it was common practice that male children were breastfed longer than females. Artificial feeding was not a widespread practice in Spain, however, wet nursing was relatively common among individuals of a high socio-economic status. What little information there is on breastfeeding in nineteenth century Spain does comes from historical sources, with no studies directly addressing the topic. There is a need for further bioarchaeological investigation to shed new light on the topic and to mitigate the potential biases of historical writings.

Chapter 3: The Bioarchaeology of Breastfeeding and Weaning

3.1 Introduction

Biological anthropology and bioarchaeology alike have a long-standing interest in studying weaning behavior among humans and non-human primates due to its importance in infant survival and population growth. There is a great wealth of information that can be gathered about populations, modern or past, through the study of weaning. The study of weaning and infant feeding is of great interest to bioarchaeologists and has become a widely studied area of bio-anthropological inquiry. Recent advancements in the field have introduced high resolution studies of individual weaning patterns and early life histories through serial analysis of tooth dentine. Since these advancements, patterns and timing of weaning can now be studied and understood at an individual level through longitudinal data, in contrast to using single-point data from multiple individuals to infer the weaning pattern.

This chapter will detail the methods used by bioarchaeologists to investigate weaning in the past. Specifically, the different isotopic methods used to infer patterns of infant feeding and weaning will be discussed, as well as how analyses have shifted from large-scale studies to a focus on individual variation in weaning. Before detailing the methods used to study weaning, a review of why bioarchaeologists are interested in studying weaning in the past will be provided to contextualize the methods discussed. This chapter will also provide an overview of the methodological basis of stable isotope analyses and stable isotope geochemistry.

3.2 Definitions and terminology

There are issues and inconsistencies in the terminology used by anthropologists studying weaning, thus it is important to define a number of terms before moving forward (cf. Dettwyler and Fishman, 1992). First, weaning is understood as a complex and multifaceted process, as opposed to a single event. The weaning process begins with the introduction of non-breastmilk foods and ends with the total cessation of breastmilk consumption (Herring et al., 1998). Non-breastmilk foods, also known as weaning or complementary foods, refer to any foods other than breastmilk consumed by a child during the weaning process (Sellen, 2007). Further, there have been issues in defining age categories among subadults. Throughout this thesis an infant refers to an individual from birth to one year of age, while a child refers an individual from one year of age to puberty (Scheuer and Black, 2000). Halcrow and Tayles (2008) break this down further, with early childhood referring to one to five years of age.

3.3 Infant feeding and childhood

3.3.1 Infant feeding and infant health

During the formative years of childhood, diet has an immense effect on overall health, growth, and survival. Immediately after birth, infants are in a state of rapid growth and have increased nutritional requirements in the first six months of life (Lewis, 2007; Scott and Halcrow, 2017). This is why breastmilk is so crucial for infant survival, especially when water sources are not safe or infant formula is not accessible. Human breastmilk provides infants with essential nutrients that fuel their growth and development

during this initial period, and also provides infants with passive immunity (discussed below). Due to the composition of breastmilk, infants are able to survive solely on breastmilk for the first six months of life (Jay, 2009; Van Esterik, 2002). The timing of exclusive breastfeeding does not universally follow a rule; rather, cross-culturally, the duration of exclusive breastfeeding can vary dramatically (Howcroft, 2013; Jay, 2009). According to Filteau (2000), it is also during these formative months that human infants are at the highest risk for disease, because their immune systems are not well developed (Filteau, 2000). By consuming breastmilk exclusively, infants are avoiding any potential contaminants in non-breastmilk foods that can lead to disease and an increased risk of mortality (Filteau, 2000). Between the importance of breastmilk in providing infants a large portion of their required nutrients, and the role of breastfeeding in protecting infants from contamination, it is clear why an increased understanding of breastfeeding is crucial.

The importance of breastmilk to infants does not end with its crucial role in supplying essential nutrients for growth and development. Breastmilk also contains a suite of immunological factors that act to protect infants from bacterial, parasitic, and other infections during their first few months, commonly referred to as 'passive immunity' (Miller, 2018; Palmquist, 2018; Scott and Halcrow, 2017). Goldman (1993) presents three factors of breastmilk that aid in supporting infant immune systems; antimicrobial agents, anti-inflammatory agents, and other immunomodulating bioactive compounds. It is due to the passage of these agents from the mother that breastfeeding infants and children have a passive immunity to a number of pathogens, including *Salmonella* and *Escherichia coli*. Further, the fats and carbohydrates within breastmilk have unique properties that aid in the

maturation and development of the infant gut microbiome (Goldman, 1993; Miller, 2018). These nutrients protect infants from diarrheal infections, one of the leading causes of infant mortality in developing countries (Filteau, 2000). Both in terms of nutrition, and immune-support, the importance of breastmilk for infant and childhood survival is evident.

While breastmilk is able to exclusively provide the nutrients and immuno-support to infants, this can only be maintained for around the first six months of life (Humphrey, 2010; Jay, 2009; Sellen, 2007). After this period, the nutritional demands of infants grow and change from what can be provided solely by breastfeeding. This results in the need for complementary foods to be introduced into the diet. However, as mentioned previously, incorporating other foods during early life, puts the infant at risk for disease due to the potential of food contamination, particularly in areas of poor sanitation (Filteau, 2000). Because weaning foods have the potential to harm infant and young children, it may seem beneficial to continue breastfeeding exclusively to prevent exposure to new, and possibly harmful foods. However, doing so would then inhibit growth and development, as the individual would no longer be receiving the nutrients its body requires (Katzenberg et al., 1996; Lewis, 2007). This results in what Rowland and colleagues (1978) refers to as the weanling's dilemma, the situation where continued breastfeeding will slow growth, but introducing complementary foods dramatically increases the risk of disease.

Studies of infant morbidity and mortality in past populations can be greatly informed by the incorporation of weaning studies. Because the cessation of exclusive breastfeeding, and in turn, the introduction of weaning foods, is accompanied by a suite of dangers for infants and young children, the beginning of the weaning process causes a great

deal of stress. This may manifest itself in the skeleton through the presence of stress markers, such as enamel hypoplasias (Jay, 2009). It is, however, important to note that weaning is only one potential cause for indicators of stress, rather the entire micro- and macro-environment of the infant must be considered (cf. Katzenberg et al., 1996:180). Due to the increased susceptibility to disease during weaning, it is generally correlated with an increase in infant/childhood mortality in archaeological populations where sanitation standards were low, formula might not have been known or accessible, or medical care was unavailable (Dittmann and Grupe, 2000; Katzenberg et al., 1996). For example, through a correlation of infant age-at-death, stress markers, and weaning estimates, Dittmann and Grupe (2000) show that weaning was likely the cause of increased infant mortality and morbidity at the Medieval (500 – 700 AD) German site of Wenigumstadt. This shows how archaeological studies of weaning can lead to more well-rounded and further contextual understanding of infant health and death in the past.

The effects of infant feeding practices and weaning do not stop after infancy, rather, they continue to influence individuals throughout their lives. The effect of infant and childhood nutrition is a major factor influencing adult health and development, a concept now known as the Developmental Origins of Health and Disease (DOHaD) (Grummer-Strawn et al., 2014). Jwa and colleagues (2014) provide an example of this in their longitudinal study of breastfed, formula fed, and mix fed Japanese children. Their results show that adolescent obesity is significantly less common in breastfed individuals (Jwa et al., 2014). Wilson and colleagues (1998) show similar findings in their analysis of breastfeeding practices in Dundee, Scotland. They found that by extending the exclusive

breastfeeding duration beyond four months, children were more likely to experience health benefits into adulthood (Wilson et al., 1998). The study of the long-term effects of breastfeeding is complex. To understand them it is important to engage with as many breastfeeding variables as possible, such as duration, the timing of complementary food introduction, and presence of mixed-feeding (i.e. the combined feeding of breastmilk and infant formula or other animal milks) (Thompson, 2012). As studies of weaning develop, both in modern and archaeological populations, the understanding of how infant feeding practices influence infant and even adult health grows. Understanding the factors behind infant morbidity and mortality is essential if we hope to understand demographic patterns of past populations.

3.3.2 Infant feeding and population demographics

Having looked at the effect of infant feeding patterns on childhood health and mortality, the topic of population demography can be discussed. One particularly daunting task ascribed to bioarchaeologists is that of recreating demographic profiles of past populations through the study of archaeological demography. Andrew Chamberlain defines paleodemography as "the investigation of the structure and dynamics of past human populations using the broad spectrum of evidence provided by the traces of human activities and remnants of material culture in the archaeological record" (2009:175). While this field of study is immensely complex and often relies on a multidisciplinary approach, it is important to discuss how the study of weaning and infant feeding practices can contribute to understanding paleodemography as there are a number of factors associated with weaning that have an influence on demographics.

As discussed previously, how and when a child is weaned, and what makes up the weaning diet, has a profound effect on infant morbidity and mortality – two key factors in understanding population demography. Creating demographic profiles of ancient populations is far from a simple task, with the field continually being critiqued for its methods and theoretical frameworks (cf. Bocquet-Appel and Masset, 1982; Petersen et al., 1975; Wood et al., 1992). While weaning studies do not provide answers to all of these critiques, they are able to shed light on particular areas of paleodemography. One such issue that paleodemographers face is understanding age-specific mortality distributions in populations (Schurr, 1998). Schurr (1998) proposes that isotopic studies of weaning may be used in unison with paleodemographic measures of fertility and paleodietary studies in order to contextualize and better understand mortality patterns, particularly surrounding infant mortality.

Another area in which studies of weaning can contribute to the paleodemographic conversation is through better understandings of the inter-birth interval. By studying the human inter-birth interval, bioarchaeologists can begin making inferences about fertility in the past. Weaning has a clear relationship with fertility. Due to the hormones produced during lactation and the stimuli from the physical act of suckling, a woman is less likely to become pregnant while breastfeeding (Jay, 2009). The duration of breastfeeding then has a direct effect on fertility and birth spacing. Longer periods of breastfeeding result in decreased fertility and longer birth spacing, while short breastfeeding periods can lead to increased fertility as the inter-birth interval is reduced (Schurr and Powell, 2005). Tsutaya and colleagues (2014) provide an example of how stable isotope weaning estimations can

be used in broader discussions of population fertility. Their study of seventeenth century Japan showed that the age of complete weaning was 3.1 years, thus fertility would have been relatively low especially in comparison to other contemporary societies around the globe. The authors were then able to contextualize their findings with other historical studies on fertility in Edo period Japan, where low fertility rates were hypothesized in rural areas (Tsutaya et al., 2014).

The effects of breastfeeding on mothers extend beyond the suppression of ovulation. The maternal investment associated with childbirth does not end with physically birthing a child, rather, mothers are under constant stress throughout the breastfeeding period. Lactation puts a significant metabolic demand on mothers, with few systems in place to buffer such demands (Sellen, 2007). Prolonged breastfeeding can drain mothers of their nutritional and energetic stores (Veile and Kramer, 2018). In terms of caloric demands, Lee (1996) estimates that a mother must consume 1.3 times her normal, non-pregnant, caloric intake during periods of breastfeeding. While this may be low among other primates, it is still a substantial demand on the mother.

Bioarchaeological measures of weaning are uniquely valuable in understanding weaning in situations where historical or written records are unavailable. When studying prehistoric populations, as bioarchaeologists are often tasked with, skeletal signs of weaning are the only means of understanding such patterns. However, these methods also hold value when studying historic populations. One common issue in studying historical accounts of the past is the biases of those writing them, specifically, those written by upper class males. Skeletal measures of weaning, either inferentially or isotopically, provide a

means of addressing this issue. Data gathered from these methods can be examined alongside and compared to historical data. This allows researchers to examine similarities and discrepancies between the two sets of information, providing a multifaceted analysis of weaning and infant feeding patterns.

3.3.3 Infant feeding and weaning through a biocultural lens

Weaning, and infant feeding practices in general, are culturally mediated processes. Understanding weaning through a biocultural framework can provide an insight into the social life surrounding infant feeding. A biocultural framework stresses the importance of applying both biological and cultural questions, understandings, and theories to create an in-depth understanding of the past (Goodman and Leatherman, 1998). Jay (2009) points out that breastfeeding practices have the potential of influencing how women spend their time. This can affect the subsistence contribution of women and in turn the childcare practices at play (Moffat and Prowse, 2018). Different cultural contexts can also affect the types of weaning foods being used. Isotope studies can inform researchers about the types of weaning foods consumed in different regions, and can examine whether differences exist between the diets of adults and children. Understanding differences in adult and childhood diet is important in understanding the social age of individuals, a concept that looks at age categories in their social and cultural context opposed as a strictly biological category (Halcrow and Tayles, 2008). Through the study of breastfeeding and infant and young child feeding practices, researchers may demonstrate changes, or lack thereof, in cultural norms surrounding childcare practices, and how social determinants may influence such practices (Halcrow et al., 2018). King and colleagues (2018) demonstrate that weaning practices

were highly variable in the prehistoric Atacama Desert, Chile. However, their study found that the observed variation is not linked with the time or the switch from pre-agricultural subsistence to agricultural, an event often linked with changes in breastfeeding practices. Rather, the variation is caused by mothers taking up individualistic breastfeeding and weaning practices to combat the harsh environment of the Atacama Desert.

When weaning is studied through a biocultural lens, researchers are able to bring the discussion in from broad studies of population demography, to the lived experience of children. Recent advances in isotopic analysis and microsampling make this even easier and will be discussed at length later in this chapter.

3.4 Inferential methods of studying weaning

Early bioarchaeological studies of weaning used a series of inferential paleodemographic methods to understand weaning patterns in the past. The term inferential is used as these methods did not directly study infant diet but look at patterns of infant mortality and prevalence of pathological indicators to infer when individuals were likely weaned (Schurr, 1997). The basis for these studies was related to the expected peak in mortality associated with weaning stress, as discussed in section 3.3.1. Researchers used mortality distributions of samples, or the presence of stress markers, such as linear enamel hypoplasia, cribra orbitalia, and Harris lines to create weaning charts that identified this exceptionally stressful period in childhood development (Lewis, 2007). For example, using previously built paleodemographic life tables, Clarke (1977) shows that there was a spike in mortality immediately following the weaning period and they interpret this as most likely reflective of the increased stress associated with the cessation of breastfeeding. Moggi-

Cecchi and colleagues (1994) provide an example of how the presence of enamel hypoplasia can be used to study weaning in the past. They highlight the ages at which enamel defects were more or less common, and use this information to infer the age at which weaning likely began and was completed. While not based on pathological indicators, Scott and Halcrow (2017) explore the use of dental microwear on deciduous teeth to infer infant feeding and weaning patterns. The authors argue that there is potential in this method on its own, however, they suggest the technique is most useful in unison with other methods, such as stable isotope analysis. Deciduous microwear has the ability to elucidate the actual foods that infants or children were weaned onto, answering a question potentially left open by other means of analysis (Scott and Halcrow, 2017).

Due to a series of methodological limitations, these methods are used less frequently, and when they are, it is in unison with other forms of analysis. For example, Herring et al. (1998) use palaeodemographic data from historical records in conjunction with stable nitrogen isotope data to investigate weaning in a nineteenth century historic skeletal sample from Belleville, Ontario, Canada. The most prominent limitation with these inferential methods comes from the complexity of the pathological markers used, and the general complexity of stress itself. Consider linear enamel hypoplasia, due to its multiple etiologies, Schurr (1997) argues that to infer weaning from its presence is greatly flawed. This is the case for all stress markers used to infer weaning patterns. Similarly, using mortality distributions to infer weaning has issues as well. There are numerous factors that might increase infant mortality, be they social or environmental; due to this, inferring weaning solely from mortality distributions is laden with potential issues (Howcroft, 2013).

The application of stable isotope methods has provided a more direct means to studying infant feeding and weaning patterns in archaeological populations.

3.5 Stable isotope analysis

Possibly one of the most direct ways bioarchaeologists can study weaning and infant feeding patterns is through stable isotope analysis of skeletal and dental tissues. Traditionally, stable isotope analysis is used to study diet and mobility in past populations and individuals; however, it can also be applied to understand weaning through the analysis of infant and childhood diet. Despite stable isotope analysis' introduction to biological anthropological inquiry in the late 1970s, the methods and theory that govern such analyses are older and stem from the work of physicists and chemists from the early twentieth century.

3.5.1 Composition of human bone and teeth

Before discussing the key principles of stable isotope geochemistry, it is important to first understand the composition of human bones and teeth. Human bone is composed of both organic and inorganic components. Roughly 90% of the organic component of human bone is made up of collagen; however, small amounts of non-collagenous organics are present (White and Folkens, 2000). This organic matrix of bone constitutes approximately 30 - 40% of a modern bone's dry weight, whereas the amount of collagen in archaeological samples is significantly less, varying from 0 – 22% (Hoppe et al., 2003; Schwarcz and Schoeninger, 2012). Collagen extracted from bone provides a source for organic carbon and nitrogen, two elements commonly used in studies of past diet. The inorganic matrix of

bone is made up of the mineral, hydroxyapatite, constituting the remaining 60-70% of a bone's dry weight. Hydroxyapatite (or bone carbonate), provides bioarchaeologists with inorganic carbon, as well as oxygen and strontium to study diet and mobility. In comparison to collagen, carbonate is more susceptible to diagenetic alteration; minerals from the soil leach into the bone, resulting in isotope values that reflect the soil instead of human diet (Koch et al., 1997). Assessing diagenesis is essential when carrying out stable isotope analysis regardless of the tissue used and will be discussed in Chapter Five of this thesis.

Throughout an individual's life their skeletal tissue is constantly remodeling; old bone is resorbed, and new bone is laid down. The timing and rate of remodeling is not yet fully understood as it is highly variable between periods of life, individual bones, and areas of a single bone (e.g. cortical vs. trabecular bone) (Schroeder et al., 2009; Sealy et al., 1995). Due to this, isotope data from adult bone are difficult to define in terms of a time frame; it is generally understood that they represent the last 10 - 20 years of an individual's life (Price et al., 2000; Sealy et al., 1995). Research by Hedges and colleagues (2007) demonstrates that the age and sex of an individual has a significant influence on the turnover rates. They found that sub-adult bone remodeled quicker than adult bone, and that turnover rates in females were higher than in males. The remodeling of human bone has implications for studies of weaning in the past. Changes in isotope values associated with shifts in diet are not immediately seen in bone, due to the rate of remodeling. Because of this delay in the incorporation of isotope signals into bone, obtaining accurate estimates on the timing and duration of weaning from bone is difficult.

Human teeth are made up of two tissues, highly mineralized enamel and the more organic dentine. Enamel is the material that covers the crown of each tooth; it is made up of 96% hydroxyapatite, with the remaining 4% composed of non-collagenous proteins (Gutiérrez-Salazar and Reyes-Gasga, 2003). Due to this, only inorganic materials can be sampled from dental enamel. Tooth dentine, however, is more similar to bone and is composed of hydroxyapatite and collagen, 70% and 30% by weight, respectively. Dentine makes up the bulk of a tooth including its roots. The presence of a collagenous component allows researchers to analyze dentine for carbon and nitrogen isotope ratios.

While bones are constantly remodeling throughout an individual's life, teeth develop during infancy and childhood and do not remodel. Tooth dentine begins to develop *in utero* and forms through a two-stage process. First, an organic matrix, composed of collagen and other non-collagenous proteins, is secreted, then mineralization occurs as hydroxyapatite is deposited (Hillson, 2014). Secretion and mineralization occur directionally away from the enamel-dentine junction (EDJ) at a rate of roughly 4-6μm per day (Beaumont et al., 2013). The earliest forming dentine is found in the tooth crown, while the last dentine to form is found in the root apex. Upon tooth completion, secondary dentine slowly begins to form in the pulp chamber (Hillson, 1996). Secondary dentine is predominantly found in adult teeth and, in general, becomes more abundant with age (Zilberman and Smith, 2001). Arora et al. (2014) note that deciduous teeth may possess a degree of secondary dentine, however, only after the tooth has completed its formation. Since primary dentine does not remodel, the isotope values from teeth represent dietary information from the individual's infancy and childhood as that is when the tooth was

formed. The incremental growth of tooth dentine is key to the methods applied in this thesis and will be discussed later in this chapter.

3.5.2 Isotopes and elements

The term isotope refers to the forms of a single element that differ in the number of neutrons and, in turn, atomic weight. Isotopes are found in two forms, stable and radioactive. Radioactive isotopes, such as carbon 14 (^{14}C), undergo a process of decay, from one isotope into another (^{14}C into ^{14}N), over time; this time is referred to as the isotope's half-life. Due to this decay, such isotopes are considered unstable. Stable isotopes, however, are non-radioactive and do not decay, making them incredibly useful in the study of past diet. Isotopes occur in nature in varying abundances; carbon, for example, has two naturally occurring stable isotopes, ^{12}C and ^{13}C. Carbon-12 is the most abundant, making up 98.3% of the earth's carbon, while ^{13}C comprises only 1.07% (Hoefs, 2015). In general, the lightest isotope is the most abundant.

Stable isotope analysis works on the basis that as chemical reactions occur, the isotope ratio of a given material will vary due to the fact certain isotopes of a single element react at different rates (Katzenberg, 2008). For example, the isotope ratio of carbon will vary between the atmosphere and a plant, as it photosynthesizes CO_2. Carbon-12 is preferentially taken up by the plant during photosynthesis because it requires less energy (through a process called kinetic isotope fractionation). The result is that plants are typically depleted in ^{13}C relative to environmental carbon (discussed further in Section 3.4.4). Another change will occur as an animal consumes the plant and the carbon is

incorporated into the animal's tissue. Through an understanding of isotope fractionation, bioarchaeologists are able to study what humans were consuming in the past.

3.5.3 Reporting isotope values

When studying stable isotopes, researchers are interested in the ratio of two isotopes of a single element (e.g. $^{13}C/^{12}C$, $^{15}N/^{14}N$). In order to increase precision in the measurement of isotope ratios, the δ (delta) notation was introduced to the field (Sharp, 2017). The delta notation does not report the absolute abundance of each isotope, rather, it reports the relative abundance in relation to an international standard. The following equation shows how δ values are calculated:

$$\delta \text{ in } ‰ = \frac{R_{sample} - R_{standard}}{R_{standard}} * 1000$$

Equation 3.1: Theoretical calculation of δ values in ‰.

R is the ratio of heavier isotopes to lighter isotopes (e.g. $^{13}C/^{12}C$). The delta notation is expressed using the ‰ (per mil) sign. Specifically, when studying diet, researchers are interested $\delta^{13}C$ and $\delta^{15}N$ values, represented in the following equations:

$$\delta^{13}C = \frac{^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{standard}}{^{13}C/^{12}C_{standard}} * 1000$$

Equation 3.2: Calculation of $\delta^{13}C$ values, expressed in ‰.

$$\delta^{15}N = \frac{^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard}}{^{15}N/^{14}N_{standard}} * 1000$$

Equation 3.3: Calculation of $\delta^{15}N$ values, expressed in ‰.

Using a set of internationally recognized standards when determining isotope ratios allows for the comparison of δ values between different laboratories. For stable carbon isotopes, the first international standard was the calcareous fossil, PeeDee Belemnite (PDB). However, supplies of this initial standard have been completely depleted. Since then, Vienna, PeeDee Belemnite (VPDB), a standard calibrated to the same ratio as the original fossil, has become the recognized standard. The reference sample used for stable nitrogen analysis is atmospheric N_2, referred to as AIR.

3.5.4 Carbon isotopes

The first isotopes that researchers used to conduct studies of past diet were ^{12}C and ^{13}C. The use of carbon to study human diet did not develop within the field of anthropology; rather, it was first used by plant biologists studying photosynthetic pathways and radiocarbon physicists interested ^{14}C dating (van der Merwe, 1982). The first application of carbon isotope analysis to archaeological samples was in the late 1970s by J. C. Vogel and Nikolaas J. van der Merwe. Due to the expected higher $\delta^{13}C$ value of maize (because it is a C_4 plant and uses a different photosynthetic pathway that produces higher $\delta^{13}C$ values) and a well established knowledge of isotope fractionation, these seminal papers used $^{13}C/^{12}C$ ratios to trace the spread of maize in the Americas (van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1977).

Today, bioarchaeologists study carbon isotopes found in bone collagen and carbonate, as well as in tooth enamel and dentine. Carbon enters these tissues through the foods we eat, and through isotope fractionation, the $\delta^{13}C$ values of human tissues allow researchers to study the diet of past populations. Carbon isotopes are first fractionated

during plant photosynthesis of atmospheric CO_2. There are three different photosynthetic pathways that plants use, resulting in unique ratios of ^{13}C to ^{12}C. The three pathways are C_3, C_4, and CAM; plants are categorized based on these three pathways.

Different photosynthetic pathways result in distinct $\delta^{13}C$ values due to the way plants fixate carbon. In C_3 plants, the initial product in the breakdown of CO_2 is a three-carbon molecule, while in C_4 plants, it is a four-carbon molecule (Schwarcz and Schoeninger, 2012; van der Merwe, 1982). This results in low $\delta^{13}C$ values in C_3 plants (-20 to -35‰), and high $\delta^{13}C$ values in C_4 plants (-9 to -14‰) (Katzenberg, 2008). C_3 plants are the most common around the globe; examples include wheat, rice, and most fruits and vegetables. C_4 plants, while not so abundant, include essential crops such as maize, millet, and sorghum (Schwarcz and Schoeninger, 2012). Crassulacean acid metabolism, or CAM, plants have $\delta^{13}C$ values that overlap with and fall between both C_3 and C_4 plants due to the fact they utilize both photosynthetic pathways (O'Leary, 1988). Examples of CAM plants include succulents, pineapple, and other desert plants.

When humans and other animals consume plants, carbon isotopes are fractionated a second time. Early carbon isotope studies and controlled feeding experiments demonstrated a predictable increase in $\delta^{13}C$ values between plant and consumer (Ambrose and Norr, 1993; DeNiro and Epstein, 1978). Van der Merwe and Vogel (1977) noted a +5.3‰ increase between diet and tissue in grazing ungulates. While there is no universally accepted shift for humans, it is generally estimated to be around 3 – 6‰ (Ambrose and Norr, 1993; van der Merwe, 1982; DeNiro and Epstein, 1978). These values must be taken into consideration when analyzing the $\delta^{13}C$ values of human tissue. Diet to tissue isotopic

spacing varies between animals and plants consumed, but also between tissues in the body. Bone collagen and carbonate have different $\delta^{13}C$ values within the same individual. This is attributed to collagen and carbonate representing different components of one's diet; collagen represents the dietary proteins consumed, while carbonate represents the whole diet (Ambrose and Norr, 1993; Krueger and Sullivan, 1984; Lee-Thorp et al., 1989).

Bioarchaeologists use the $\delta^{13}C$ value of bones and teeth to determine the proportion of C_3 and C_4 plants in the diet. As some of the earliest studies have shown, this allows researchers to determine the spread of certain crops, such as maize (Vogel and van der Merwe, 1977), as well as providing general dietary information regarding C_4 plant consumption. Through the study of stable carbon isotopes, researchers are also able to study the relative dietary abundance of marine and terrestrial proteins. This line of inquiry is based on an observed, roughly 7‰ difference in $\delta^{13}C$ values between marine carbonate and atmospheric CO_2 (Chisholm et al., 1982). Due to this, a notable difference in $\delta^{13}C$ values can be seen in mammals consuming terrestrial diets compared to those consuming marine diets.

3.5.5 Nitrogen isotopes

Shortly after researchers discovered the value of stable carbon isotopes in the study of past diet, DeNiro and Epstein (1981) demonstrated the potential of stable nitrogen isotope analysis in dietary studies. Similar to carbon, nitrogen has two naturally occurring stable isotopes, ^{14}N and ^{15}N, the ratio of which (^{15}N /^{14}N) provides the bases for dietary studies. The majority of the world's nitrogen is in the form of N_2 in the atmosphere; however, only a few organisms fix N_2 directly, such as blue/green algae, and certain

legumes through symbiotic bacteria (Schoeninger and Moore, 1992). Most terrestrial plants acquire their nitrogen from soil containing nitrates as a result of the breakdown of organic matter. In marine systems, nitrogen is introduced through bacterial denitrification which, in general, results in more positive $\delta^{15}N$ values in marine organisms (Schoeninger and Moore, 1992). These early studies of stable nitrogen isotopes also discovered an enrichment of ^{15}N in each step up the food chain, resulting in the increase of $\delta^{15}N$ values as one moves up trophic levels (Minagawa and Wada, 1984). This is referred to as the trophic level effect (TLE). It is generally accepted that each step up the food chain is associated with a 3‰ increase in $\delta^{15}N$ values, this is true of herbivores consuming plants and carnivores consuming other animals (Katzenberg, 2008).

In a bioarchaeological context, researchers are interested in the $\delta^{15}N$ value of human bone and tooth collagen; unlike carbon, nitrogen is not found in the inorganic matrix of bones or teeth. One application of stable nitrogen isotope analysis to the study of paleodiet is the use of the TLE to determine the trophic level at which individual consumers feed; this can shed light on the types and quantity of animal protiens consumed. Another use of $\delta^{15}N$ values and TLEs in bioarchaeology is to reconstruct weaning practices, as this is a crucial concept to this thesis, it will be discussed in detail later in this chapter. Nitrogen can also help distinguish marine and terrestrial protein consumption. Because marine food chains are considerably longer than terrestrial ones, animals that consume marine resources have higher $\delta^{15}N$ values when compared to terrestrial consumers. Schoeninger and DeNiro (1984) show that animals with a terrestrial diet typically have $\delta^{15}N$ values less than 9‰, while those with a marine diet have values greater than 15‰. Individuals and populations

consuming exclusively marine proteins have $\delta^{15}N$ values up to 20‰ (Richards and Hedges, 1999).

Typically, $\delta^{13}C$ and $\delta^{15}N$ values are plotted against each other to optimize the ability to interpret the data. Figure 3.1 provides an example of hypothetical $\delta^{13}C$ and $\delta^{15}N$ values plotted against each other to distinguish unique isotope values associated with different organisms. Due to the fact increased $\delta^{13}C$ values are indicative of both a C_4 heavy diet and a dependence on marine proteins, looking at both carbon and nitrogen isotopes allows researchers to distinguish the two when in areas of expected C_4 consumption (Pate, 1994).

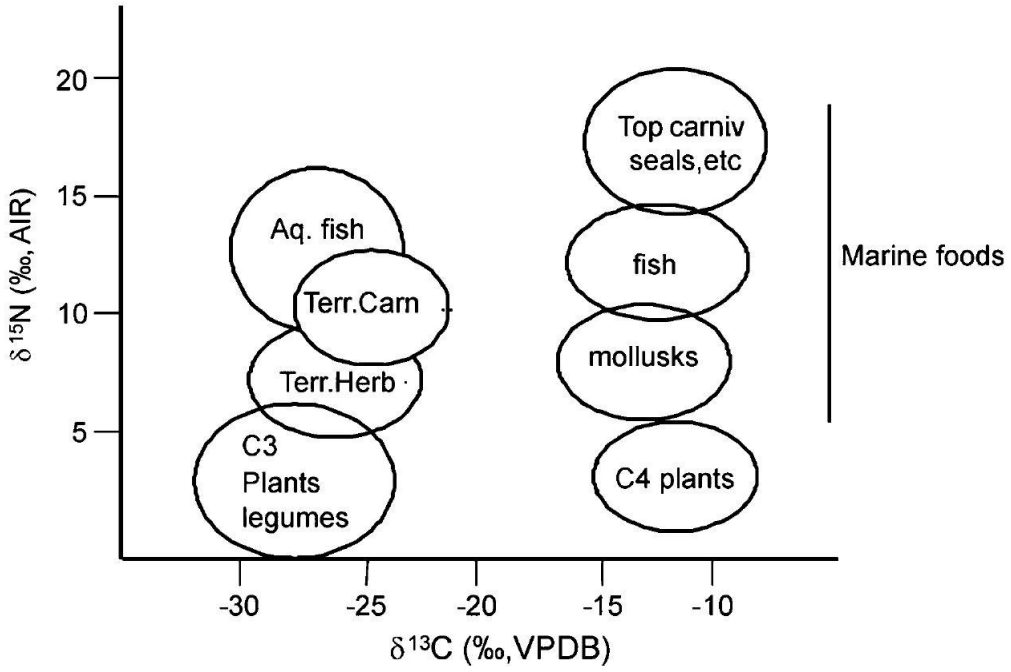


Figure 3.1: Bivariate graph showing the average $\delta^{15}N$ vs. $\delta^{13}C$ values of various organisms. Reprinted from Schwarcz and Schoeninger, 2012:732.

As well as reflecting diet, research has shown that nitrogen isotopes values can also be influenced by factors including nutritional stress and disease. Malnourishment and

diseases associated with diet can result in $\delta^{15}N$ values shifts. Fuller and colleagues (2005) used hair keratin from living individuals to demonstrate that, in periods of nutritional stress associated with pregnancy, $\delta^{15}N$ values increased for the duration of the episode. Neuberger et al. (2013) demonstrate similar findings in their study of hair keratin from recently deceased individuals with known periods of starvation or malnourishment prior to their death. Their study shows an increase in $\delta^{15}N$ values in all but one individual (n = 16) as well as a diagnostic decrease in $\delta^{13}C$ during periods of elevated $\delta^{15}N$ and, in turn, nutritional stress. The reason behind the increase in $\delta^{15}N$ during periods of stress is the body being in a catabolic state. When the body does not receive sufficient dietary protein, it may resort to recycling existing bodily tissues, resulting in a second isotope fractionation (and trophic level effect) (Reitsema, 2013). It is this second fractionation of nitrogen that results in $\delta^{15}N$ values being increased during periods of nutritional stress.

3.6 Stable isotope analysis and weaning

Since its inception in the 1980s, stable isotope analysis has become an increasingly common method to study weaning and infant feeding practices in the past. The ability to use stable isotope analysis to study breastfeeding and weaning in modern mother-infant pairs was first demonstrated by Fogel and colleagues (1989), in which the authors noted an increase in $\delta^{15}N$ values of nail clippings amongst babies who were breastfed. This increase is due to the fact nursing infants feed one trophic level above their mothers, because infants are consuming a product of the mother's body, essentially a carnivorous act resulting in the TLE (Jay, 2009). In the same study, Fogel and colleagues (1989) applied this method to an archaeological sample. Using estimated age-at-death, the authors observed high $\delta^{15}N$ values

among individuals they hypothesized were breastfeeding and lower $\delta^{15}N$ values among individuals who they interpreted as going through the weaning process. This relationship between breastfeeding infants and maternal $\delta^{15}N$ values was confirmed in a modern study by Fuller and coworkers (2006).

Stable isotope data have now been used to understand weaning patterns from a variety of time-periods and locations, such as Roman Italy (Prowse et al., 2008), Roman Egypt (Dupras et al., 2001), Iroquoian Ontario (Katzenberg et al., 1993), and Medieval England (Mays et al., 2002). These studies elucidate weaning patterns through the study of $\delta^{15}N$ values in bone collagen in sub-adult remains. In order to investigate weaning trends, researchers are required to conduct isotopic analyses on bone from a large sample of individuals, after age-at-death estimation is complete. It is necessary for the sample to have an adequate representation of infant and children's remains as the $\delta^{15}N$ values of those individuals are representative of that age. In the end, researchers create charts that show the distribution of $\delta^{15}N$ by age, where higher $\delta^{15}N$ among infants in the first years of life would be indicative of breastfeeding, and the lower values seen in older individuals are indicative of the gradual cessation of breastfeeding and introduction of complementary foods.

While these methods have resulted in a wealth of information on weaning in past populations, they are not without their limitations, the first being the nature of the sample required for this kind of analysis. Bioarchaeological assemblages are often fragmentary in nature, thus finding a sample large enough and with an adequate representation of infant and child remains can be difficult. This limits the number of sites and samples that bioarchaeologists can use stable isotope analysis on to study weaning patterns. With these

methods, it becomes impossible to study early sites that may be represented by only a few individuals.

Another more pressing issue with the nature of the assemblages required to conduct these analyses is that fact that non-surviving infants are the target of analysis (Beaumont et al., 2013). This stems from the issue of selective mortality that is discussed by Wood and colleagues (1992) in their seminal publication *The Osteological Paradox*. This is the issue that skeletal assemblages, in general, are made up of non-survivors; that is, a group of 20-25 year-olds in a skeletal assemblage does not necessarily represent living 20-25 year-olds in that population as the sample consists only of individuals who have died. This becomes an issue in weaning studies because we must then ask if these children, the targets of isotope analysis, died due to weaning or feeding behavior that caused premature death, thus biasing the weaning patterns recreated for the group.

Another drawback to this approach is the lack of precision in estimating weaning patterns. Because such studies rely on bone tissue for analysis, precision is difficult to obtain. Since bone continues to remodel during life at varying rates throughout the skeleton, dietary change associated with the different steps of weaning may not immediately be reflected in skeletal tissue (Eerkens et al., 2011). This results in reduced precision when estimating weaning patterns in a population. Another limitation is the fact the method relies the creation of population level weaning patterns. Thus, it is impossible to study variation in weaning patterns at an intra-population level. As Eerkens and colleagues (2011: 3102) write, “we cannot test whether there is greater investment in, for example, females versus males, low versus high status, or tall versus short individuals.” This is all of

great importance if we hope to better understand individual variation in weaning and the lived experience of infants and children. An alternative method of sampling dentine for isotope weaning studies has been undertaken to resolve some of these issues.

3.7 Tooth dentine and weaning

More recently, bioarchaeologists have begun using stable isotope analysis of tooth dentine to study weaning. As discussed previously, teeth do not remodel during life, and develop sequentially from crown to root. Because dentine secretion and mineralization occur at a predictable rate and at known ages, paired with advances in stable isotope technology, bioarchaeologists have found unique ways to study weaning and breastfeeding practices, including the study of intra-individual variability in diet and weaning at an individual level. Stable isotope analysis of tooth dentine began with the bulk sampling of dentine and moved to include serial sampling of dentine, which is where this high resolution, individual level analysis can be seen. These earlier bulk dentine studies are based on the comparison of dentine $\delta^{15}N$ values from either multiple different teeth, or from tooth dentine and bone collagen. Wright and Schwarcz (1999) sampled crown dentine from three teeth per individual (M1, PM4, and M3), each representing a different age range, and used the corresponding $\delta^{15}N$ and $\delta^{13}C$ values to investigate weaning behaviour at the site of Kaminaljuyu in Guatemala. Elevated $\delta^{15}N$ values in first molars, with respect to pre-molars and third molars, indicate that solid protein foods were introduced around the age of two. Subsequently, Dupras and Tocheri (2007) demonstrated the potential using bulk dentine samples to studying weaning through their analysis of all deciduous and all permanent teeth from sub-adults and adults at Roman era Kellis, Egypt. Their study found

that deciduous dentition $\delta^{15}N$ was higher by 2‰ over permanent dentition, showing evidence of breastmilk consumption; since each tooth develops over a unique time span, they were able to deduce a dependence on breastmilk until roughly three years of age. The incorporation of, and movement towards dentine, to study weaning has mediated the issue of isotope delay and turnover rates in bone, as well as providing an outlet to study individuals who survived childhood, which will be discussed further in section 3.7.2.

Soon after, researchers began serial sectioning tooth dentine based on the developmental layers of dentine. The basis for this method is to collect samples for stable nitrogen and carbon analysis from the collagen within tooth dentine, in incremental layers that follow the natural formation of the tooth as closely as possible. This results in multiple isotope values per individual, which can be plotted against the tooth development chronology to observe possible weaning patterns or feeding practices in general. These weaning curves are similar to those that were discussed previously, in relation to bulk isotopic analysis of bone, but now at an individual level, as opposed to a sample level.

3.7.1 Serial sampling methods

Serial sampling, or microsampling, of dentine began with the analysis of faunal teeth, where it was found that dentine could be sampled along annual growth lines to observe dietary change (e.g. Balasse et al., 2001; Evacitas et al., 2016; Zazzo et al., 2010). By the early 2000s, bioarchaeologists, inspired by these faunal studies, began to use dentine microsampling on human teeth. Using the Medieval English site of Wharram Percy (900 – 1500 AD), Fuller and colleagues (2003) applied dentine microsampling techniques to deciduous second molars, permanent canines, and third molars (n = 37), detecting evidence

of the weaning process in individual teeth, as well as general intra-individual dietary variation. Isotope results from deciduous molars, along with rib collagen, showed the age of complete weaning to be around two years old. Since this foundational study, a number of other researchers have applied dentine microsampling to archaeological samples and continue to refine the methods. There are two main methods used to study dentine incrementally, one built from the methods used by Fuller et al. (2003), and the other using a cardpunch to microsample dentine at different areas of the tooth.

The first and more commonly used method to microsample human dentine comes from the methods employed by Fuller and colleagues (2003), which were more recently revised by Beaumont et al. (2013). This technique is based on incrementally sampling thin sections of human dentine and associating each sample with an estimated, yet precise, age range; this allows researchers to assign dietary changes with approximate age. Ultimately, this can help us understand the age at which individuals began to consume complimentary foods and when breastfeeding stopped entirely. Researchers using this method either approach sampling by embedding the root in plaster and slicing it in roughly one millimeter sections, or the tooth dentine is partially demineralized, and the resulting collagenous model is sectioned using a scalpel (Beaumont et al., 2013). Collagen is then extracted from these thin sections using standard methods of collagen extraction; pure collagen is then run on a mass spectrometer to measure the carbon and nitrogen isotopic ratios within the samples.

A great deal of progress has been made in regard to this method. In the first study of its kind by Fuller et al. (2003), they were able to produce three to four incremental samples

per tooth. Less than a decade later, Eerkens and colleagues (2011) used these techniques to obtain between five and ten samples per tooth. More recently, this technique has been further refined and has shown that it is possible to get up to 20 samples per tooth, depending on size and preservation (Beaumont et al., 2013; 2014; Beaumont and Montgomery, 2015). Each advancement of this method is associated with an increase in serial samples taken from a single tooth, which results in a higher resolution understanding of the individual's diet. The current limitation is that samples are becoming increasingly small and more difficult to analyze on a mass spectrometer, which require a specific amount of collagen (0.5 – 1mg) for accurate analysis, so further progress is dependent upon advancements in isotope ratio mass spectrometry.

Since different teeth form at different periods throughout one's life, each tooth represents a different age range. Beaumont and Montgomery (2015) provide a detailed breakdown of the different age ranges associated with each tooth, and how this can be applied to the microsampling of human dentine. Due to the fact different teeth provide different time frames, this technique has a number of applications. They have been used to investigate the timing of weaning and diets in post-Medieval London (Henderson et al., 2014), the Atacama Desert (King et al., 2018), Byzantine Greece (Kwok et al., In Press), and pre-historic California (Eerkens et al., 2011). Other applications include studies of individual dietary variation over time, extending beyond the weaning period. Eerkens and colleagues (2016) use incremental sampling to show the different exploitation patterns of brackish-water foods in a late Holocene site located in California, both between individuals and periods of life. Another study using this technique outside of the context of weaning is

Beaumont and Montgomery's (2016) analysis of victims of the nineteenth century Great Irish Famine. The authors use isotopic microsampling alongside historical records to assess variation in dietary patterns associated with this period of great stress, and the effect of intervention in the form of the introduction of maize.

The second well established methodology for microsampling human dentine was first introduced by Burt and Garvie-Lok in 2013. In contrast to sectioning a whole tooth for analysis, this method uses a cardpunch to sample small cylinders of dentine at predetermined locations within the tooth. In order to do so, one half of a tooth, with the enamel removed, is mounted for histological examination where the location of the neonatal line is measured, then the other half of the tooth is demineralized. Samples are then taken from the demineralized tooth, using the cardpunch, in three locations, before the neonatal line, near the neonatal line, and at the apex of the root (Burt and Garvie-Lok, 2013). Because of the timing of tooth formation, isotopic data from these three locations can provide information on breastfeeding and weaning in the past.

While the amount of research using this method is less substantial, it has been shown to be a valuable tool in assessing childhood dietary patterns. Using a sample of modern deciduous teeth, Burt and Amin (2014) examined variation in infant feeding practices in a modern Canadian sample. The authors showed that this method is capable of picking up breastfeeding signals and can differentiate breastfed from bottle-fed individuals. The first archaeological application of this methods was carried out by Burt in 2015 on a late Medieval sample from the UK. This study showed that children likely began the weaning process around two years of age, but this was not homogenous throughout the

sample (Burt, 2015). Outside of the analysis of weaning, the data also showed that the diet of weaned children was similar to the diet of older children and adults in the population, contrary to what might have been expected (Burt, 2015).

3.7.2 Benefits of dentine microsampling

The recent increase in popularity of microsampling approaches is likely due to the benefits associated with this new higher resolution understanding of individual dietary patterns. Many of the benefits to approaching the study of weaning through dentine microsampling directly answer the issues discussed previously associated with other isotopic methods of studying weaning.

As previously mentioned, a major issue associated with studies of weaning that apply a population-level distribution approach, is that they rely on studying non-surviving individuals. A number of researchers argue that dentine microsampling provides a means of addressing this issue (Beaumont et al., 2013; Howcroft, 2013; King et al., 2018; Tsutaya and Yoneda, 2015). Sampling tooth dentine allows us to study weaning through individuals who have survived childhood, as opposed to those who died during this period (Beaumont et al., 2013). Since tooth dentine does not remodel, teeth from adult individuals can be studied to infer weaning patterns if certain teeth are present. By applying techniques that utilize incremental sampling of tooth dentine, researchers are able to overcome this barrier and begin to understand the weaning experience of children who survived childhood.

Other benefits to these approaches include the ability to look at variation in weaning patterns. There is no reason to suspect weaning is a homogenous activity; it is a biocultural

process shaped by numerous factors. Using serial studies of tooth dentine, individual weaning histories can be analyzed and compared with others in the same sample. Due to this, it is increasingly possible to study the factors that influence breastfeeding and infant feeding practices through the study of intra-population variation. Such analyses could be based around status, sex, migrant status, or numerous other factors. Outside of weaning studies, the high resolution individual dietary histories provided by the methods allow for the increased understanding of individual life-histories. This makes it increasingly possible to ask more in-depth questions about life in the past (Burt, 2015).

3.8 Summary and limitations

Weaning has long been of interest to biological anthropologists and bioarchaeologists alike, and studies of this pivotal period in an individual's life continue to grow. Whether it is through studies of modern individuals or past populations, biological anthropology, and bioarchaeology specifically, can contribute significantly to the broader study of weaning and the effects of different infant and young child feeding practices. Such studies may inform researchers about infant health, population demography, and cultural norms and taboos. Bioarchaeological studies of weaning often approach such research through a biocultural lens, understanding weaning as both a biological and cultural process. Through biocultural studies of weaning, we can further understand weaning as a culturally mediated practice.

While microsampling tooth dentine may resolve some of the critiques pertaining to other methods of studying weaning, they are not without limitations. Currently, incremental sampling of dentine is not able to sample dentine precisely along growth lines due to their

conical shape. Because of this, each sample is representative of slightly overlapping layers of growth. Future research would benefit from developing methods to sample dentine along growth lines to mitigate this issue, but as of yet sampling and isotope ratio mass spectrometer technology is not yet at a level where this is possible. Another issue, expressed clearly in Henderson and colleagues' (2014) study, has to do with assigning ages to each microsample taken from a single tooth. Due to inter-individual variability in tooth formation, it is difficult to assign age ranges to each tooth, which will then be divided to create an age for each microsample (Henderson et al., 2014). Traditionally, this is done using eruption charts, but to move forward with more accurate estimates, researchers will need to better understand the timing of dentine formation.

Stable isotope analysis has been a powerful catalyst in the field of bioarchaeology, and its contribution to studies of weaning have allowed the bioarchaeology of breastfeeding to develop into an increasingly large field of study. Until the advent of skeletal-based weaning studies, children were largely left out of the archaeological and historical records. Bioarchaeologists are more commonly collaborating with medical anthropologists and medical practitioners to work on understanding this complex biologically and culturally-shaped process. With advancements in stable isotope technologies and the introduction of dentine microsampling to human dentition, bioarchaeologists are able to create higher resolution understandings of human diet over time than ever before. This provides the opportunity to study the individual weaning histories of infants and children, both the non-survivors and those who survived the weaning process. Future researchers would benefit from the application and further development of these techniques. Methods are becoming

increasingly standardized and less time-consuming to carrying out, making them more applicable to address any questions around weaning and infant and young child feeding.

Chapter 4: Vitamin D Deficiency and Bioarchaeology

4.1 Introduction

Vitamin D plays a key role in the development and remodeling of the skeleton, whether you take it in the form of child-friendly fortified Gummy Bears, standard capsules, or synthesize it directly from the sun. Vitamin D deficiency can result in a number of pathological conditions, two particular diseases that arise are rickets and osteomalacia. Both are metabolic bone diseases, which, in severe or prolonged cases, result in skeletal deformities due to the disruption of bone growth and remodeling. In the field of paleopathology, rickets is used to define the skeletal lesions found in sub-adults, where a distinction is made between active and healed cases; while osteomalacia, is used to discuss lesions found in adults (Brickley et al., In Press). Bioarchaeologists have a longstanding interest in the skeletal manifestations of vitamin D deficiency and have contributed greatly to understanding the diseases associated with this deficiency. Recently, it has become more common to frame paleopathological research on vitamin D deficiency within a bioculturally informed framework, recognizing the various social, cultural, political, and economic variables that can influence susceptibility and spread of these conditions (Brickley et al., 2014; Giuffra et al., 2015; Veselka et al., 2017). This chapter will first discuss the importance of vitamin D and its synthesis into the human body, before explaining the current clinical definitions and diagnostic methods of vitamin D deficiency. Finally, the paleopathological study of vitamin D deficiency will be discussed where the diagnostic criteria of rickets and osteomalacia will be highlighted. Throughout, this chapter will highlight the cultural factors that influence vitamin D deficiency around the world.

4.2 The role of vitamin D in the body

The primary function of vitamin D in the human body is to ensure adequate calcium and phosphorus levels, which are responsible for bone growth and development (Holick, 2006). Sufficient levels of vitamin D are required to regulate and promote the absorption of calcium in the intestines and kidneys (Bikle et al., 2013; Bouillon et al., 2008). According to Holick (2004; 2006), when sufficient levels of vitamin D are not met, only 10-15% of dietary calcium is absorbed; with sufficient levels of vitamin D, around 30% of dietary calcium is absorbed. During pregnancy and lactation, calcium absorption is doubled in mothers due to the increased calcium requirements associated with pregnancy and breastfeeding (Cross et al., 1995; Fudge and Kovacs, 2010; Holick, 2004). Prolonged failure to maintain adequate levels of vitamin D, and in turn calcium and phosphorus, results in delayed or poor mineralization of the organic bone matrix (Brickley and Ives, 2008; Holick, 2006). In severe cases, this leads to rickets in infants and children, and osteomalacia in adults.

4.3 Vitamin D intake

There are three ways in which vitamin D enters the human body, ultraviolet radiation from sunlight, foods rich in vitamin D, and supplementation. Ultraviolet radiation provides the most abundant source of vitamin D as very few foods contain significant amounts. There are two forms of vitamin D; vitamin D_2, which is synthesized by plants, and vitamin D_3, which is synthesized by mammals (Wagner and Greer, 2008). Throughout this thesis, vitamin D without any subscripts refers to both vitamins D_2 and D_3. Unlike other vitamins, vitamin D is technically a hormone in the steroid family that is initially inactive

until it goes through a process of synthesis in the human body (Bikle et al., 2013; Brickley and Ives, 2008).

4.3.1 Ultraviolet radiation

Humans acquire roughly 90 to 100% of their required vitamin D through exposure to sunlight, specifically, through the ultraviolet (UV) radiation produced from the sun (Holick, 2003). The sun emits three forms of UV radiation, UVA, UVB, and UVC. It is through exposure to the UVB rays that the process of vitamin D synthesis begins in the human body. Upon contact with skin, UVB rays convert 7-dehydrocholesterol, which is within the epidermis, into previtamin D_3 (Bikle, 2012; Chen et al., 2007; Holick et al., 2007). Due to its unstable nature, previtamin D_3 is then converted into vitamin D_3, which is bound to the vitamin D binding protein (DBP) and moved from the skin into the circulatory system (Bikle, 2012; Holick, 2006). The vitamin D_3 then separates from the DBP and moves into the liver where hydroxylation occurs turning it into 25-hydroxyvitamin D (25(OH)D), this is the form of vitamin D that is clinically-measured in the bloodstream to diagnose deficiency (Holick, 2006; Wagner and Greer, 2008). A second stage of hydroxylation occurs once the 25(OH)D reaches the kidney, resulting in its conversion to 1,25-dihydroxyvitamin D (1,25(OH)2D), the biologically active form of vitamin D that aids in calcium and phosphorus homeostasis (Brickley and Ives, 2008; Holick, 2006).

There are a number of variables that may influence the level of vitamin D an individual absorbs through UVB radiation; these include geographic, biological, and cultural variables. Geographic location is a key determinant for the production of vitamin D; ultraviolet radiation (UVR) varies based on the solar zenith angle which is influenced

greatly by latitude (Chaplin and Jablonski, 2009). Equatorial and nearby regions have the highest levels of UVR, which correlates to the highest levels of vitamin D producing UVB (Kimlin, 2008). As one moves further away from the equator, the amount of effective UVB radiation is lowered. Kimlin (2008) notes that at around 40° latitude there is a marked seasonal difference in UVB and vitamin D production – by 90° vitamin D producing UVB is only available roughly four months out of the year. This puts individuals living in higher latitudes at an increased risk for vitamin D deficiency.

Biologically, skin pigmentation is one of the most significant variables affecting vitamin D production. Melanin, the pigment responsible for giving human skin and hair its color, acts as a natural sunscreen, absorbing UVR to prevent damage to underlying tissue and in turn limiting the production of vitamin D (Chen et al., 2007; Jablonski and Chaplin, 2013). Higher concentrations of epidermal melanin (e.g., highly pigmented skin) results in reduced UVB intake and previtamin D_3 production when compared to lighter skin in an identical setting (Chaplin and Jablonski, 2009; Chen et al., 2007; Holick, 2003; Mithal et al., 2009). This is exacerbated in cases where individuals with dark skin are living at high latitudes where exposure to UVB is restricted.

Finally, there are cultural factors that influence vitamin D intake through UVB radiation. Cultural practices and beliefs surrounding clothing, time spent outside, beauty, and exposure to sunlight have a large impact on epidermal vitamin D production. In certain regions of the world where conservative attire, including secular or religious skin coverings and veils, are more common, there is a direct correlation between prevalence of conservative attire, and vitamin D insufficiency and deficiency. In a study of Turkish

women, Guzel and colleagues (2001) report significantly reduced levels of 25(OH)D amongst women wearing full-body covering religious clothing in comparison to a control group. Similar findings are reported throughout the Middle East, India, and Northern Africa, with evident differences between men and women, as veiling is most common amongst women (e.g. Malik et al., 2018; Sachan et al., 2005; van Schoor and Lips, 2011). Low levels of 25(OH)D in these areas may also be attributed to cultural practices that encourage women to stay indoors and abstain from outdoor activities.

Sociocultural practices that result in limited exposure to UVB radiation are not restricted to religious practice and belief. Practices such as swaddling infants and sun avoidance due to the known carcinogenic properties of over-exposure to sunlight also result in reduced exposure to UVB and are common around the globe (Brickley et al., 2014; Mithal et al., 2009). Thus understanding religious practices, beliefs around sun exposure, and cultural ideals of childcare are essential when studying vitamin D deficiency as they may directly influence disease susceptibility.

4.3.2 Dietary sources of vitamin D and dietary supplements

Unsurprisingly, since the vast majority of vitamin D in the human body is produced through exposure to sunlight, diet plays only a small role in maintaining the required levels of vitamin D. While only vitamin D_3 is produced through UVB radiation, both vitamin D_2 and D_3 can be obtained through diet. Vitamin D_2 is found in plants and produced through the exposure of ergosterol in yeast to UVR (Holick et al., 2008; Wagner and Greer, 2008). Vitamin D_3 is produced naturally in mammals and found in oily fish (Holick et al., 2008). Once vitamin D enters the body through dietary sources it follows the same path as vitamin

D produced in the skin. It is picked up by the DBP and moved to the liver and kidneys where it is eventually converted it into active 1,25(OH)2D.

Source of vitamin D	Amount of vitamin D
Dietary items	
Atlantic salmon, raw (100g)	1200 IU (30 µg)
Cod liver oil (5mL)	500 IU (12.5 µg)
Free range chicken eggs, raw (100g)	183.2 IU (4.58 µg)
Chicken flesh and skin, raw (100g)	60 IU (1.5 µg)
Beef meat, ~15% fat, raw (100g)	28 IU (.7 µg)
Whole cow's milk, 3.5%, unfortified (100g)	4 IU (.1 µg)
Fortified foods	
Fortified milk (100g)	~44 IU
Fortified orange juice (100g)	~44 IU
Fortified butter (100g)	~50 IU
Fortified breakfast cereal	~ 100 IU (variable between cereals)
Infant feeding items	
Breastmilk, human (100g)	3.2 IU (.079 µg)
Infant formula powder, fortified (100g)	330 IU (8.25 µg)
Sun exposure	
Exposure to UVB radiation, ~7-11 minutes, arms, face, and neck exposed	1000 IU*

Table 4.1: Common sources of vitamin D and the associated amount of vitamin D. µg to IU was converted following the ratio of 1µg/40IU. Data were collected from Frida Food Data (National Food Institute, 2015) and Holick (2007). *Caucasian adult in Toronto, Canada during summer months (Gill and Kalia, 2015).

Despite plants and animals producing vitamin D, very few food items naturally contain vitamin D and those that do often contain very low levels. However, there are exceptions to this, oily fish (e.g., salmon) and cod liver oil are foods that contain high levels of vitamin D. Some animal byproducts, such as eggs, also contain vitamin D albeit in much smaller amounts. Table 4.1 presents the vitamin D content of a selection of common foodstuffs compared to other sources of vitamin D. According to Frida Food Data (National Food Institute, 2015), an online database that provides nutritional information on a large

variety of foods, the vast majority of foods containing significant amounts of vitamin D are fish and fish-based products. In areas where the annual hours of sunlight are low, past populations may have been more dependent on vitamin D-rich foods in order to maintain sufficient vitamin D levels. In Scotland, where UVB producing sunlight is only available for 3-4 months a year, people throughout history have demonstrated consistent use of fish, predominantly cod and herring, which, alongside exposure to the minimal UVB radiation, was likely able to aid in the maintenance of adequate levels of vitamin D (Chaplin and Jablonski, 2013).

Today, in the developed world, a number of foods are commonly fortified with vitamin D in order to maintain adequate levels of 25(OH)D and keep rickets controlled. Throughout North America and Europe the most commonly fortified foods include milk, children's cereal, and margarine (Holick et al., 2011). Fortified foods would not have been available to past populations; thus, it is important to consider the potential vitamin D-rich foods that would have been available for consumption when studying vitamin D deficiency in the past.

The importance of sufficient dietary calcium in preventing rickets and osteomalacia also must be acknowledged. As mentioned previously in this chapter, vitamin D and calcium work together to ensure proper skeletal development. Insufficient calcium levels have been shown to exacerbate the symptoms of vitamin D deficiency, and in extreme situations can cause rickets itself (Holick, 2006; Pettifor, 2007). Sachan and colleagues (2005) suggest that a lack of dietary calcium may be at the root of high vitamin D

deficiency prevalence rates in tropical areas such as India, where UVB radiation is abundant.

4.4 Vitamin D and breastfeeding

As discussed previously in Chapter Three, infants and children are in a state of rapid growth which requires adequate vitamin D and calcium to ensure proper mineralization. Due to the critical nature of the relationship between infant development and vitamin D status, researchers have long had an interest in studying infant vitamin D intake and the associated role of breastmilk. As early as 1930, Hess noted that breastfeeding plays a key role in preventing infantile rickets. Their research found that breastfeeding infants were less likely to be vitamin D deficient, and in cases where they were deficient, the severity was reduced. Hess (1930) also states that the effectiveness of breastmilk in combating rickets is dependent on the mother's vitamin D status. This has been a crucial topic of recent research; in general, human breastmilk is not particularly high in vitamin D (see Table 4.1), however, there is a degree of variability based on maternal vitamin D status (Thiele et al., 2013; Wagner and Greer, 2008). Hollis and colleagues (2015) demonstrated in their study that mothers supplemented with 6400 IU of vitamin D each day were able to provide sufficient vitamin D to their infants without the need to supplement the infants directly. This study backs up Hess' (1930) observation from nearly 90 years ago, showing that breastfeeding may play a role in maintaining infant vitamin D levels.

Conversely, other research has shown that without maternal supplementation or high maternal vitamin D levels, the consumption of breastmilk is unable to solely provide an infant with enough vitamin D (Misra et al., 2008; Specker, 1994). Even in cases where

mothers are vitamin D sufficient, human breastmilk will provide an infant with less than 100 IU of vitamin D per day, significantly less than what is recommended (Hillman, 1990). In vitamin D deficient mothers, the vitamin D content of the breastmilk is reduced even further putting the infant at a severe risk for rickets. Ward and colleagues' (2007) study of rickets in Canadian children found that 94% of individuals diagnosed with rickets were exclusively breastfeed and not supplemented at the time of diagnosis. The authors state that exclusively breastfed infants have an increased risk of rickets, particularly in high latitude regions (Ward et al., 2007). Overall, it is generally accepted that, due to the low amount of vitamin D in breastmilk, it is insufficient in providing infants and young children adequate amounts of vitamin D (Elidrissy, 2013; Holick, 2017; Wagner and Greer, 2008).

However, it is also worth noting that the amount of vitamin D provided through breastmilk is essentially based on maternal vitamin D levels, thus it can be influenced by the same geographic, biological, and cultural variables. Due to this, the vitamin D content of an individual's breastmilk will vary from person to person. Women who are more prone to vitamin D deficiency are less likely to provide sufficient vitamin D through breastmilk alone. In order to ensure infants are acquiring enough vitamin D, supplementation is recommended. According to the American Academy of Pediatrics, exclusively breastfed infants should be supplemented with 400 IU of vitamin D orally, each day, ensuring adequate vitamin D levels without potentially harming the child with exposure to sunlight (Thiele et al., 2013). In the United States, infant formula is required to contain between 40 to 100 IU of vitamin D per 100 kcal, providing infants with sufficient vitamin D without external supplementation (Misra et al., 2008). While fortified infant formula and oral

vitamin D supplementation may be common today in certain parts of the world, it is not universally available. The introduction of vitamin D fortified foods into regions where vitamin D deficiency is common has resulted in reduced rates of rickets. However, similar to life in the past, children in some rural regions do not receive commercial infant formula or supplements and rely on sunlight and breastmilk to supply sufficient vitamin D (Craviari et al., 2008).

During the period of weaning, children are at an increased risk for vitamin D deficiency. As discussed, the majority of an infant's vitamin D will come from infant formula, breastmilk, or supplementation. Upon being introduced to weaning foods, an infant may lose their source of vitamin D unless they are being weaned onto fortified foods or foods high in vitamin D, continually supplemented, or exposed to sunlight with their skin uncovered. To combat any potential risk, supplementation of 400 IU per day is encouraged during the weaning process and into childhood (Wagner and Greer, 2008). Throughout history, children would not have been weaned onto fortified foods, rather the weaning diet would have been highly variable based on class, geographic location, and time period (Fildes, 1995). Weaning foods in eighteenth and nineteenth century Europe, while still variable, would often be low in vitamin D, commonly consisting of animal milk, meat broths, and cereal-based pap (Fildes, 1986). Through their study of post-Medieval Dutch remains, Vesleka et al. (2015) show that poor weaning foods likely played a key role in the pathogenesis of rickets amongst infants at the Beemster site.

4.5 Clinical description of vitamin D deficiency

Within the clinical literature, vitamin D deficiency is defined and diagnosed through the level of circulating 25(OH)D in blood serum, although the levels required for deficiency are debated. It is generally accepted that circulating 25(OH)D levels should be around <20 ng/mL in order to diagnosed as 'deficient' (Cashman et al., 2016; Holick, 2017; Wagner and Greer, 2008). Hollis (2005), however, argues that vitamin D deficiency should be defined by 25(OH)D levels less than 32 ng/mL (80 nmol/L) as that is when negative effects become evident (e.g., reduced bone mineral density). Clinically, there is also a differentiation between vitamin D deficiency and vitamin D insufficiency; a number of scholars have argued that insufficiency starts at around <20 ng/mL, while deficiency starts at <10 ng/mL (Rosen, 2011; Thacher and Clark, 2011). Accordingly, it is when serum levels are <10 ng/mL that vitamin D begins to severely affect the skeleton. Clinicians have also set out to determine the required vitamin D intake necessary to maintain normal physiological function and keep serum levels out of the insufficient/deficient range. While there is no universal recommended intake, general guidelines exist for various groups, predominantly based on age. It is recommended that infants until one year of age get at least 400 IU of vitamin D daily unless their mothers are heavily supplemented (Holick et al., 2011). After the first year, 600 IU is recommended and is sufficient until old age. Situations do arise that cause this number to shift. If signs of infantile vitamin D deficiency arise, it may be dealt with through increased vitamin D supplementation (Holick, 2017). As well, it is recommended that elderly individuals have a daily intake of at least 800 IU of

vitamin D, others have pushed this number higher, depending on the specific needs of the individual (Kolarczyk et al., 2017; Rosen, 2011).

4.5.1 Rickets

The main metabolic bone disease that results from vitamin D deficiency is rickets. As stated previously, rickets is the skeletal manifestation of vitamin D deficiency found in children; today, it is most prevalent amongst children between three and 18 months of age (Pettifor, 2004; Wagner and Greer, 2008). Rickets occurs from vitamin D deficiency due to the reduced level of calcium absorption associated low levels of serum 25(OH)D. Once levels reach a deficient state, parathyroid hormone (PTH) is secreted causing the release of calcium and phosphorus in bone to maintain circulating levels (Özkan, 2010). This decrease in calcium and phosphorus then leads to the decrease in bone mineralization that is characteristic of rickets. In living humans, a physical examination is common to diagnose rickets; physicians look for delayed fontanel closure, enlargement of wrists, delayed tooth eruption, and craniotabes, the thinning or softening of cranial bones (Misra et al., 2008; Özkan, 2010). In extreme cases, a protrusion of the sternum, as well as, bowing of the lower and upper limbs may be observed (Holick, 2006). Radiographic and laboratory tests are also a key part in the diagnosis of rickets. X-rays can be used to look at cortical thinning of long bones and impairment of the metaphyses of the knee and wrist (Özkan, 2010). Finally, testing blood serum 25(OH)D levels can confirm a diagnosis.

While rickets is predominantly caused by vitamin D deficiency, it can also be caused by a dietary deficiency of calcium. This concept, that calcium deficiency could cause rickets, was not always accepted; however, in the 1970s research showed that

children living in tropical areas with high exposure to sunlight were developing rickets (Pettifor, 2004). Eventually, after further research demonstrated sufficient serum 25(OH)D levels in rachitic individuals with high UVB exposure, it became a more accepted etiology. Since then, it has been important to consider access to dietary calcium when looking at cases of rickets, especially in regions of abundant UVB radiation.

4.5.2 Osteomalacia

While this thesis is only concerned with rickets, it is important to briefly discuss osteomalacia. Just as rickets affects the development and mineralization of bone in children, osteomalacia affects the mineralization of remodeling adult bone. While the underlying pathogenesis of the two conditions is nearly identical, their symptoms and diagnosis are different. Osteomalacia is often first noticed due to muscle weakness and bone pain. Diagnosis is, in part, based on the radiographic presence of Looser's zones, which are stress fractures (pseudofractures) caused by insufficient calcium and phosphorus in bone, and low serum calcium, vitamin D, and phosphatase levels (Bhan et al., 2010). For certain diagnosis, however, a bone biopsy is potentially required. In general, cases of osteomalacia do not result in severe bone deformity like rickets, but they do result in a build-up of unmineralized osteoid, increasing bone mass while decreasing the mineralized component (Leali et al., 2009). The build-up of unmineralized osteoid weakens the bone causing the development of pseudofractures and increasing the susceptibility to complete fractures. In extreme cases, bone and muscle deformities of the pelvis and hip may develop resulting in a difficulty walking and a 'waddling gait' (Brickley and Ives, 2008; Holick, 2006).

4.6 The bioarchaeology of vitamin D deficiency

The presence of skeletal markers associated with rickets and osteomalacia have allowed bioarchaeologists, or more specifically, paleopathologists to study these diseases in the past, allowing for an improved understanding of the disease process. While the first medically documented discussion of rickets was from Daniel Whistler in 1645 (Rajakumar, 2003), the paleopathological study of rickets has provided evidence of probable rickets and vitamin D deficiency as far back as 24,000 BP (Brickley and Ives, 2008). According to Holick (2003), rickets has long been associated with the Industrial Revolution; it was endemic throughout most northern European and North American cities by the 19th century. However, paleopathological studies of rickets have demonstrated that the disease is found outside of this specific context, and is linked to a number of social, economic, and cultural variables. For example, it has been observed in a rural, non-industrialized, Dutch community (Veselka et al., 2015), a 17th century French church site (Schattmann et al., 2016), a group of Neolithic Scottish burials (Armit et al., 2015), and in the upper-class Medici family of 16th and 17th century Italy (Giuffra et al., 2015). There is no doubt rickets and vitamin D deficiency were present prior to this or in different regions of the world. Seeing vitamin D deficiency manifest in the skeleton is a sign of severe and prolonged vitamin D deficiency. Short durations of deficiency, which would have negative health effects on modern populations, cannot be seen in the skeletal record (Snoddy et al., 2016) although new microscopic methods have been developed to detect vitamin D deficiency even when skeletal changes are not evident (see below). In acute cases of vitamin D deficiency, it may not be skeletally recognizable; similarly, since vitamin D deficiency is

often not fatal, individuals that survive childhood rickets may not have discernable skeletal deformation (Brickley et al., 2017). It is important to remain mindful of this fact when studying vitamin D deficiency in the past, through the skeletal record we only see a small portion of the individuals that may have been affected by vitamin D deficiency or insufficiency.

4.6.1 Diagnosing rickets in human skeletal remains

Macroscopic examination of the available skeletal remains is usually the first step in diagnosing vitamin D deficiency. The most commonly used criteria for diagnosing vitamin D deficiency at a macroscopic level are presented by Ortner and Mays (1998) and Mays et al. (2006). When studying rickets in the past, a distinction can be made between active and healed rickets. Active rickets refers to cases where the individual would have died while still rachitic, often infants or young children, while healed rickets refers to cases where the skeletal lesions associated with rickets have healed, often seen in older children or adults (Ortner and Mays, 1998). In general, rickets is diagnosed based on the observed porosity of the cranial and post-cranial skeleton, flaring of the long bone metaphyses, long bone bending, and unusual curvature of the ilium (Mays et al., 2006). According to Mays and colleagues (2006), instances of vitamin D deficiency that lacked porous cortices and growth plate abnormalities were considered healed. Table 4.2 provides a list of all the diagnostic criteria commonly used in the paleopathological study of rickets. The actual diagnosis of rickets based on these features must be done carefully and while remaining mindful of differential diagnoses. A number of these features are also associated with other diseases,

thus it is through the presence of multiple features and consideration of the biocultural context that rickets can be diagnosed with any degree of certainty.

Feature
Cranial vault porosity [1, 2]
Orbital roof porosity [1, 2]
Deformed mandibular ramus [1, 2]
Rib deformity [1, 2]
Costochondral rib flaring [1, 2]
Costochondral rib porosity [1, 2]
Ilium concavity [1, 2]
Deformed lower limbs [1, 2]
Deformed upper limbs [1, 2]
Flaring of the long bone metaphyses [1, 2]
Thickining of the long bone metaphyses [1, 2]
Porosity of the long bone metaphyses [a, 1, 2]
Superior flatting of femoral metaphysis [1]
Coxa vara [1]
Porosity/roughening of growth plate [a, 1]

Table 4.2: A list of the diagnostic criteria for rickets. [a] This feature is only found in active cases of rickets. [1]Mays et al. (2006); [2] Ortner and Mays (1998)

As briefly discussed in previous sections of this thesis, rickets can be seen through radiographic imaging. Mays et al. (2006) applied radiographic analysis to paleopathological samples and established a protocol for their use in the field. The main radiographic features examined are coarsening of the trabecular bone, cortical bone thinning, tunneling of the cortical bone, and thickening of the cortical bone on the concave surface of the bending deformities. These radiographic methods show great potential in the study of incomplete skeletal material as it allows the examination of internal structures when macroscopic

features are rendered unusable due to taphonomic alteration (Mays et al., 2006). Similar to macroscopic features, these must be considered alongside other features as none are diagnostic on their own.

Another new method that has is being used to examine vitamin D deficiency in the past is the study of interglobular dentine (IGD). Developed by D'Ortenzio and colleagues (2016), the technique uses scanning electron microscopy or histological examination of tooth dentine to look for mineralization defects found within the developmental layers of the tooth. Their study has demonstrated the potential of using IGD in archaeological samples to examine periods of vitamin D deficiency by highlighting the presence and severity of IGD in cases of known vitamin D deficiency. Due to the fact that teeth do not remodel throughout life, the episodes of vitamin D deficiency can be associated with age range based on the location of the IGD (D'Ortenzio et al., 2016). This enables the study of healed rickets in cases where all other evidence may be missed and allows for the application of a life course approach.

4.7 Conclusions

Vitamin D deficiency, and its manifestation as rickets and osteomalacia are very complex conditions that are influenced by a suite of socio-political, cultural, geographical, and biological variables. If blood serum levels of vitamin D drop low enough, calcium and phosphorus homeostasis is impaired which can cause a number of adverse effects including skeletal defects due to improper mineralization of osteoid. Through infancy and childhood, vitamin D is crucial to ensure proper bone growth and development, insufficient vitamin D results in the characteristic bowing of the limbs among other health consequences. Due to

the importance of vitamin D to infant development, there has been a good deal of clinical research on the relationship between vitamin D deficiency and breastfeeding. While it is true that, in general, breastmilk contains low levels of vitamin D, in certain situations exclusively breastfed infants can maintain adequate vitamin D levels. While clinical research on this topic is high, there has been no bioarchaeological study of the relationship between breastfeeding and vitamin D deficiency. Bioarchaeology's ability to study populations that experienced high rates of vitamin D deficiency and lived prior to the widespread use of fortified foods or formula provides a unique and valuable perspective on this topic.

Chapter 5: Materials and Methods

5.1 Materials

5.1.1 The Crypt at the Church of the Trinitarias

In 2015, archaeologists uncovered an infant cemetery in a crypt below the church of the Trinitarias in Madrid, Spain, while searching for the remains of the famous Spanish writer, Miguel de Cervantes. The church of the Trinitarias is located in the heart of old town Madrid, and was built in the seventeenth century (Figure 5.1). The entire infant cemetery was exhumed, and a total of approximately 450 individuals were found. All individuals from the cemetery were sub-adults, between the ages of birth and six years. The burials were located in the floors and niches of the crypt, interred in both coffins and without. Based on this variety in burial styles (i.e. niche/coffin/floor), the children buried here likely represented a range of different socioeconomic statuses (Ríos et al., 2016). The archaeologists noted a high prevalence of rickets among all individuals interred in the cemetery, estimated to be present in at least 60% of the sample (Ríos et al., 2016). Through analysis of archival records and the clothing found within the burials, the cemetery was dated to the nineteenth century (Ríos et al., 2015, 2016). Currently, there is no published bioarchaeological work analyzing the remains exhumed from this cemetery.

5.1.2 The sample

In order to investigate the relationship between breastfeeding, weaning practices, and vitamin D deficiency in the Trinitarias cemetery, 14 teeth from 12 individuals interred at the Trinitarias cemetery were selected for stable isotope analysis of dentine. Samples

were collected by M. Brickley from the crypt and brought to Canada for analysis. All of the teeth analyzed for this thesis were deciduous molars and incisors. The 14 teeth were selected based on the presence and length of the tooth's root. To maximize the information gathered from a single tooth it was important to select deciduous teeth with roots that were near completion. For most of the individuals sampled, their permanent dentition was still early in its formation thus the teeth had little dentine and consist predominantly of a crown with no root, limiting the sampling potential. As well, while permanent teeth allow for the analysis of those who survived childhood, they develop over a longer period and have reduced precision when microsampling dentine – the age range associated with each sample in permanent teeth is often larger than in deciduous teeth.

Presence of osteological signs of vitamin D deficiency, in this case, rickets, was also considered when selecting samples for this thesis. Of the 12 individuals used in this study, nine show clear signs of vitamin D deficiency (MAD 05, 06, 07, 08/09, 10, 14, 15, 16, 17), while three do not (MAD 01/02, 03, 04). The diagnosis of vitamin D deficiency was carried out by the Spanish team in charge of excavating the crypt. Diagnosis followed the criteria presented by Ortner and Mays (1998) and more specifically, Mays et al. (2006) (discussed in detail in section 4.6.1). In order for an individual to be diagnosed with vitamin D deficiency, three of the 16 features presented by Mays and colleagues (2006) had to be present (Aranzadi, n.d.). Through the analysis of breastfeeding and weaning patterns seen in rachitic and non-rachitic individuals, paired with the historical data presented in Chapter Two, this thesis will compare the patterns between the two groups within this sample and discuss any apparent trends.

Figure 5.1: Aerial photograph of Madrid with a yellow star showing the location of the Church of the Trinitarias. Insert shows a map of Spain with the province of Madrid highlighted in yellow. Base map from ArcMap 10.1. Source: Esri

5.1.3 Age estimation

Within bioarchaeology, age estimation techniques used on sub-adult remains examine the development of bones and teeth (Beach et al., 2010). As the majority of skeletal and dental development occurs early in life, age estimates of younger individuals are typically more precise than those of adults. When studying sub-adults, dental development and eruption patterns are an accurate and precise method of estimating an individual's age. Studies have shown that tooth development is stable and is less influenced by environmental factors than skeletal development (Elamin and Liversidge, 2013; Cardoso, 2007). Additionally, the highly mineralized nature of teeth offers a degree of protection from post-mortem alteration (Santana et al., 2017).

While a preliminary age estimation was carried out by the Spanish archaeologists who excavated the sample, to ensure accuracy and consistency in interpreting the isotopic results, secondary age estimates were also carried out using tooth development patterns. The primary reason for age assessment is to estimate the terminal age for each tooth being sampled. Both the age of growth initialization and termination is essential in mapping individual changes in isotopic ratios over time. Terminal age estimates for each of the 14 teeth sampled in this thesis were carried out using the development charts and data provided by the London Tooth Atlas (AlQahtani et al., 2010). For teeth that had completed development, the age of tooth completion was used to determine the terminal age for that tooth. When the tooth root was still developing, it was scored based on the development categories presented by Morrees and colleagues (1963), further used by AlQahtani et al. (2010). Each developing tooth was assigned an age based on the most likely age for that

stage of development. The estimated age at death for each individual is provided alongside other sample information in Table 5.1.

Sample ID	Original ID	Tooth Type	Estimated Age at Death	Vitamin D Deficient?
MAD 01/02	N12 - 1	i1, m1	2.5 years	No
MAD 03	N5 - 1	m1	6.0 years	No
MAD 04	ScIV - 16	m1	3.5 years	No
MAD 05	Sc4 - 15	i1	1.5 years	Yes
MAD 06	Sc4 - 12	m1	2.5 years	Yes
MAD 07	Sc1 - F2 - 18	i1	1.5 years	Yes
MAD 08/09	Sc1 - F2 - 5	i1, m1	2.5 years	Yes
MAD 10	Sc1 - F1 - 2	m1	3.5 years	Yes
MAD 14	Sc4 - 79	m1	3.5 years	Yes
MAD 15	Sc4 - 42	m1	2.5 years	Yes
MAD 16	Sc4 - 12	m1	3.5 years	Yes
MAD 17	N23 - 2	m1	3.5 years	Yes

Table 5.1: Sample information including estimated age at death and vitamin D status

5.2 Methods

5.2.1 Tooth sectioning

The first step in preparing the teeth for isotope analysis was to embed and section each tooth. Sectioning was required to allow for the creation of histological slides that will be analyzed for the presence of interglobular dentine (after D'Ortenzio et al., 2016). Prior to sectioning, it was essential to embed each tooth in epoxy to ensure they did not fragment during the sectioning process. Buehler EpoThin was used to embed each tooth. After the epoxy set, teeth were sectioned into two halves using a Buehler IsoMet1000 slow speed saw fit with a diamond wafering blade; one half was used for isotope analysis and one was retained. Molars were section along the mesio-distal plane, exposing the buccal aspect of

the tooth, while incisors were sectioned along the labio-lingual plane. Halves containing the largest section of root were selected for isotope analysis.

5.2.2 Removal from epoxy

Following the procedures laid out in France et al. (2011), teeth were removed from the epoxy before continuing with demineralization. First, any loose epoxy was removed manually, when possible. Each embedded tooth was then soaked for 48 hours in 100% acetone, changing the acetone after the first 24 hours and manually removing epoxy if possible. The study conducted by France and colleagues (2011) demonstrated that 100% acetone allows for the easy removal of epoxy resins without influencing the isotopic ratios in any way. The tooth and epoxy block were soaked in 15mL of acetone per gram of the epoxy block's total weight to ensure that the sample remained submerged during soaking. After soaking for 48 hours, the tooth was manually removed from the epoxy. In cases where small amounts of epoxy remained adhered to the tooth, a third, six-hour, soak in acetone occurred. Each tooth was then rinsed in distilled water before being ultrasonicated for a total of 15 minutes. Distilled water was changed after each five-minute increment. The final step was to dry the teeth at 80 degrees Celsius for 24 hours in an oven.

5.2.3 Preparation for demineralization

The next step was to carefully remove the enamel from each tooth. For a number of the teeth, the enamel was loose and could be removed manually with a pair of fine-tip tweezers. In cases where the enamel was secure, a Dremel hand-drill fitted with a diamond-tip drill bit was used to remove enamel. The drill bit was soaked in .25M hydrochloric acid (HCl) and rinsed with distilled water between each tooth to ensure there was no cross-

contamination. While enamel was not analyzed as part of this thesis, the enamel removed from each tooth was reserved for potential future analyses.

The teeth were then cleaned of any surface contaminants. Using the diamond-tip drill bit, any cementum adhered to the root was gently removed along with any other debris. Teeth were then submerged in distilled water and washed in an ultrasonic bath for a minimum of 15 minutes; distilled water was changed every five minutes. After being removed from the ultrasonic bath, the teeth were dried in a 60 degree Celsius oven overnight. Once dry, each tooth was weighed in order to calculate collagen yields after the collagen extraction.

5.2.4 Demineralization and microsampling

Teeth were demineralized following the Longin (1971) method, later modified by Chrisholm et al. (1982). Each tooth was placed in a labeled centrifuge tube and pre-soaked in .001M HCl for five minutes before beginning an initial one hour .25M HCl soak. After the hour, each sample was lightly agitated, and the acid was decanted. The tubes containing the teeth were filled with .25M HCl. The samples soaked for between two and four months in .25M HCl, with the acid being refreshed each day for the first six weeks and every second day thereafter. Each tooth remained submerged in HCl until the tooth was demineralized, caution was exercised to ensure the teeth did not over-demineralize and loose their original shape.

Once a sample completed demineralization, it was rinsed with distilled water three times to cleanse it of residual HCl. The sample then began a 30 minute .1M sodium hydroxide (NaOH) soak to remove any humic and fulvic contaminants. A recent study has

shown the benefits of NaOH soaks opposed to ultrafiltration when removing humic and fulvic contaminants (Szpak et al., 2017). Following the 30-minute soak, samples were rinsed in distilled water four times, and once in .001M HCl, agitating the sample during each rinse. These steps ensure the sample was brought back to a neutral pH. Following these rinses, the tooth was ready for microsampling and collagen extraction.

The dentine microsampling techniques used here are based on the methods detailed in Beaumont et al. (2013); specifically, "Method 2: sectioning after demineralization", provided a framework for the methods applied in this thesis. This method has been proven successful in a number of other projects (e.g., Beaumont et al., 2014; Beaumont and Montgomery, 2016; Henderson et al., 2014). Following demineralization, the length of each tooth was measured, and test tubes were labeled in preparation for each microsample. On a non-slip cutting surface, each tooth was sectioned using a sterile blade, beginning at the crown and moving down the root. The number of samples collected from each tooth varied based on tooth length (Table 5.2). To ensure enough collagen was collected, microsample length averaged around three millimeters. Each section was then placed in a labeled glass tube for hot water extraction.

To extract the collagen from the demineralized tooth sections each tube was filled with five mL of .001M HCl, covered with two layers of plastic wrap, and sealed with tape. Samples were then placed in an oven at 90º Celsius overnight to liquefy the collagen. The next day samples were removed from the oven and the liquid, now containing collagen, was decanted into a weighed glass vial. The tube containing the tooth section was filled again with five mL of .001M HCl, sealed, and placed in the oven again overnight. The

second batch of collagen containing liquid was then added to the first, and they were placed in a 60 degree Celsius oven until dry. Repeating the hot water extraction process ensures as much collagen as possible is being extracted. Once dried, the vial containing the collagen was weighed again to provide a collagen yield for each tooth.

Sample ID	Tooth Type	Number of Microsamples	Length of Microsamples
MAD 01	Central incisor	5	3.0mm
MAD 02	First molar	4	3.0mm
MAD 03	First molar	4	3.0mm
MAD 04	First molar	4	3.5mm
MAD 05	Central incisor	3	3.0mm
MAD 06	First molar	4	2.5mm
MAD 07	Central incisor	4	3.0mm
MAD 08	Central incisor	3	3.0mm
MAD 09	First molar	4	2.5mm
MAD 10	First molar	4	2.5mm
MAD 14	First molar	4	2.5mm
MAD 15	First molar	4	2.5mm
MAD 16	First molar	3	3.0mm
MAD 17	First molar	4	3.0mm

Table 5.2: Tooth samples with the number and length of microsamples

Approximately one milligram of collagen from each sample then had to be weighed into tin cups for analysis on the mass spectrometer. Normally collagen can be powdered and placed into tin cups for analysis in the mass spectrometer, this was not possible here due to the small weight of each sample. In order to get the collagen into the tin cups, the dried collagen was first be rehydrated in .5mL of distilled water. Then, using an Eppendorf pipette, 40µL of the rehydrated collagen was pipetted into the tin cups after their weight had been recorded. The tin cups containing liquid collagen were heated on a hotplate at 60º Celsius until dry. The process of adding more rehydrated collagen and drying was repeated

until enough collagen was collected in each tin cup. Once the target one milligram of collagen was reached, tin cups were folded for analysis.

In order to analyze the isotope data each serial section taken from each tooth needed an assigned age. This was done following the methods detailed in Beaumont and Montgomery (2015) and was based on the age estimation techniques discussed in section 5.1.3. The starting age of development (*in utero*) was recorded for each tooth alongside the age of tooth completion, this allowed for the recording of the total growth duration for each tooth. Total growth duration was then divided by the number of samples taken from each tooth, resulting in the growth duration of each sample. Starting at the age of growth initialization, the sample growth duration is added to create an age range for each serial section. The age ranges were then averaged to provide a mean age for each sample. For example, the deciduous first molar, on average, develops for 2.4 months before birth and forms until 3.5 years of age providing a total growth duration of 44.4 months. If four samples are taken from this tooth, each sample represents 11.1 months of growth. For the first sample, 11.1 is added to the initialization age of -2.4 months, which yields an age range of -2.4 – 8.7 months for the sample. 11.1 is then added to estimate the age range for the subsequent sample, this continues until age ranges are provided for all sections.

5.2.5 Mass spectrometry and instrumentation

After being packed into tin cups, samples were sent to the G.G. Hatch Stable Isotope Laboratory in Ottawa, Ontario, for mass spectrometry analysis. Samples were run on a Delta Advantage Isotope Ratio Mass Spectrometer (IRMS) interfaced to a Vario EL Cube Elemental Analyzer (EA). Standards (AIR and VPDB) and samples, packed in tin

cups, were loaded into the EA and combusted at 1,800º Celsius. The CO_2 and N_2 gases produced from combustion were then carried by helium through a series of oxidizing and reducing chemicals before being separated using a purge and trap absorption column. Separate gases were then sent to the IRMS where isotope ratios were generated. G. G. Hatch reports that analytical precision is ± 0.2‰ based on internal laboratory standards.

5.2.6 Assessment of sample integrity

Despite the ability of bone and tooth collagen to preserve in a burial environment, it is essential when carrying out stable isotope analysis to assess samples for potential diagenetic alteration. Alteration occurs due to the complex burial environment a sample is exposed to when interred; organic and inorganic compounds containing nitrogen and carbon may contaminate bone and tooth samples, in turn influencing the isotope values (Schwarcz and Schoeninger, 1991).

Researchers commonly assess the integrity of collagen samples through three methods: atomic carbon to nitrogen ratios (C:N ratio), collagen yields, and carbon and nitrogen specific concentrations (Schwarcz and Schoeninger, 1991). Studies on the use of the C:N ratio, as a signifier for diagenetic alteration, state if the C:N ratio of a sample falls outside the acceptable range of 2.9 – 3.6, this is considered an indication of diagenesis (DeNiro, 1985). Other researchers have proposed acceptable ranges of 2.6 – 3.4 (Schoeninger et al., 1989) or 3.1 – 3.5 (van Klinken, 1999). Atomic C:N ratios on their own are not sufficient for assessing sample integrity, as samples within these acceptable ranges are not necessarily free of potential diagenetic alteration (Schwarcz and Schoeninger,

1991). However, atomic C:N ratios are useful in conjunction with other methods of diagenetic assessment.

Collagen yields (%coll) indicates the ratio of extracted collagen in relation to the dry bone or dentin sample, according to this formula:

$$\frac{weight\ of\ collagen}{weight\ of\ sample\ pre\text{-}demineralization} * 100 = \%coll$$

Equation 5.1: Collagen yield

Since the tooth was demineralized prior to sectioning, the collagen yield was calculated by summing the weight of collagen collected from each section on a tooth-by-tooth basis. This value was used in the *weight of collagen* section of equation 5.1. The use of collagen yields to assess sample integrity is to ensure the bone or tooth is preserved enough to yield suitable organic collagen. A threshold of >1.0 %coll is generally accepted, while above 5.0 %coll is indicative of good preservation (van Klinken, 1999; Schwarcz and Schoeninger, 1991). Finally, the individual carbon and nitrogen concentrations (%C and %N, respectively) of the extracted collagen can be used to determine sample integrity. While human collagen should measure to be roughly 35% carbon by weight (van Klinken, 1999), only values less than 3% are considered suspicious (Ambrose, 1993). The nitrogen content of human collagen is characteristically between 11% – 16%; however, like carbon, values have to be below 1% to be considered potentially diagenetically altered (Ambrose, 1993; van Klinken, 1999). To assess any potential diagenetic alteration in the samples looked at in this thesis, collagen yields, atomic C:N ratios, and %C and %N concentrations were observed. The results are reported in Chapter Six.

Chapter 6: Results

6.1 Introduction

In this chapter of the thesis, the stable carbon and nitrogen isotope data from the Madrid serial tooth sections are presented. Sample integrity is first discussed to show that all samples are free of potential diagenetic alteration. Individual isotope profiles for each tooth are then presented, first by nitrogen then by carbon. Finally, $\delta^{15}N$ and $\delta^{13}C$ data are presented together, followed by a discussion on inter-tooth variation in the same individual.

6.2 Sample integrity

Before presenting the isotope results, it is first essential to ensure that all samples that have been analyzed are not influenced by any diagenetic alteration. Chapter Five discussed the three criteria commonly used to assess sample integrity: C:N ratios, collagen yields, and individual carbon (%C) and nitrogen (%N) percentages. The carbon to nitrogen ratio (C:N) of each tooth section was calculated and is presented in Table 6.1. The range of the C:N ratios is 3.15 – 3.37 with a mean value of 3.24. All samples fall within the generally accepted range of 2.9 – 3.6 presented by DeNiro (1985), as well as the more restricted range of 3.1 – 3.4 by van Klinken (1999). The C:N ratios indicate that there is likely minimal to no diagenetic alteration within the samples.

The %C and %N concentrations of each sample were examined and the values are presented alongside the C:N ratios in Table 6.1. None of the samples had values outside of the accepted %C and %N thresholds. The mean value for carbon was 35.0% and for nitrogen was 12.6%, fitting within the expected 35% for carbon and within the 11% – 16%

range for nitrogen (van Klinken, 1999). Based on the %C and %N values found in these samples, diagenesis is unlikely.

Sample ID	% carbon	% nitrogen	C:N ratio
MAD 01.1	37.9	13.4	3.31
MAD 01.2	*	*	*
MAD 01.3	33.3	12.1	3.21
MAD 01.4	31.2	11.2	3.24
MAD 01.5	33.6	12.0	3.26
MAD 02.1	34.4	12.6	3.20
MAD 02.2	32.4	12.0	3.16
MAD 02.3	34.9	12.9	3.15
MAD 02.4	35.1	12.8	3.20
MAD 03.1	36.6	13.5	3.17
MAD 03.2	37.3	13.6	3.20
MAD 03.3	35.5	12.7	3.26
MAD 03.4	39.1	14.3	3.19
MAD 04.1	36.9	13.5	3.18
MAD 04.2	39.0	14.3	3.18
MAD 04.3	32.5	12.0	3.17
MAD 04.4	34.7	12.7	3.18
MAD 05.1	35.2	12.2	3.37
MAD 05.2	34.6	12.6	3.19
MAD 05.3	35.3	12.8	3.21
MAD 06.1	35.4	12.8	3.23
MAD 06.2	35.8	12.9	3.24
MAD 06.3	36.4	13.1	3.24
MAD 06.4	34.4	12.4	3.24
MAD 07.1	31.7	11.4	3.24
MAD 07.2	32.5	11.7	3.24
MAD 07.3	34.4	12.5	3.22
MAD 07.4	34.0	12.1	3.28
MAD 08.1	31.1	11.4	3.20
MAD 08.2	38.0	13.9	3.19
MAD 08.3	32.5	11.8	3.21
MAD 09.1	30.6	11.0	3.25
MAD 09.2	34.8	12.4	3.28

MAD 09.3	34.4	12.3	3.26
MAD 09.4	33.8	12.0	3.29
MAD 10.1	37.1	13.4	3.24
MAD 10.2	38.6	14.0	3.21
MAD 10.3	38.5	13.8	3.25
MAD 10.4	35.2	12.3	3.34
MAD 14.1	37.5	13.3	3.28
MAD 14.2	28.0	9.8	3.34
MAD 14.3	35.8	12.8	3.27
MAD 14.4	35.4	12.4	3.33
MAD 15.1	35.1	12.6	3.25
MAD 15.2	30.2	10.8	3.26
MAD 15.3	40.7	14.6	3.25
MAD 15.4	33.8	12.0	3.29
MAD 16.1	41.4	14.8	3.26
MAD 16.2	39.0	14.0	3.25
MAD 16.3	31.4	11.0	3.32
MAD 17.1	34.0	12.0	3.30
MAD 17.2	34.2	12.0	3.32
MAD 17.3	33.5	12.0	3.26
MAD 17.4	35.0	12.6	3.25
Mean (*n = 53)*	**35.0**	**12.6**	**3.24**

Table 6.1: % carbon and % nitrogen concentrations for each sample, alongside the atomic C:N ratio.

The amount of collagen within a given sample is another means of assessing the degree of potential diagenetic alteration. Following the steps in Chapter Five, the collagen yield was calculated for each tooth sampled. Because teeth are demineralized prior to sectioning, collagen yields were calculated for each tooth rather than for each sample (see section 5.2.6), this is consistent with previous dentine microsampling studies (c.f. Beaumont et al., 2013). Table 6.2 provides the collagen yield (%coll) for each tooth included in this study. Collagen yields ranged from 10.4% to 20.9% with a mean value of

15.3%, greatly surpassing the minimal requirement of 1% and the increased requirement of 5%, which is considered to represent good preservation (Schwarcz and Schoeninger, 1991; van Klinken, 1999). Based on the three criteria presented above, none of the samples were deemed likely to have undergone diagenetic alteration, allowing all samples to be included in further analyses. However, one sample, MAD 01.2 was lost during preparation and was not included in the analysis.

Sample ID	Tooth type	% collagen
MAD 01	Incisor	14.0
MAD 02	Molar	11.9
MAD 03	Molar	13.2
MAD 04	Molar	14.4
MAD 05	Incisor	20.5
MAD 06	Molar	20.9
MAD 07	Incisor	11.2
MAD 08	Incisor	16.9
MAD 09	Molar	10.4
MAD 10	Molar	12.6
MAD 14	Molar	20.7
MAD 15	Molar	17.2
MAD 16	Molar	16.4
MAD 17	Molar	13.2
Mean (*n*=14)		**15.3**

Table 6.2: Collagen yields for each tooth sampled for analysis

6.3 Stable isotope results

6.3.1 Nitrogen

Of the 53 samples prepared for analysis from the 14 Madrid teeth, all yielded sufficient collagen for analysis. Table 6.3 provides the $\delta^{13}C$ and $\delta^{15}N$ data for all tooth sections from all individuals. Amongst all samples, the $\delta^{15}N$ values are highly variable. They ranged from 11.7‰ to 16.9‰, with a mean value of 14.2‰.

Sample ID	Estimated mean age in Months	$\delta^{13}C$ (‰) (VPDB)	$\delta^{15}N$ (‰) (AIR)
MAD 01.1	-0.3	-17.8	12.9
MAD 01.3	13.2	-17.4	13.4
MAD 01.4	19.9	-17.6	12.9
MAD 01.5	26.6	-18.3	11.9
MAD 02.1	1.7	-17.4	13.6
MAD 02.2	9.8	-17.5	13.6
MAD 02.3	17.9	-17.8	13.4
MAD 02.4	26.0	-18.1	12.5
MAD 03.1	3.2	-17.6	13.5
MAD 03.2	14.3	-17.8	13.6
MAD 03.3	25.4	-18.2	12.7
MAD 03.4	35.5	-18.3	14.3
MAD 04.1	3.2	-18.3	13.5
MAD 04.2	14.3	-17.8	14.3
MAD 04.3	25.4	-18.2	12.0
MAD 04.4	35.5	-18.3	12.7
MAD 05.1	0.0	-18.2	14.6
MAD 05.2	7.2	-17.2	15.2
MAD 05.3	14.4	-17.5	14.3
MAD 06.1	1.7	-17.5	15.2
MAD 06.2	9.8	-17.3	15.5
MAD 06.3	17.9	-17.9	15.9
MAD 06.4	26.0	-18.3	16.0
MAD 07.1	-0.9	-17.2	15.3
MAD 07.2	4.5	-16.9	16.4

MAD 07.3	9.9	-16.8	16.9
MAD 07.4	15.3	-17.0	16.4
MAD 08.1	2.0	-17.4	14.5
MAD 08.2	13.2	-17.6	14.2
MAD 08.3	24.4	-18.0	13.0
MAD 09.1	1.7	-17.3	14.9
MAD 09.2	9.8	-17.9	13.9
MAD 09.3	17.9	-18.2	12.7
MAD 09.4	26.0	-18.5	11.7
MAD 10.1	3.2	-17.4	15.5
MAD 10.2	14.3	-17.4	15.7
MAD 10.3	25.4	-18.0	14.6
MAD 10.4	35.5	-18.8	13.5
MAD 14.1	3.2	-16.6	16.0
MAD 14.2	14.3	-17.3	14.9
MAD 14.3	25.4	-17.1	14.3
MAD 14.4	35.5	-17.7	12.7
MAD 15.1	1.7	-17.2	13.9
MAD 15.2	9.8	-17.4	14.3
MAD 15.3	17.9	-17.7	13.0
MAD 15.4	26.0	-18.3	12.4
MAD 16.1	5.0	-17.2	15.3
MAD 16.2	19.8	-17.8	15.9
MAD 16.3	34.6	-18.7	16.1
MAD 17.1	3.2	-16.9	13.8
MAD 17.2	14.3	-17.3	14.6
MAD 17.3	25.4	-17.4	14.3
MAD 17.4	35.5	-17.6	13.1
Mean (*n* = 53)		**-17.7**	**14.2**

Table 6.3: Stable carbon and nitrogen isotope data for all samples (*n* = 53).

Figure 6.1 shows that variability in $\delta^{15}N$ values is not isolated to a single age group, however, the range of values is greatest around the age of three years (i.e. 36 months). As well, this figure shows that $\delta^{15}N$ values are higher within the first 10 months of age,

specifically between 5 and 14.9 months. Values tend to become lower as age increases, with some exceptions. A comparison of means using a Mann-Whitney U test showed that the $\delta^{15}N$ value of dentine developed from eight months *in utero* to 14.9 months of age was significantly higher than dentine developed from 15 to 35.5 months of age ($U = 194.5$, $p = 0.006$).

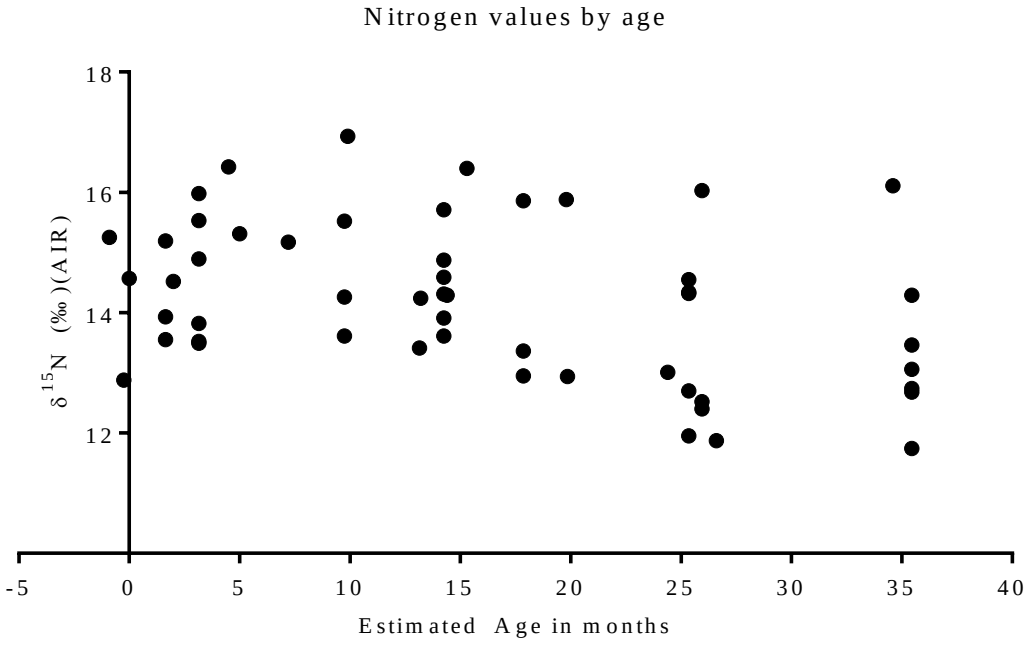
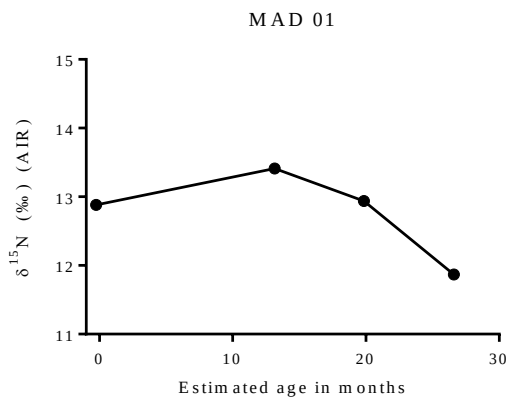
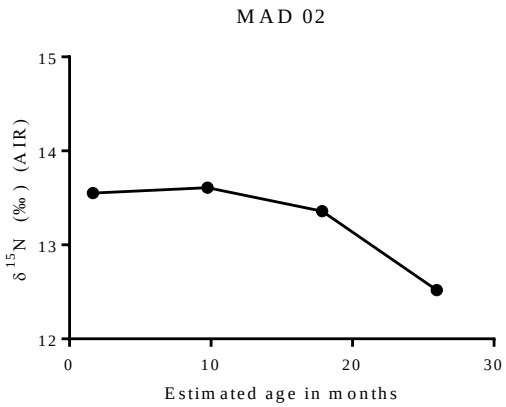


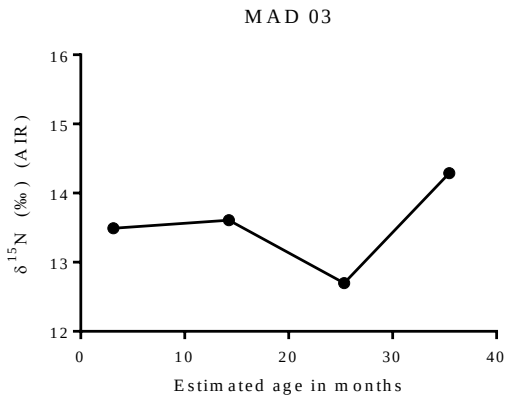
Figure 6.1: Scatter of all samples ($n = 53$) plotting $\delta^{15}N$ against estimated age in months

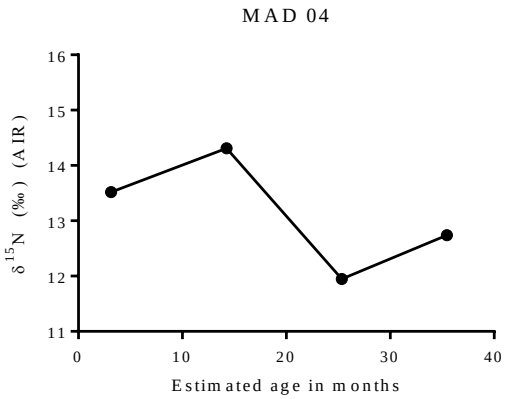
Nitrogen isotope data for the serial section of each individual tooth are plotted alongside the estimated mean age for each section in order to create isotopic profiles for each tooth. These charts are presented in Figure 6.2. From these individual profiles, two trends in the data emerge. The first trend is present in two individuals (MAD 06 and 16), seen as an increase in $\delta^{15}N$ values as the individual ages, with no discernible decrease at

any age. The second trend is a gradual decrease in $\delta^{15}N$ values over time (observed in MAD 01, 02, 04, 08, 09, 10, 14, 15, 17). A number of individuals that fit into this second trend do, however, display a small increase in $\delta^{15}N$ in the first year of life. This is likely due to the maternal isotopic signal found in the occlusal most section of dentine (formed *in utero*) blending with the dentine formed after birth enriched in ^{15}N due to breastfeeding. Individuals MAD 03, 05, and 07 have isotope profiles that do not fit into either of these observed trends. MAD 03 shows steady $\delta^{15}N$ values in the first year of life, followed by a decrease at around two years old and a sharp increase between two and three years of age. MAD 05 shows a $\delta^{15}N$ increases in the first six months, followed by a decrease by 14 months of age. Data from MAD 05 are limited as only three samples were obtained and the sample time span is limited. MAD 07 has a pattern similar to the first trend noted (i.e. a general increase in $\delta^{15}N$ values with age); however, there is a decrease in $\delta^{15}N$ values between 10 and 15 months of age. Explanations for the variability observed in each of these samples will be discussed in the next chapter.

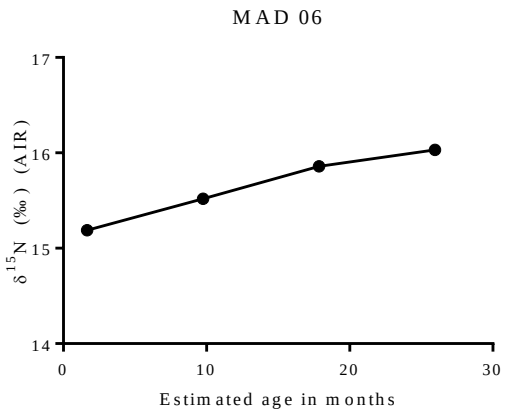


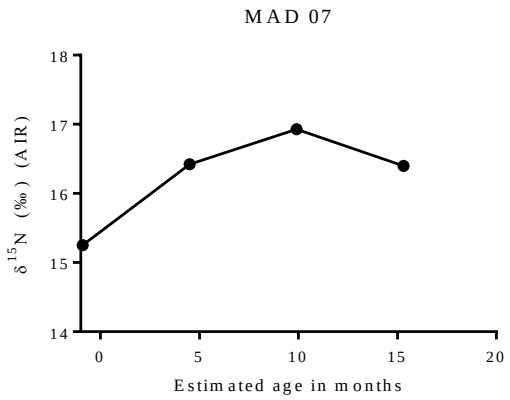



MAD 03
δ15N (‰) (AIR)
Estimated age in months


MAD 04
δ15N (‰) (AIR)
Estimated age in months


MAD 05
δ15N (‰) (AIR)
Estimated age in months


MAD 06
δ15N (‰) (AIR)
Estimated age in months


MAD 07
δ15N (‰) (AIR)
Estimated age in months


MAD 08
δ15N (‰) (AIR)
Estimated age in months

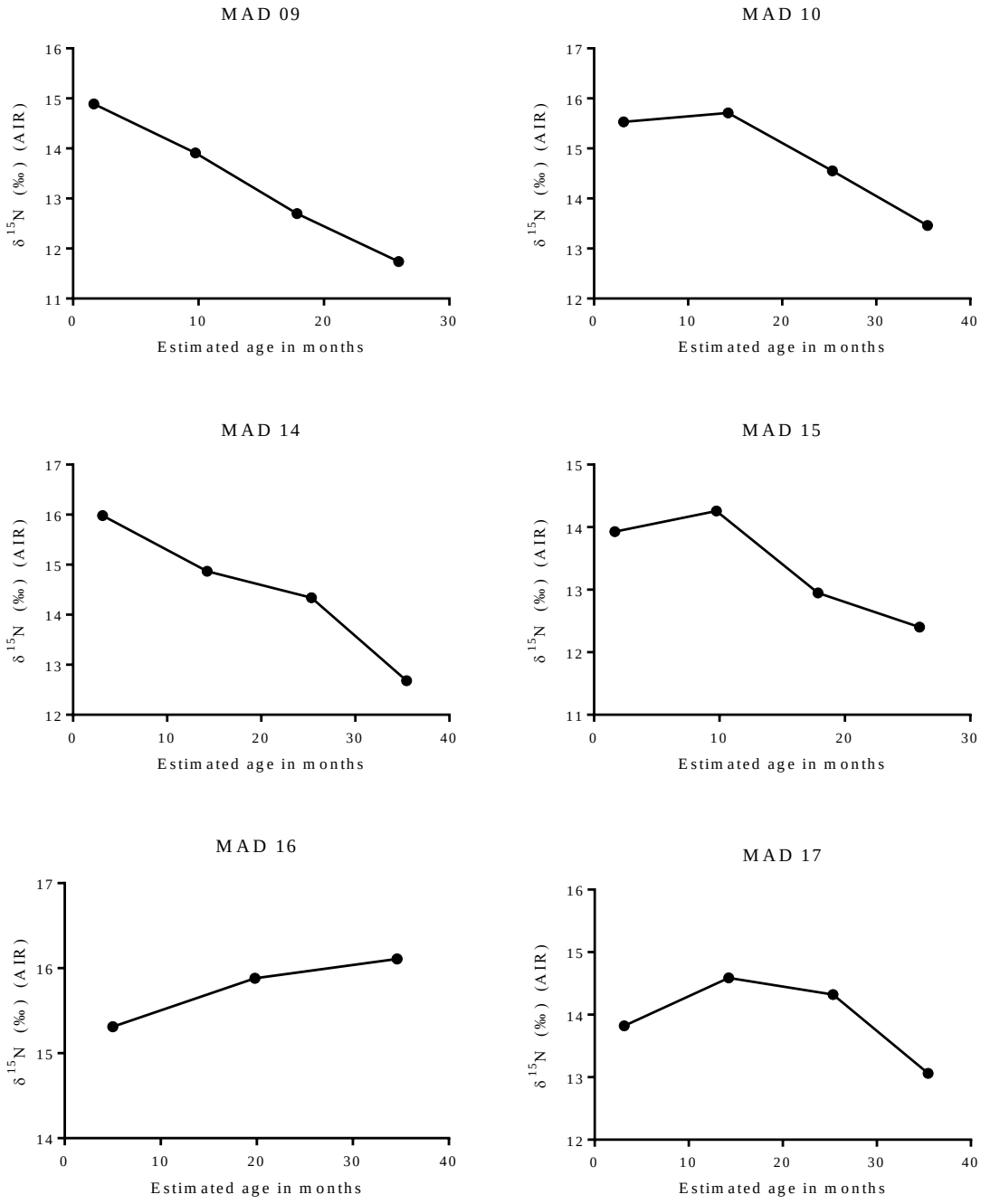


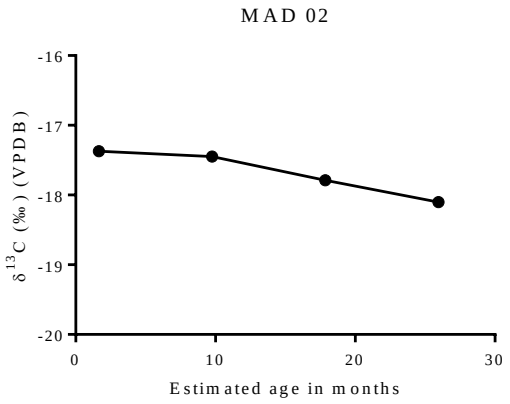
Figure 6.2: Individual nitrogen isotope histories for all individual teeth sampled. Nitrogen isotope data are plotted against the approximate mean age in months for each tooth section.

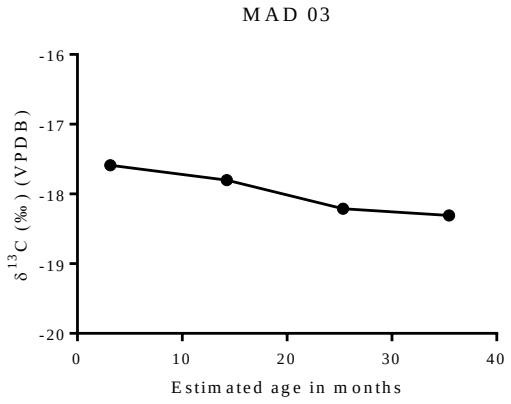
6.3.2 Carbon

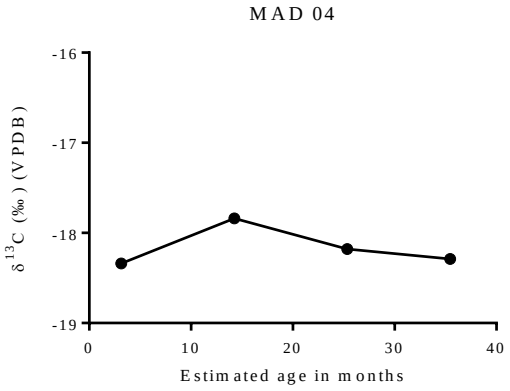
In comparison to nitrogen values, carbon isotope values show considerably less variation. The isotopic data are presented in Table 6.3, $\delta^{13}C$ values range from -18.8‰ to -16.6‰ with a mean value of -17.7‰. When considering breastfeeding behaviour, less variation is observed in $\delta^{13}C$ values as carbon undergoes a smaller trophic level effect of ~1‰ during breastfeeding in contrast to the ~3‰ shift observed in $\delta^{15}N$ values (Fuller et al., 2006). Similar to $\delta^{15}N$ values, the $\delta^{13}C$ values from dentine developed between eight months *in utero* to 14.9 months of age are significantly higher than values obtained from the 15 to 35.5 month tooth sections ($U = 52.5$; $p = 0.000$).

Individual carbon profiles for each tooth sampled are provided in Figure 6.3. Eight of the teeth (MAD 02, 03, 08, 09, 10, 15, 16, and 17) demonstrate a trend in which $\delta^{13}C$ values steadily decrease as age increases. Similar to this pattern seen in nitrogen profiles, it suggests evidence of breastfeeding followed by weaning. MAD 01, 04, 05, 06, and 07 all display a slight increase in $\delta^{13}C$ values around 10 – 12 months of age, followed by decreasing $\delta^{13}C$ values as age progresses. One profile (MAD 14) shows a unique pattern not seen in any other individual, where $\delta^{13}C$ values decrease in the first 12 months, increase throughout the second year, and finally decrease by age three.

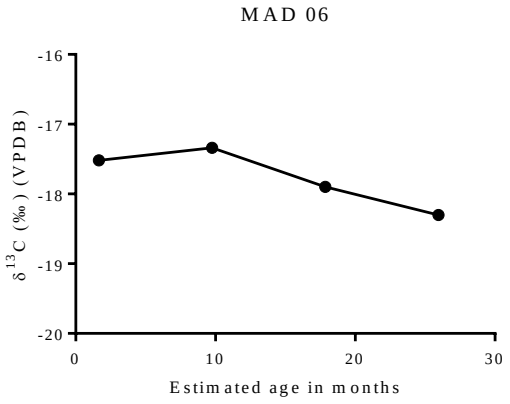

MAD 01
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
10
20
30
Estimated age in months

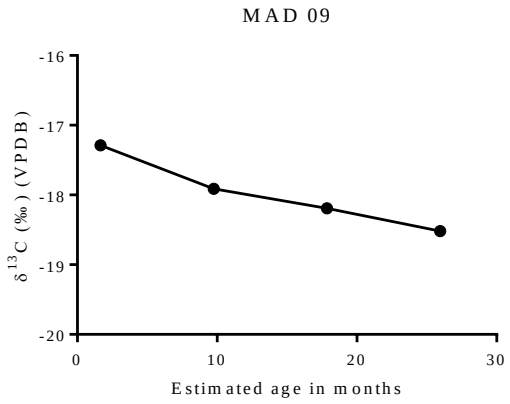

MAD 02
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
10
20
30
Estimated age in months

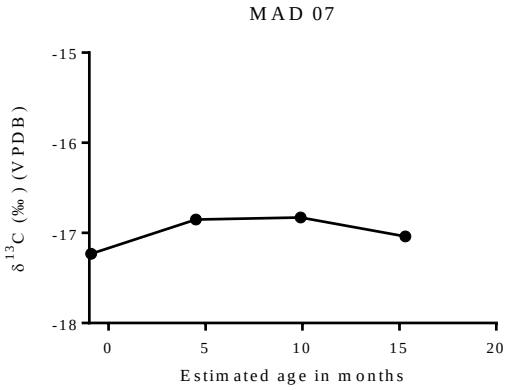

MAD 03
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
10
20
30
40
Estimated age in months

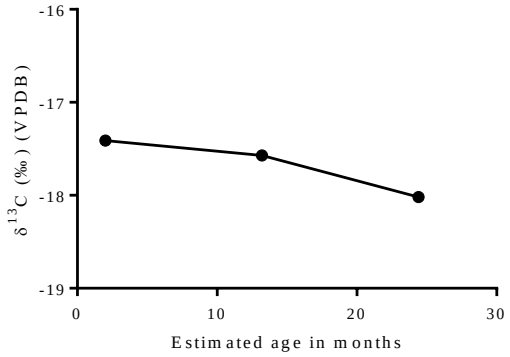

MAD 04
δ13C (‰) (VPDB)
-16
-17
-18
-19
0
10
20
30
40
Estimated age in months

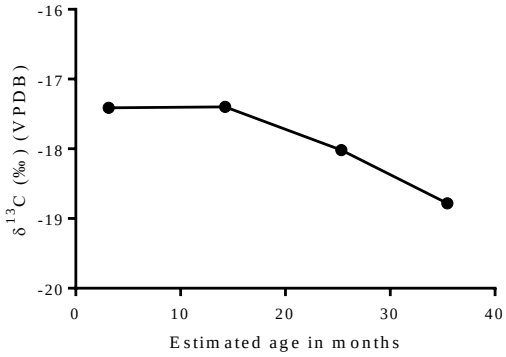

MAD 05
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
5
10
15
20
Estimated age in months

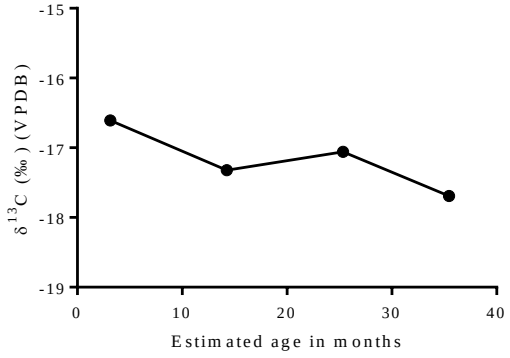

MAD 06
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
10
20
30
Estimated age in months

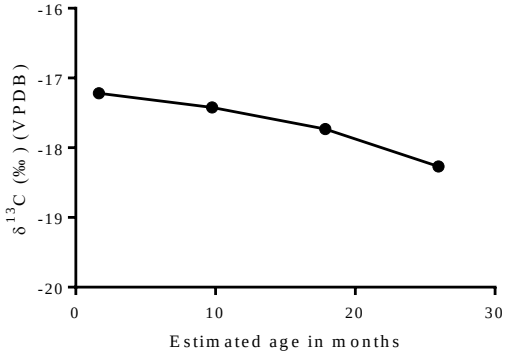

MAD 09
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
10
20
30
Estimated age in months


MAD 07
δ13C (‰) (VPDB)
-15
-16
-17
-18
0
5
10
15
20
Estimated age in months


MAD 08
δ13C (‰) (VPDB)
-16
-17
-18
-19
0
10
20
30
Estimated age in months


MAD 10
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
10
20
30
40
Estimated age in months


MAD 14
δ13C (‰) (VPDB)
-15
-16
-17
-18
-19
0
10
20
30
40
Estimated age in months


MAD 15
δ13C (‰) (VPDB)
-16
-17
-18
-19
-20
0
10
20
30
Estimated age in months

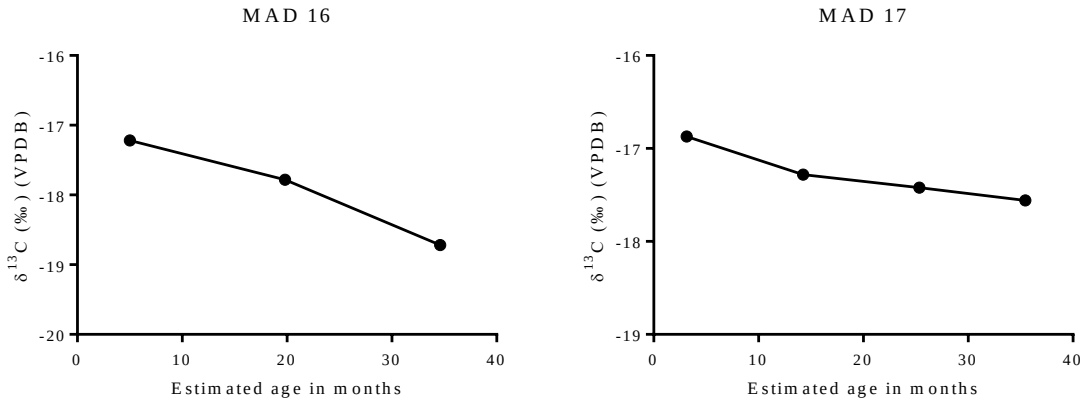


Figure 6.3: Individual carbon isotopic histories for all individual teeth sampled. Carbon isotope data are plotted against the estimated mean age in months for each sample

6.4 Carbon and nitrogen integration

While nitrogen and carbon isotope profiles for each tooth have been examined individually, it is important to consider how the two isotopes vary together. Due to the enrichment effect breastfeeding has on both $\delta^{15}N$ (~3‰) and $\delta^{13}C$ (~1‰) values, one would expect $\delta^{13}C$ and $\delta^{15}N$ values to exhibit similar patterns as age increases, with breastfeeding and weaning trends being more pronounced in the nitrogen values. While this trend is, in general, seen in the teeth sampled in this thesis, there are outliers. Figure 6.4 shows the carbon and nitrogen profiles for MAD 01, one of the individuals that demonstrates the expected similarities between the nitrogen and carbon patterning, that is, an increase in $\delta^{13}C$ and $\delta^{15}N$ values after birth followed by a gradual decline in $\delta^{13}C$ and $\delta^{15}N$ values that suggests the weaning process had started. For MAD 01, the peak $\delta^{13}C$ and $\delta^{15}N$ values occur at approximately 13 months of age, after which time they start to decline. MAD 01, 02, 05, 07, 08, 09, 10, 14, and 15 all demonstrate this similar pattern. In two of the teeth sampled (MAD 06 and 16) an inverse relationship is seen between the $\delta^{13}C$ and $\delta^{15}N$

values, as demonstrated by MAD 16 in Figure 6.5. In both these individuals, the $\delta^{15}N$ values increase with age, while $\delta^{13}C$ values decrease with respect to age.

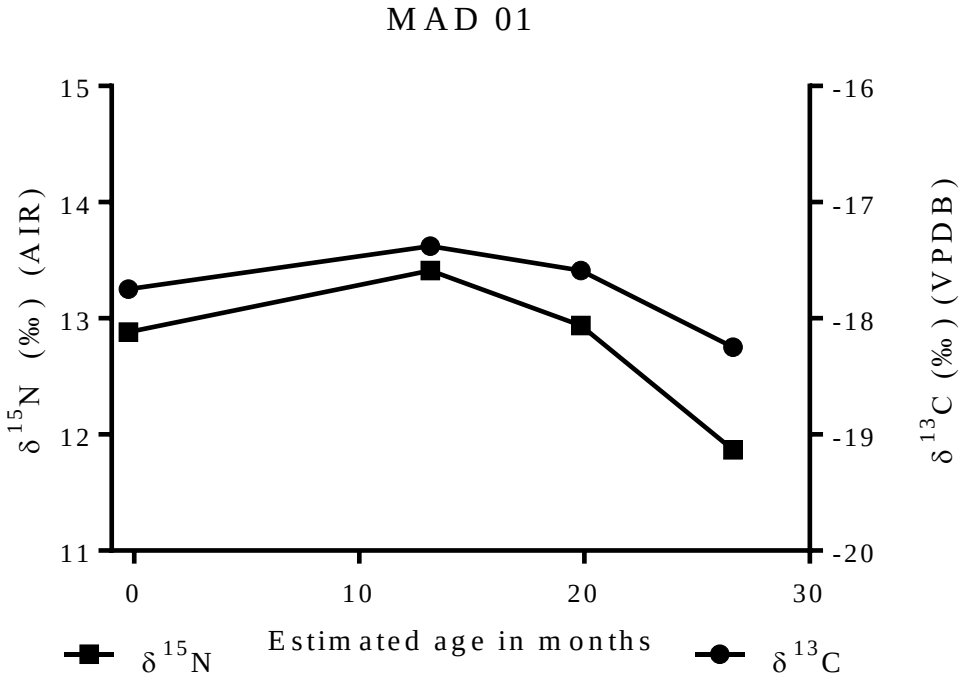


Figure 6.4: Combined carbon and nitrogen isotope profile for MAD 01. Both $\delta^{13}C$ and $\delta^{15}N$ data are plotted against the mean estimated age in months.

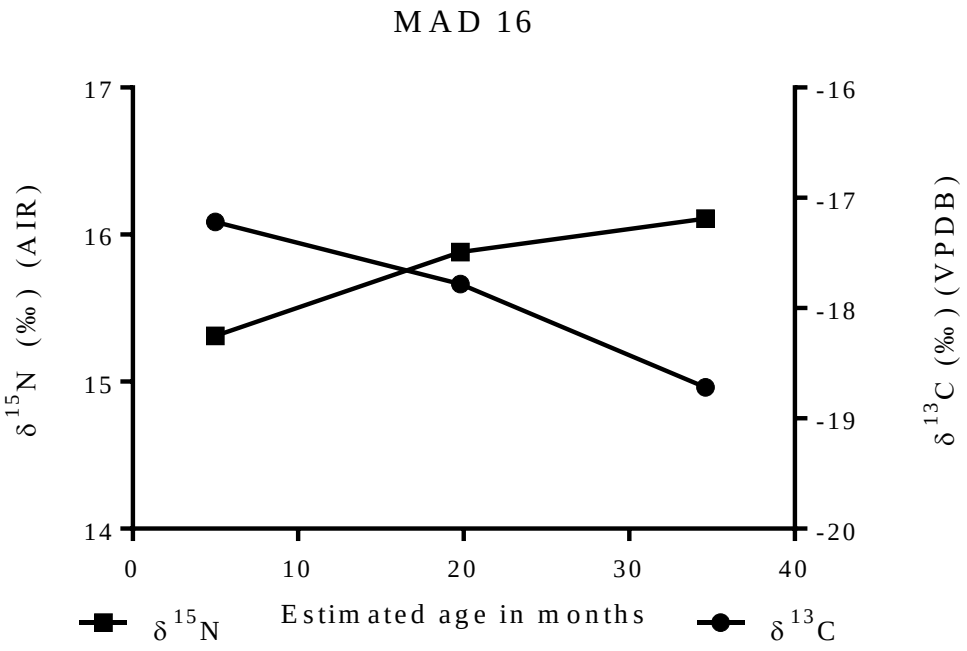


Figure 6.5: Combined carbon and nitrogen isotope profile for MAD 16. Both $\delta^{13}C$ and $\delta^{15}N$ data are plotted against the mean estimated age in months.

The remainder of the combined carbon and nitrogen profiles demonstrate unique patterns that do not fit into those exemplified by MAD 01 and MAD 16. The multi-isotope profile for MAD 03 (Figure 6.6) appears to follow no discernible pattern. Initially, $\delta^{15}N$ values undergo a slight increase while $\delta^{13}C$ decreases, however, the magnitude of both these changes is minimal (>0.5‰). This is followed by a paired decrease in carbon and nitrogen values. Finally, between the ages of two and three, $\delta^{13}C$ decreases again, while $\delta^{15}N$ values sharply increase.

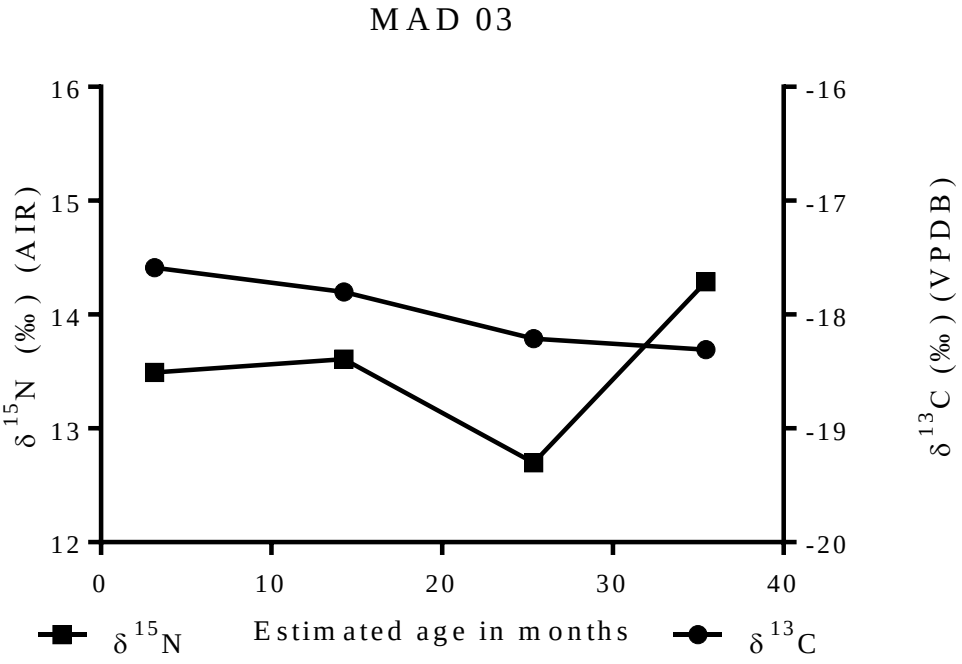


Figure 6.6: Combined carbon and nitrogen isotope profile for MAD 03. Both $\delta^{13}C$ and $\delta^{15}N$ data are plotted against the mean estimated age in months.

For MAD 04 (Figure 6.7), both carbon and nitrogen experience a rise then fall in the first two years of life, however, during the last year of development, $\delta^{13}C$ values continue to fall as $\delta^{15}N$ slightly increases. The final tooth, MAD 17 (Figure 6.8), shows that while carbon values steadily decrease with age, nitrogen values increase in the first year and decrease thereafter.

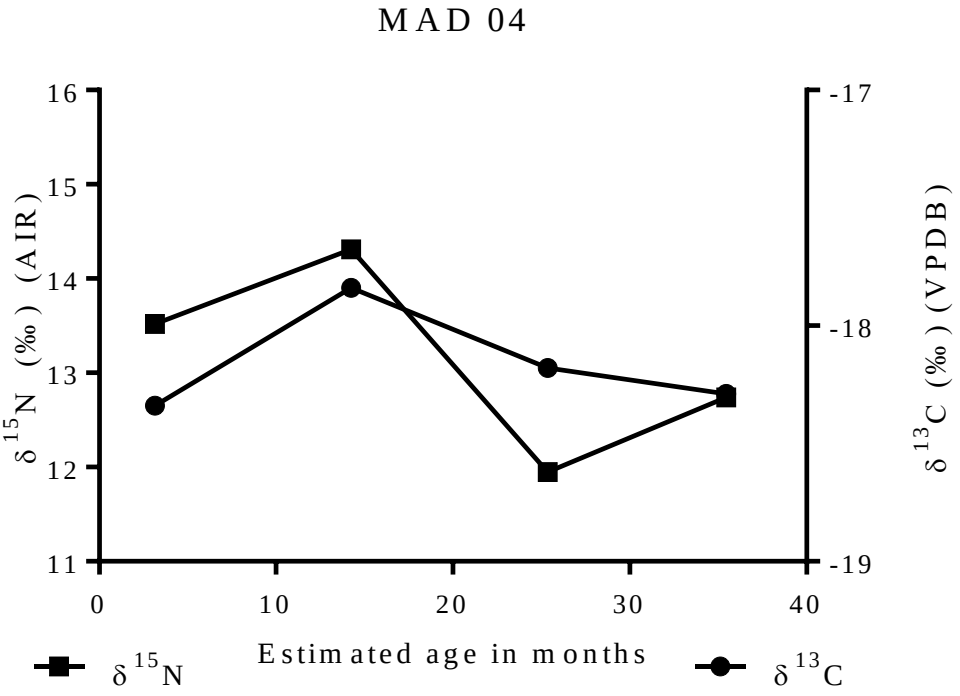


Figure 6.7: Combined carbon and nitrogen isotope profile for MAD 04. Both $\delta^{13}C$ and $\delta^{15}N$ data are plotted against the mean estimated age in months.

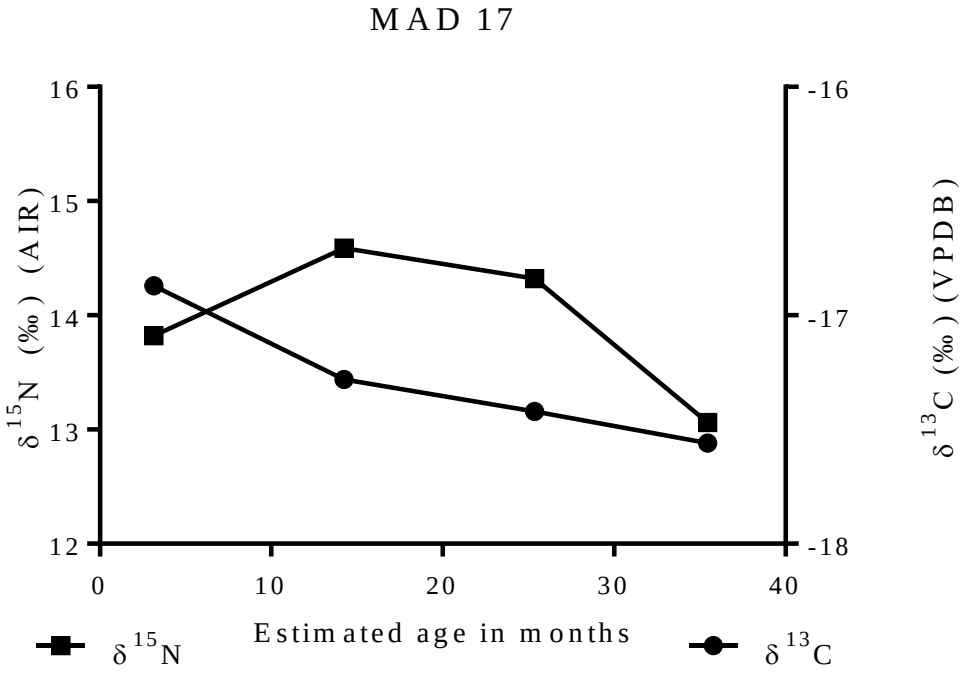


Figure 6.8: Combined carbon and nitrogen isotope profile for MAD 17. Both $\delta^{13}C$ and $\delta^{15}N$ data are plotted against the mean estimated age in months.

6.5 Multi-tooth analysis

For 10 of the individuals sampled as part of this thesis, only one tooth was selected for isotope analysis, however, two individuals had both their deciduous central incisor and deciduous first molar sampled for isotope analysis. This was to extend the timeframe, due to the fact incisors finish development before the first molar (2.5 and 3.5 years, respectively), as well as allow for the examination of inter-tooth variation in $\delta^{15}N$ and $\delta^{13}C$ patterns. Figures 6.9 and 6.10 present combined $\delta^{13}C$ and $\delta^{15}N$ plots for each individual.

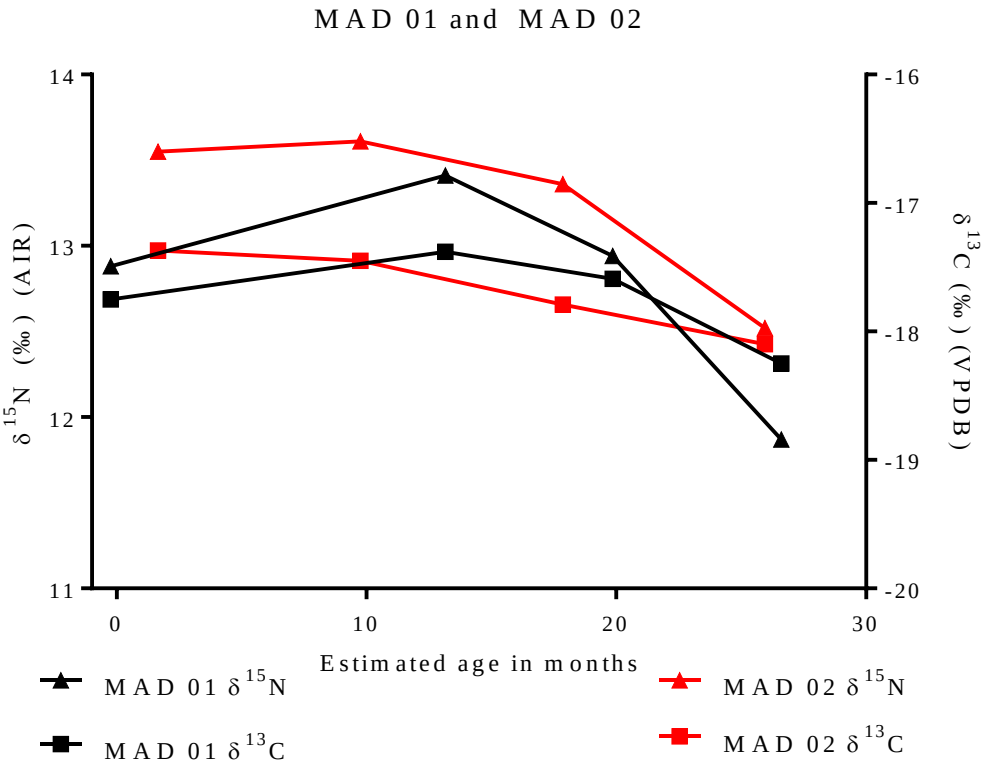


Figure 6.9: Carbon and nitrogen data for MAD 01 and MAD 02, from one individual, plotted together against approximate age in months. MAD 01 represents di[1], MAD 02 represents dm[1].

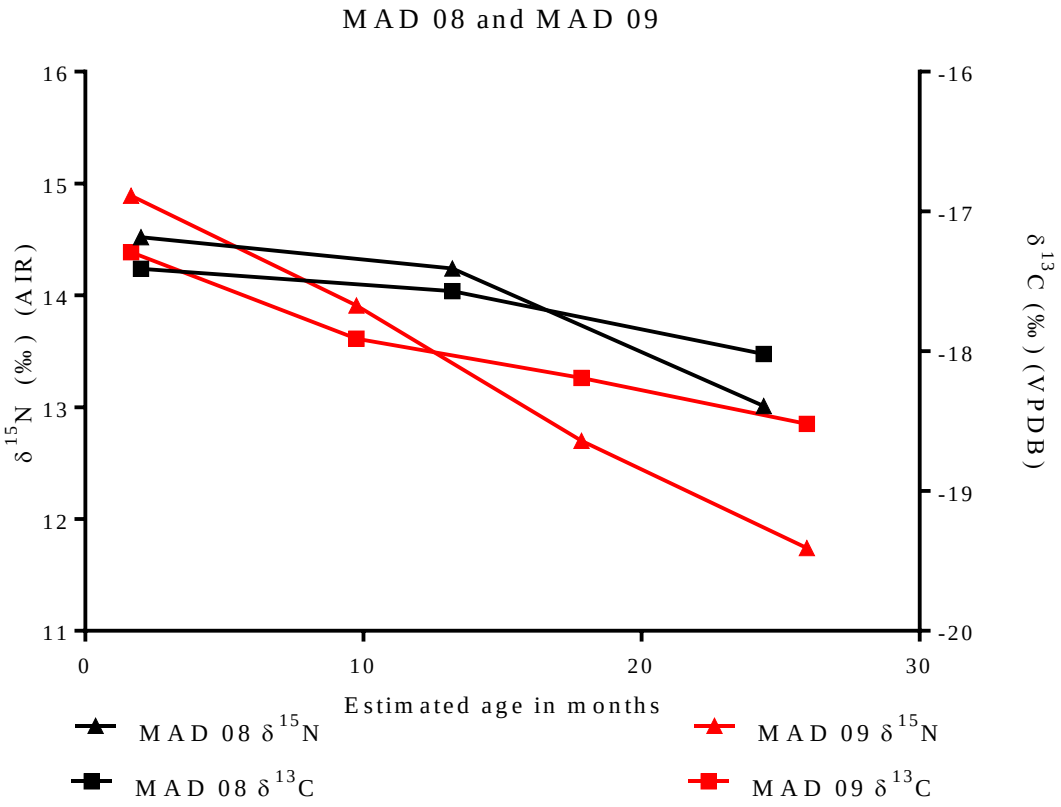


Figure 6.10: Carbon and nitrogen data for MAD 08 and MAD 09, from one individual, plotted together against approximate age in months. MAD 08 represents di[1], MAD 09 represents dm[1].

The individual represented by MAD 01 and MAD 02 has a consistent $\delta^{15}N$ pattern between the two teeth, both increasing initially before decreasing between remaining samples. The $\delta^{13}C$ patterns slightly vary between the two teeth, MAD 01 increasing at first before continuing to decrease, while MAD 02 strictly decreases with age. Serial sample MAD 01.1 (deciduous incisor) represents the approximate mean age of -0.3 months with a $\delta^{15}N$ value of 12.9‰, while sample MAD 02.1 (deciduous molar), approximately aged 1.7 months, has a $\delta^{15}N$ value of 13.6‰, showing a variation of 0.7‰. This variation is likely caused by the earlier age of growth initialization in deciduous incisors, which results in the

maternal nitrogen value influencing the infant value. Samples MAD 01.5 and MAD 02.4 show a total variation of 0.6‰ with approximate mean ages being 26.6 and 26.0 months, respectively. The variation in the remaining serial samples is lower and can be attributed to the gradual decrease in $\delta^{15}N$ associated with weaning, or variation associated with measurement error. The carbon isotope values are more closely grouped, with the earliest forming dentine samples showing the greatest degree of variation, just as seen with the $\delta^{15}N$ values; the total variation between these first samples is 0.4‰ and can again be attributed to maternal carbon values impacting infant values. All other serial samples show a variation of 0.1‰ – 0.2‰, which is likely due to the lack of overlap in mean approximate ages and/or variability due to measurement error.

The individual represented by MAD 08 and MAD 09 shows consistent patterning in both $\delta^{13}C$ and $\delta^{15}N$ values, that being a steady decline in values with age (Figure 6.10). Both with carbon and nitrogen, the variation between the two teeth is low; for $\delta^{15}N$ the variation ranges from 0.3‰ to 0.4‰, while $\delta^{13}C$ ranges from 0.1‰ to 0.3‰. Again, the greatest variation in $\delta^{15}N$ values is in the earliest samples, possibly showing the influence of maternal nitrogen, but since the shifts in nitrogen values are so small this may also be influenced by measurement error.

6.6 Conclusions

The stable isotope data presented in this chapter shows that the individuals interred at the Trinitarias crypt demonstrate nitrogen and carbon enrichment and patterns of change that are indicative of breastfeeding and eventual weaning. Greater variation is seen in the $\delta^{15}N$ values as expected due to the known ~3‰ enrichment associated with breastfeeding.

Carbon isotope profiles also indicate the likely presence of breastfeeding, often demonstrating patterns similar to those seen with $\delta^{15}N$ values. There are, however, a small number of isotope profiles (MAD 03, 04, 06, and 16) that do not fit the expected pattern associated with breastfeeding and weaning. The examination of $\delta^{13}C$ and $\delta^{15}N$ in unison has shown that variation is present between carbon and nitrogen patterning which can be explained by a number of different scenarios, outlined in the Chapter Seven.

Chapter 7: Discussion

7.1 Introduction

This chapter integrates the stable isotope results presented in Chapter Six with the historical and archaeological context for breastfeeding and weaning in nineteenth century Spain. First, the individual isotope profiles will be discussed and interpreted allowing for the creation of individual feeding histories. The practices and views surrounding breastfeeding in nineteenth century Madrid, Spain, will be discussed using these feeding histories and the historical literature. Since many of the individuals interred in this crypt had skeletal evidence of rickets, the stable isotope results will be combined with existing data on vitamin D deficiency to assess any correlation between the two. Finally, the limitations of this research will be presented and discussed.

7.2 Individual infant and childhood feeing histories

Before beginning to look at the trends in breastfeeding practices that occurred in nineteenth century Madrid, this section presents the feeding histories for each individual. This aims to contextualize the numbers and trends seen in Chapter Six and allows for the individual determination of breastfeeding and weaning histories. Table 7.1 provides a summary of the weaning trends observed in each individual alongside their vitamin D deficiency diagnosis.

Sample ID	Age at start of weaning (in months)	Age when weaning was complete (in months)	Skeletal evidence of rickets?
MAD 01/02	10	Died during	No
MAD 03	14	*	No
MAD 04	14	36	No
MAD 05	7	Died during	Yes
MAD 06	10	Died during	Yes
MAD 07	10	Died during	Yes
MAD 08/09	*	Died during	Yes
MAD 10	14	36	Yes
MAD 14	*	36	Yes
MAD 15	10	Died during	Yes
MAD 16	*	*	Yes
MAD 17	14	*	Yes

Table 7.1: Individual weaning histories summarized, and their vitamin D status based on the finding by Ríos et al. (2016), * indicated that an age estimate was not possible due to unusual $\delta^{15}N$ and $\delta^{13}C$ patterns (e.g. stress-induced $\delta^{15}N$ increases).

7.2.1 MAD 01/02

The teeth MAD 01 and MAD 02 represent a single individual, MAD 01 is a deciduous central incisor, while MAD 02 is a deciduous first molar. Based on an increase in $\delta^{15}N$ values from birth to around 13 months of age, it is likely that this individual was breastfed during the first year of life. The steady decline in both $\delta^{13}C$ and $\delta^{15}N$ (Fig. 7.1) values in the remaining dentine sections indicate that this child likely began the weaning process after 14 months of age. This individual was estimated to be approximately 2.5 years of age at the time of death. The total decline in $\delta^{15}N$ values from one year of age to death is 1.7 ‰. While this is close to the 2‰ – 3‰ offset expected with weaning, it is likely that this individual died before weaning was complete. Carbon isotope values, however, decline 0.9‰ between one year and death. This is consistent with the hypothesis that this individual began consuming less breastmilk by 10 - 13 months of age and was still

weaning at the time of death. The respectively sharper, nearly 1‰, decrease in $\delta^{13}C$ values is potentially caused by carbon isotopes declining earlier than nitrogen values (Fuller et al., 2006). As well, the $\delta^{13}C$ values from MAD 01 show an initial increase between birth and 13 months, while values from MAD 02 decline immediately. This is most likely due to the fact that deciduous incisors begin development before molars, thus the first sample from MAD 01 contained more *in utero* dentine, thus reducing the $\delta^{13}C$ value of the first sample.

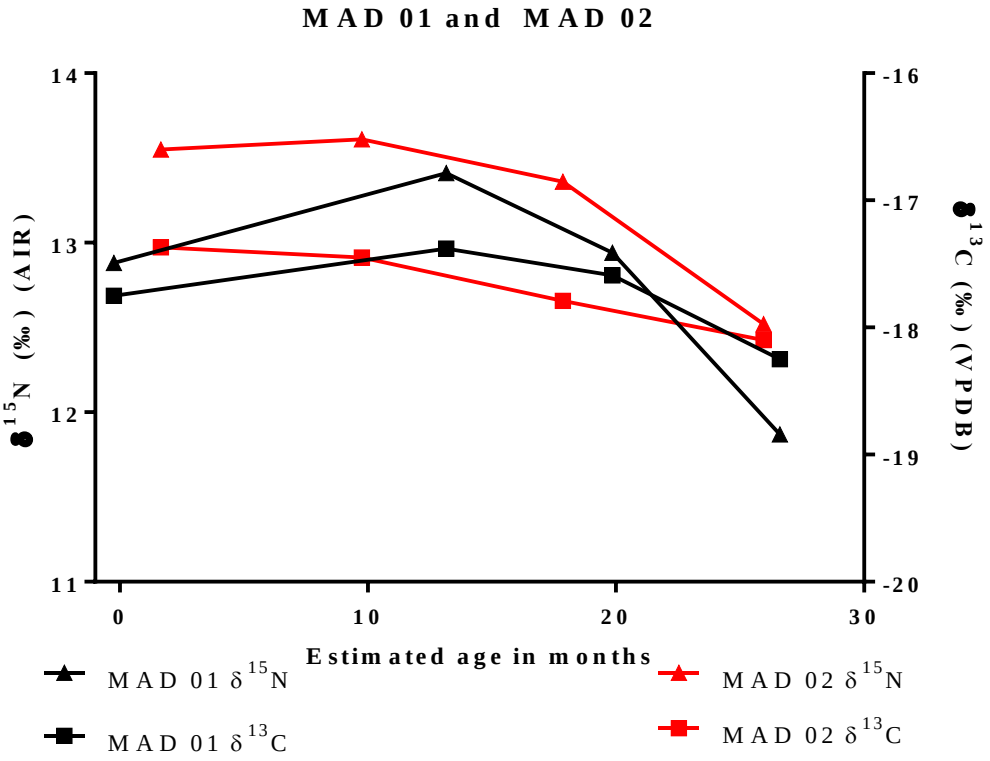


Figure 7.1: Carbon and nitrogen isotope profile for MAD 01 (deciduous central incisor) and MAD 02 (deciduous first molar). Isotope data are plotted against estimated age in months.

7.2.2 MAD 03

This individual, represented by a deciduous first molar, shows a slight increase in $\delta^{15}N$ values from birth to approximately 14 months (by 0.1‰), followed by a 0.9‰ decrease in $\delta^{15}N$ values by 24 months. MAD 03 then demonstrates a unique spike in $\delta^{15}N$ by 35.5 months (roughly three years), increasing by 1.6‰. While the decrease in $\delta^{15}N$ is lower than would be expected during weaning, this can be attributed to a number of causes. First, it could be due to the practice of initial mixed feeding or early supplemental feeding, but still with some contribution of breastmilk to the infant's diet. Research carried out by Fuller and colleagues (2006) demonstrated that individuals who were simultaneously breastfed and bottle-fed displayed a decrease in $\delta^{15}N$ associated with weaning that was reduced to roughly 1‰. However, the spike in $\delta^{15}N$ at age three also provides insight to the minimal decline in nitrogen values associated with weaning. It is most likely that the cause of the spike in $\delta^{15}N$ is stress related. In contrast to chronic stress, it appears as though this individual experienced a period of stress between the ages of two and three years, during or succeeding the weaning process. Stress is a more likely cause of this spike due to the magnitude of the increase and the lack of change in $\delta^{13}C$ values; if the change were dietary, there would be an associated increase in $\delta^{13}C$.

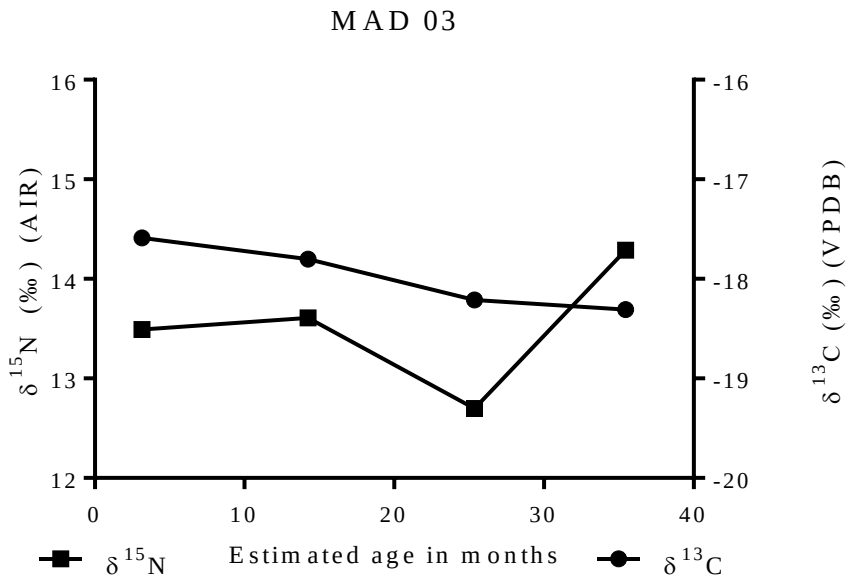


Figure 7.2: Carbon and nitrogen isotope profile for MAD 03. Isotope data are plotted against estimated age in months.

The increase in $\delta^{15}N$ seen in this individual is consistent with patterns seen in studies on the effect of nutritional stress on nitrogen isotope values. The magnitude of the increase here is 1.6‰, while on the higher end of their range (0.17 – 1.93‰), this fits within the values reported in Neuberger et al.'s (2013) study of modern human hair in cases of known malnourishment and starvation. In this study, individuals showed, on average, a 0.54‰ increase in $\delta^{15}N$ values during periods of nutritional stress. Similarly, MAD 03 demonstrates the decrease in $\delta^{13}C$ alongside the increasing $\delta^{15}N$. This has also been seen before in incremental dentine samples from individuals associated with the nineteenth century Great Irish Potato Famine, in the work of Beaumont and Montgomery (2016). Their study found that the $\delta^{15}N$ values of children increased prior to the introduction of famine relief foods (maize), and they explain that the most likely cause of this increase is

nutrition stress, as long as no corresponding increase in $\delta^{13}C$ is present. The increase in $\delta^{15}N$ associated with stress is caused by the body being in a state of catabolism; without enough protein, the body draws protein from its own tissue, where a second trophic level effect occurs resulting in higher $\delta^{15}N$ values.

7.2.3 MAD 04

MAD 04 demonstrates the expected pattern for a breastfed and weaned individual. The highest $\delta^{15}N$ value is at around 12 months, indicating this individual likely acquired a significant amount of their nutrition from breastmilk in the first year of life (Fig. 7.3). The following 2.4‰ decrease in $\delta^{15}N$ shows weaning occurred between the first and second year. A slight increase of 0.7‰ in $\delta^{15}N$ by the age of three could be related to stress (as discussed in section 7.2.2), or a ^{15}N-enriched post-weaning diet. Again, stress is a possible cause due to the magnitude of the increase and the corresponding $\delta^{13}C$ decrease. King and colleagues (2018) found a similar pattern in an individual from the Atacama Desert, Chile. Dietary change was ruled out due to decreasing $\delta^{13}C$ values, and stress was the most likely explanation because of the close proximity of the increase and age of death. A ^{15}N-enriched weaning diet could also account for the increase in $\delta^{15}N$, however, as just mentioned, a dietary shift would have an associated increase in $\delta^{13}C$. Maternal and population isotope data would help clarify this pattern, but since this individual died around the time this tooth finished development, it is most likely that this individual experienced a period of nutritional stress leading up to death.

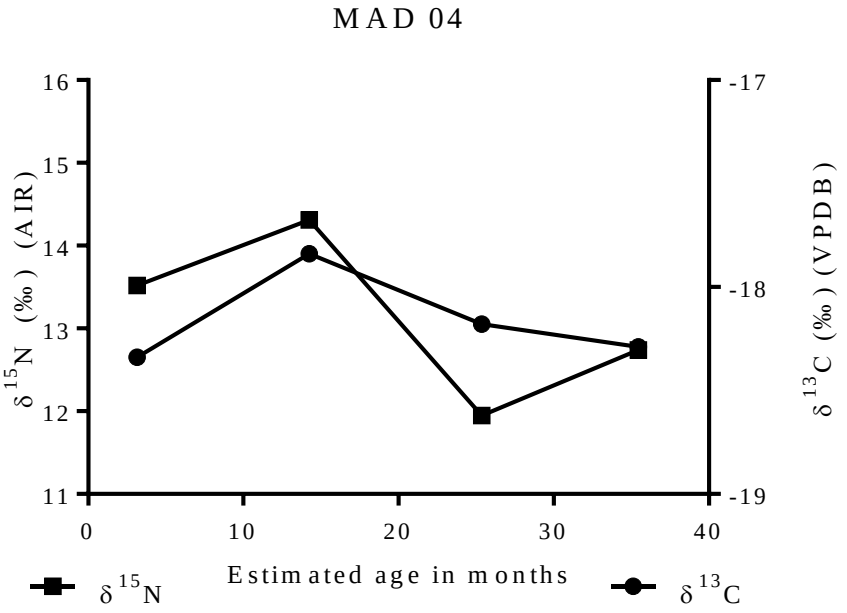


Figure 7.3: Carbon and nitrogen isotope profile for MAD 04. Isotope data are plotted against estimated age in months.

7.2.4 MAD 05

This individual died at approximately 18 months and is represented by an incomplete central incisor. The $\delta^{15}N$ values in the last two dentine sections show a decline of 0.9‰, between seven and 14 months, after reaching a peak of 15.2‰ (Fig. 7.4). This pattern suggests that breastmilk played a key dietary role until around seven months of age, at which time weaning began. Since the final dentine sample represents dentine that was developing at the time of death, and the fact the decrease in $\delta^{15}N$ value is only 0.9‰, it is possible that this individual died during the process of weaning.

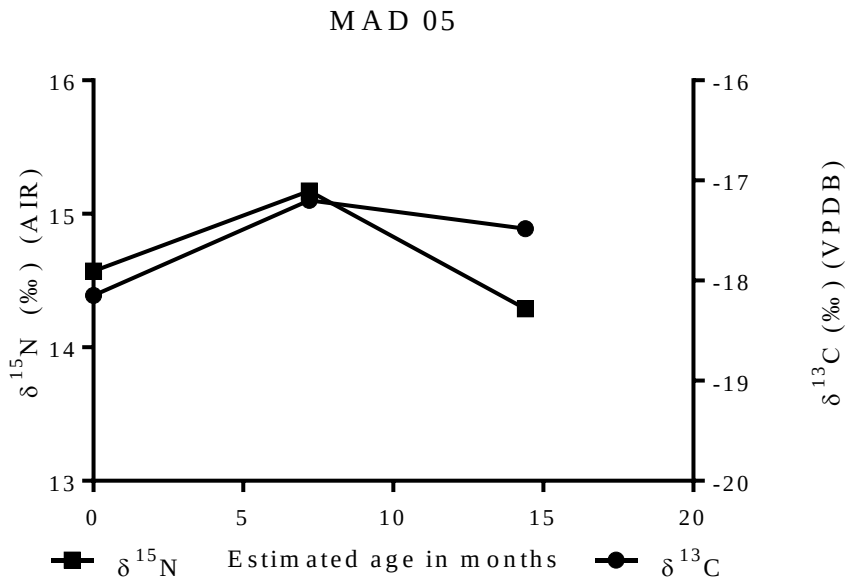


Figure 7.4: Carbon and nitrogen isotope profile for MAD 05. Isotope data are plotted against estimated age in months.

7.2.5 MAD 06

MAD 06 shows unique patterning in their $\delta^{13}C$ and $\delta^{15}N$ values, specifically that the carbon values decrease with age while the nitrogen values increase (Fig. 7.5). The $\delta^{13}C$ values show a pattern consistent with an expected weaning curve, with a decline in values beginning at around 10 months of age, marking the reduction of breastmilk consumption and increased dependence on complementary foods, and ending with this individual's death at approximately 2.5 years of age; the total decline in $\delta^{13}C$ values was 1.0‰. It is not possible to determine whether this individual had been completely weaned at the time of their death. While the unusual $\delta^{15}N$ pattern demonstrated here could be caused by the introduction of high trophic level weaning foods, the potential of stress-induced $\delta^{15}N$

enrichment must also be considered. Beaumont et al. (2015), Beaumont and Montgomery (2016), and King et al. (2018) report that increasing $\delta^{15}N$ values within incremental dentine samples that lack a corresponding increase in $\delta^{13}C$ values is likely indicative of stress-induced nitrogen enrichment in contrast to dietary change. Because $\delta^{13}C$ values decrease as the $\delta^{15}N$ values are increasing here, physiological stress and tissue catabolism is likely causing this individual's increase in nitrogen values.

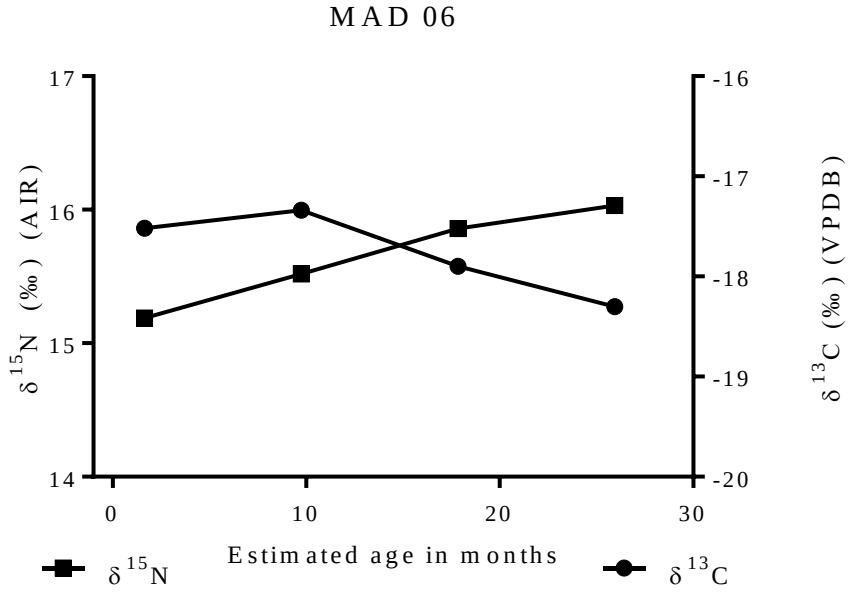


Figure 7.5: Carbon and nitrogen isotope profile for MAD 06. Isotope data are plotted against estimated age in months.

7.2.6 MAD 07

MAD 07 provides a dietary history of an individual who died around the age of 18 months. While the information is limited due to the relatively early age at death, the $\delta^{13}C$ and $\delta^{15}N$ profiles for this individual indicate that this individual was likely consuming

predominantly breastmilk until the age of 10 months, as indicated by both increasing $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 7.6). Following this increase both values begin to decline, indicating this individual likely died shortly after the initiation of weaning and introduction of complementary foods.

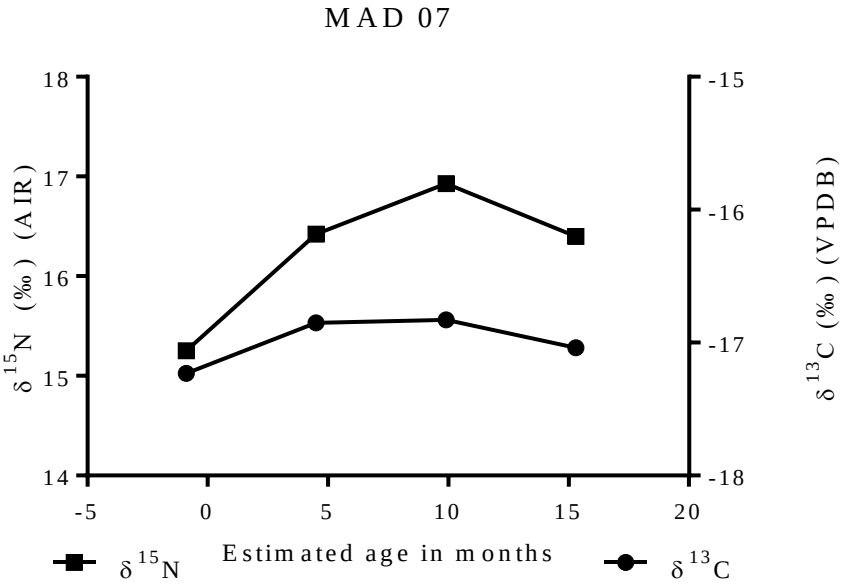


Figure 7.6: Carbon and nitrogen isotope profile for MAD 07. Isotope data are plotted against estimated age in months.

7.2.7 MAD 08/09

This individual is also represented by two teeth, a deciduous central incisor and a deciduous first molar. The $\delta^{13}C$ and $\delta^{15}N$ values of this individual begin to decrease immediately following birth (Fig. 7.7), without the expected increase in both isotopic values typically associated with breastfeeding. Decreasing values immediately following birth could be attributed to either elevated *in utero* $\delta^{15}N$ values, or a complete lack of

breastfeeding. If this was caused by a lack of breastfeeding, one would expect a sharp decrease in values, indicative of abrupt dietary change, in contrast to the gradual decrease observed here. King and colleagues (2018) note this same pattern in four individuals from the Atacama Desert where the decreasing $\delta^{15}N$ values following birth are interpreted to be linked with maternal stress. The first dentine samples from these individuals contain a portion of dentine that developed *in utero*. Dentine formed *in utero* is considered to represent short-term maternal $\delta^{13}C$ and $\delta^{15}N$ values at the time of development (Beaumont et al., 2015). Thus, if maternal $\delta^{15}N$ values are increased due to a particularly stressful pregnancy, the dentine formed during pregnancy would have higher $\delta^{15}N$ values resulting in the increase seen here. Birth would then remove the infant from the stressful environment, causing subsequent $\delta^{15}N$ values to be reflective of breastfeeding and weaning signals. Similarly, it is possible that this infant entered a state of catabolism immediately following its birth, resulting in the high $\delta^{15}N$ value observed in the early tooth sections. Without knowing the exact age in which this decrease began, it is not possible to determine which scenario is most likely. Based on the continued decrease in $\delta^{15}N$ values between 10 and 26 months with no evidence of $\delta^{15}N$ values leveling out, it is likely this individual died (around the age of 2.5) before being completely weaned off breastmilk.

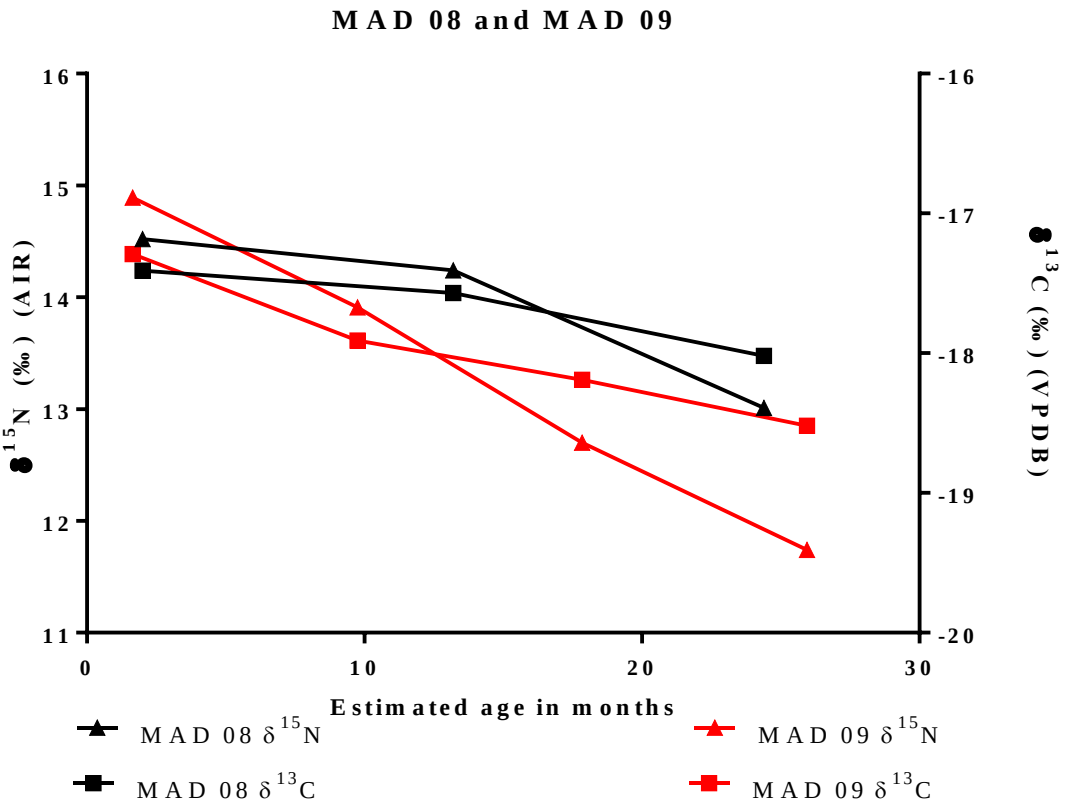


Figure 7.7: Carbon and nitrogen isotope profile for MAD 08 (deciduous central incisor) and MAD 09 (deciduous first molar). Isotope data are plotted against estimated age in months.

7.2.8 MAD 10

The serial samples from MAD 10 demonstrate the pattern expected from a breastfed then weaned individual. It is likely that breastmilk was a major dietary component during the first 14 months of life, after which it appears this individual began to be weaned and consume significantly more complementary foods. A drop of 2.4‰ in $\delta^{15}N$ and 1.4‰ in $\delta^{13}C$ between 14 months and roughly three years of age indicate that this individual was likely weaned off breastmilk entirely by the age of three (Fig. 7.8).

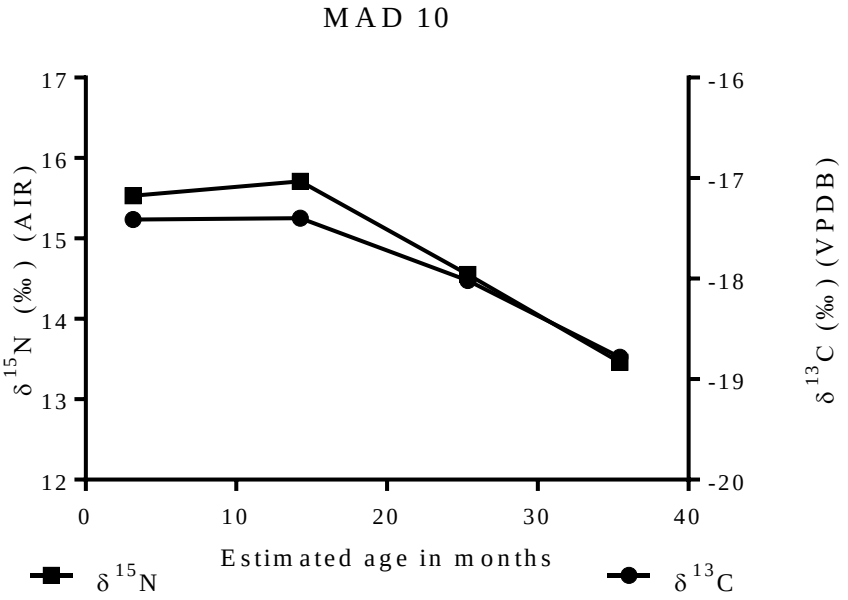


Figure 7.8: Carbon and nitrogen isotope profile for MAD 10. Isotope data are plotted against estimated age in months.

7.2.9 MAD 14

Similar to the individual represented by MAD 08 and 09, this individual has $\delta^{13}C$ and $\delta^{15}N$ values that decline from birth (Fig. 7.9), again this is likely due to high *in utero* $\delta^{15}N$ values and maternal stress. The decrease seen in both $\delta^{13}C$ and $\delta^{15}N$ over time indicates this individual was likely breastfed, however, the duration of the weaning period is difficult to assess. Due to the magnitude of the decrease in both $\delta^{13}C$ and $\delta^{15}N$, the weaning process was likely complete by the age of three.

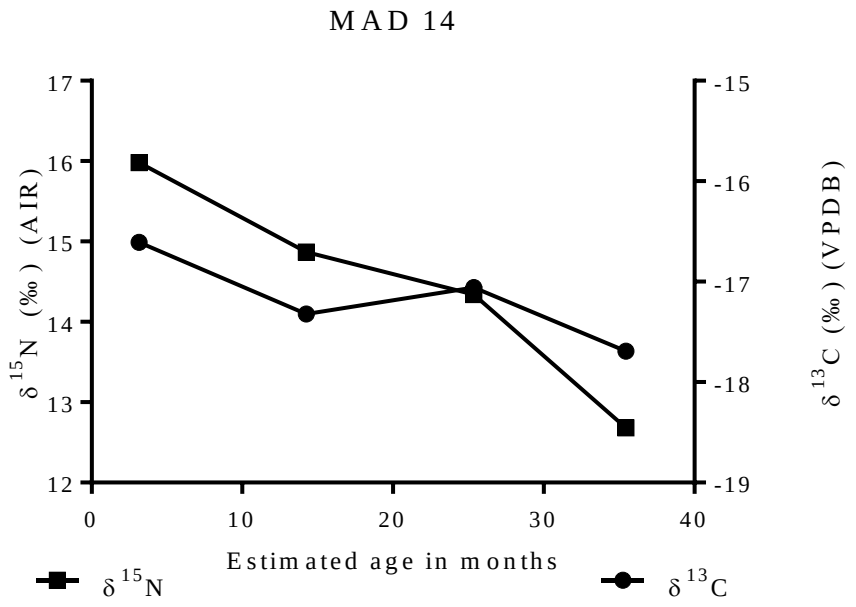


Figure 7.9: Carbon and nitrogen isotope profile for MAD 14. Isotope data are plotted against estimated age in months.

7.2.10 MAD 15

The $\delta^{15}N$ values from this individual show that breastmilk was likely the dominant nutritional source until around 10 months of age. While the decline in $\delta^{15}N$ after 10 months (1.9‰) fits within the expected range of 1.7 – 2.8‰ reported by Fuller and colleagues (2006) in their study of modern infant/mother pairs, it is difficult to say whether weaning was complete by the age of two, or if it was still ongoing. However, the steady decline in $\delta^{13}C$ values, with a magnitude of 1.1‰, show that it is possible the weaning process was complete by the time this individual was two years old, around the age this individual died (~2.5 years).

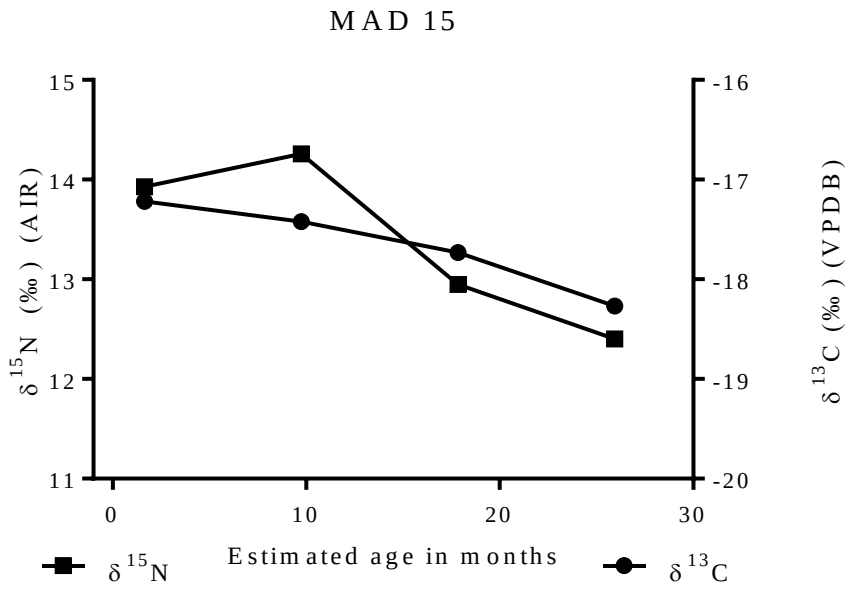


Figure 7.10: Carbon and nitrogen isotope profile for MAD 15. Isotope data are plotted against estimated age in months.

7.2.11 MAD 16

Similar to the pattern seen in MAD 06, the carbon and nitrogen values of this individual have an inverse relationship, that is $\delta^{13}C$ values decrease while $\delta^{15}N$ values increase. The carbon values show a steady decline of 1.5‰ from the initialization of tooth development to around three years of age, which is indicative of weaning. The increasing nitrogen values over the same time span, however, do not show the expected changes associated with breastfeeding and weaning. Rather, because of the inverse relationship between carbon and nitrogen here, the increasing $\delta^{15}N$ values over time are most likely associated with chronic stress during childhood.

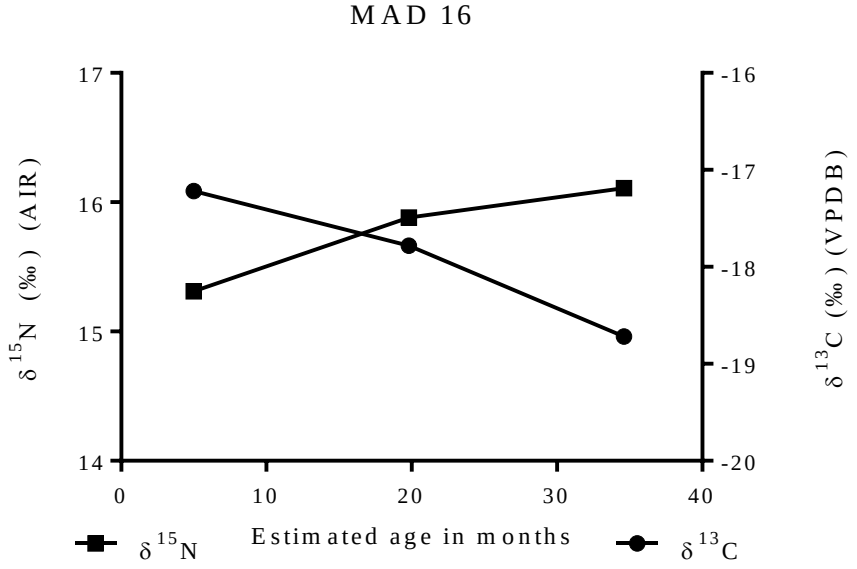


Figure 7.11: Carbon and nitrogen isotope profile for MAD 16. Isotope data are plotted against estimated age in months.

7.2.12 MAD 17

While this individual displays a normal weaning pattern in the $\delta^{15}N$ values, the magnitude of the decrease is lower than would be expected in a weaning individual (1.5‰). Since there is no plateau in the later nitrogen values, which would indicate weaning had completed and the subsequent $\delta^{15}N$ values had leveled-out to the weaned diet, it is possible that weaning continued after this tooth had finished developing, explaining the reduced magnitude of the decrease. However, isotopically distinguishable dietary variation between mother and child could also potentially cause a smaller decrease in $\delta^{15}N$ values. For example, if a child is consuming a higher trophic level weaning diet than its mother, the 2 – 3‰ decrease associated with weaning could be reduced. Finally, as noted by Fuller and colleagues (2006), it is possible that this individual was fed a combination of breastmilk

and other foods (e.g. cooked cereals or animal milk) from birth or very early in life. In their study, cases where mixed feeding was practiced, the magnitude of the $\delta^{15}N$ decrease was reduced. Without having isotope data extending further into this individual's life, or isotopic data from the mother, it is difficult to determine which scenario is more likely.

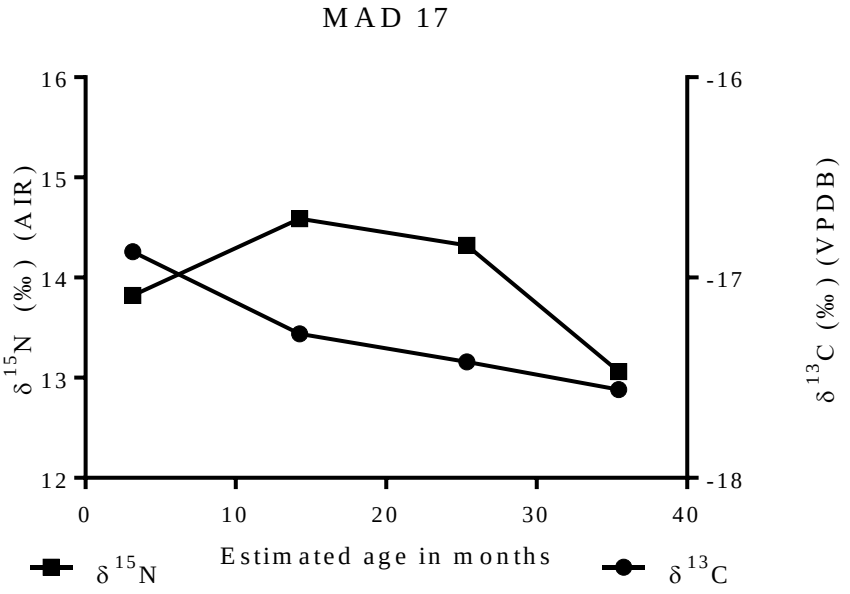


Figure 7.12: Carbon and nitrogen isotope profile for MAD 17. Isotope data are plotted against estimated age in months.

7.3 Breastfeeding trends in nineteenth century Madrid

7.3.1 Variation in breastfeeding practices

The stable isotope results provide an insight into the infant and early childhood experience in nineteenth century Madrid. As Chapter Two highlighted, breastfeeding practices throughout nineteenth century Europe were highly variable based on geographic location, the specific period, and socio-economic status. The results presented here have

shown that breastfeeding practices experienced by this group of infants and children from Madrid were similarly not homogeneous. Figure 7.13 shows $\delta^{13}C$ values plotted against $\delta^{15}N$ values for all samples. While a modest linear correlation is seen (Spearman Correlation; $r = 0.52$), there is significant scatter of the data, indicating that breastfeeding, infant feeding practices, and weaning diet were variable between individuals. If infant and young child feeding practices were homogenous in nature the correlation would be stronger and variability would be reduced. This is further supported by the scatter seen in $\delta^{15}N$ values plotted against age, seen in Figure 6.1 in Chapter Six. Such variation in breastfeeding and infant feeding practices is also seen in eighteenth – nineteenth century London, as demonstrated by Henderson and colleagues (2014) where $\delta^{15}N$ values were most variable in the first years of life. Likewise, numerous studies that apply dentine serial sampling methods outside of industrial Europe have noted considerable inter-individual variation in breastfeeding practices. Holt's (2009) Master's research examined serial dentine samples from a group of sub-adults from the Greek colony at Apollinia Pontica (coast of Bulgaria). The results of their thesis also indicate varied breastfeeding and weaning practices. A correlation of all $\delta^{13}C$ and $\delta^{15}N$ samples yielded results similar to those presented here ($r = 0.59$), as well, the age of weaning completion in their sample ranged from 12 to 36 months. In their study of Byzantine Greeks from Ancient Nemea (400 – 500 AD), Kwok and colleagues (In Press) show that most individuals were weaned off breastmilk either by the age of two (n =11) or three (n = 9), while six individuals demonstrated unique patterns, showing heterogeneity in the breastfeeding and weaning practices here. The findings presented here, along with these other examples, demonstrate

the value of studying weaning at an individual level as population level analyses mask this individual variation.

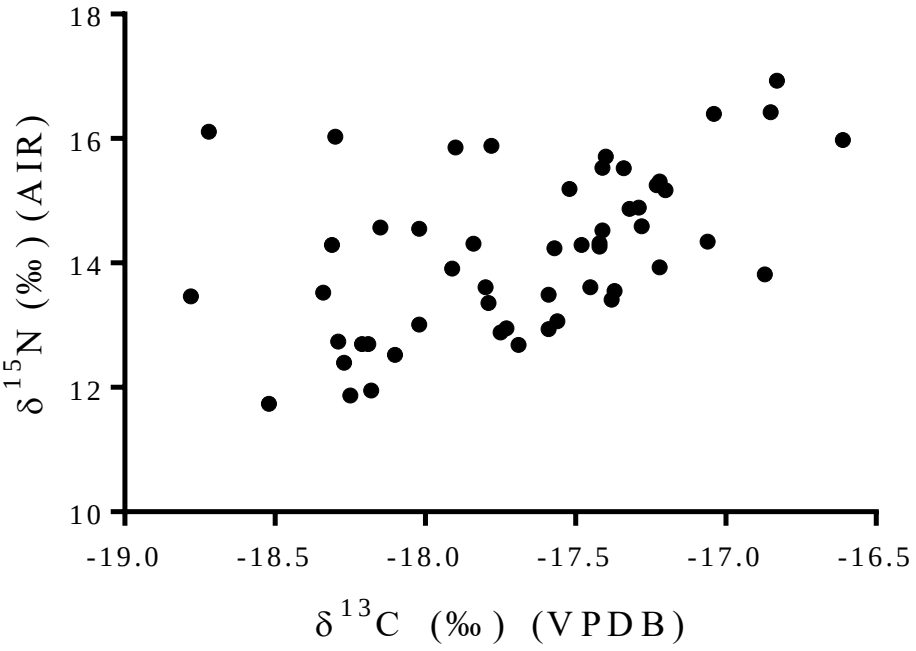


Figure 7.13: Scatter plot of $\delta^{15}N$ and $\delta^{13}C$ values for all samples.

7.3.2 Prevalence of breastfeeding

Throughout the eighteenth and nineteenth centuries, with the spread of the Industrial Revolution through Europe, it was common for mothers to not breastfeed their infants or children (the cost of which was increased infant mortality) (Fildes, 1995). This was due to women's involvement in factory work, which would separate them from their children for much of the day thus infants were either not breastfed and instead fed a grain base, often cooked in water or broth, or wet nursed. Among factory workers, whether one's

child was wet nursed or not breastfed at all was dependant on income. Since wet nurses were often those living in poverty, some factory workers could afford to hire them, however, those with reduced income would resort to feeding their child a form of cereal pap or foundling homes (Fuchs, 2005). Spain, however, was not influenced as strongly by the Industrial Revolution in comparison to other countries in Europe due to the delayed industrialization of Spain (Martinez-Carrion and Perez Castejon, 1998; Molinas and de la Escosura, 1989). This potentially would have increased the percentage of breastfed infants and lowered the percentage of wet nursed infants. While the data cannot determine who was breastfeeding the infant, this study has successfully shown that all individuals were likely breastfed at some point in their childhood. While a decrease of ~3‰ in nitrogen values is expected between the breastfeeding and weaning signals, certain individuals here demonstrated a reduced shift. It is possible that this is related to the initial mixed feeding of infants, indicating that, while the prevalence of breastfeeding is high in this sample, exclusive breastfeeding for the first months may not have been universally practiced. Kwok and colleagues (In Press) found two individuals from Byzantine Greece with similar patterns in their $\delta^{15}N$ values (that is a reduced, roughly 1‰, decrease); these individuals were also interpreted to have been fed a combination of breastmilk and animal milk in their infancy. It is, however, also possible that methodological constraints could be causing this reduced shift. Since serial sectioning results in sections containing dentine that developed over a period of time, the averaging of the $\delta^{15}N$ values over that time could result in a slight blurring of isotope values, reducing the expected shift in $\delta^{15}N$ values.

7.3.3 Onset age of weaning

As Section 7.2 demonstrated, the data collected for this project permit a discussion of breastfeeding duration and the timing of weaning. While the individual profiles provide a unique and rich insight into the variation seen in breastfeeding and infant feeding practices, the data collected here can also be used to examine general trends in timing. In 85.7% of the teeth examined, $\delta^{15}N$ values were elevated or showed the characteristic increase in $\delta^{15}N$ indicative of breastfeeding in the early-forming tooth sections, peaking between 10 and 14 months of age. This shows that, in this sample of infants from Madrid, the majority of the individuals were deriving significant amounts of their dietary protein from breastmilk until the age of 10 to 14 months, after which complementary foods became more prevalent in the diet. This is slightly later than the historical information on Spanish breastfeeding practice, which indicates that weaning was initiated between six and 11 months, as reported by Reher and colleagues (1997). Another bioarchaeological study of weaning, although in nineteenth century Italy, found the weaning process likely began around 1.5 years of age based on the prevalence of linear enamel hypoplasia (LEH) (Moggi-Cecchi et al., 1994). However, this age estimate is based on the idea that LEHs are indicative of weaning, while weaning is one cause of LEH formation, their etiology is more complex than this and care must be had when interpreting their prevalence. On the other hand, Henderson and colleagues' (2014) isotopic study of life histories in eighteenth – nineteenth century London demonstrated that weaning started at the younger age of roughly six months.

As has been discussed throughout this thesis, the general social changes, and specifically changes to the gender composition of the workforce, associated with the Industrial Revolution potentially impacted the onset age and duration of weaning. However, without data on breastfeeding and weaning patterns prior to the Industrial Revolution this can only be speculative. Historical sources show that in Britain, the age of complete weaning dropped from around 17 months in the sixteenth and seventeenth centuries, to roughly nine months in the late eighteenth centuries (Fildes, 1995). The results of Henderson et al.'s (2014) study are consistent with this change. The changes to family structure that accompanied the Industrial Revolution can help make sense of this relatively short duration of breastfeeding. While in some cases it is possible that not breastfeeding one's child became more common, in others the introduction of complementary foods occurred earlier in life, both of which would have been caused by the transition of mothers from the home into the work force, a common practice in regions of high industrialization (unlike most of Spain). When mothers were involved in factory work, breastfeeding became difficult and infants would often be wet nursed, weaned early, or not breastfed at all. Throughout the nineteenth century, Spain was not considered to be a fully industrialized country. Spain's economy was based almost entirely on agricultural work, making up around two third of the labour force throughout the entire century (Casares et al., 2000). It was not until the end of the century that even the urban centers, such as Madrid, began to see industrialization, although, the degree of this transformation was minimal until into the twentieth century (Martinez-Carrion and Perez-Castejon, 1998). It is likely that the continued dependence on an agricultural economy, in contrast to a more industrialized one,

resulted in the breastfeeding and weaning trends that are seen in this Spanish sample. These individuals were likely breastfed slightly longer before weaning began and experienced a more gradual weaning process (discussed below) due to the comparatively lower rates of factory work and in turn maternal labour involvement.

Studies on the timing of weaning in Italy have also shown extended breastfeeding. Fildes (1995) reports a slightly later complete weaning age, ranging from one to two years, while in Britain it was estimated to be around nine months. As mentioned above, Moggi-Cecchi and colleagues' (1994) LEH study of weaning in nineteenth century Florence found children began weaning off breastmilk at 1.5 years of age, with total cessation of breastmilk consumption occurring gradually after this; demonstrating a longer breastfeeding and more gradual weaning period than historical sources show. Spain and Italy were in similar economic situations during the nineteenth century. Both were slower to industrialize and had comparable economic power in comparison to Britain, France, and Germany (Molinas and de la Escosura, 1989). This thesis used stable isotope data to produce a more accurate chronology of the initiation and completion of weaning compared to Moggi-Cecchi et al.'s (1994) LEH study. They both do, however, show later onset of weaning than samples from other heavily industrialized locations. The similarly late onset age of weaning and gradual weaning process in both this Spanish sample and the Italian sample presented by Moggi-Cecchi et al. (1994), shows a possible pre-industrial trend in breastfeeding practices. Further studies on breastfeeding patterns in both earlier and later populations are needed to fully understand the effects of industrialization on breastfeeding and weaning behaviour.

7.3.4 Age of weaning completion

While the results presented here provide an insight into the presence of breastfeeding signals and the onset age of weaning, determining the duration and age in which breastmilk consumption stops is difficult with the data presented here. Since the isotope data presented in this thesis are from deciduous incisors and molars, they are limited to the development period of those teeth, until 2.5 and 3.5 years of age, respectively. To determine the age at which breastmilk consumption completely stopped there would need to be a plateau of $\delta^{15}N$ values following the decline expected with weaning. When dealing with a short time span (i.e. the first 3.5 years of life), weaning would have to be complete well before the tooth finished developing in order for the plateau to be achieved. None of the individuals studied here display a plateauing in their $\delta^{15}N$ values, however, by using the magnitude of the decrease in both $\delta^{13}C$ and $\delta^{15}N$ values, an approximate age can be provided for some individuals.

In the sample of sub-adults analyzed in this thesis, it is likely that six of the individuals (MAD 05, 06, 07, 08/09, and 15) died during the weaning process based on their estimated age at death and the incomplete evidence of weaning in their isotope data. The significance of this will be discussed in the following section; however, since the isotope data shows likely evidence of incomplete weaning, their estimated age at death (between 1.5 and 2.5 years old) shows that weaning was likely still occurring at these ages. In the individuals who survived longer, it appears that children were beginning to be completely weaned by the age of three. All other individuals have unique $\delta^{15}N$ patterns that have prevented an estimation of the age at which weaning was complete. Considering

weaning was possibly complete by the age of three in this sample, it appears children are consuming breastmilk much longer and experiencing a more prolonged and gradual weaning process than in contemporaneous Britain.

7.4 Breastfeeding and vitamin D deficiency

Of the individuals sampled as part of this thesis, 75% percent of them have skeletal evidence of vitamin D deficiency, or specifically, rickets (Table 7.1). One goal of this thesis was to determine if there was a relationship between an individual's breastfeeding pattern and their vitamin D status. Based on the individual feeding profiles discussed in section 7.2, it would appear as though no clear relationship is present. It is important to note that this hypothesis is potentially influenced by the small sample size regarding non-rachitic individuals, future studies would benefit from a more balanced sample of rachitic and non-rachitic individuals.

Since all individuals show some evidence of breastmilk consumption, it is clear there is no relationship between the mere presence/absence of breastfeeding and rickets. However, it appears as though the general weaning behaviour seen among these individuals is also consistent between both those with and without evidence of rickets. Among the individuals without skeletal evidence of rickets weaning appears to have begun at 10 to 14 months of age. Individuals with evidence of rickets show a nearly identical range (10 – 14 months), but with one outlier. It appears as though MAD 05 began weaning off of breastmilk at around seven months of age (Fig. 7.4), earlier than any other individual. Due to challenges in determining the age in which individuals finished weaning (presented in section 7.3.4) it is difficult to determine any patterns between the age of weaning

completion and their vitamin D status. Evidence of weaning being complete by the age of three is seen in both those with skeletal evidence of rickets and those without. Since data on this are limited, few claims can be made on the topic.

Four of the individuals (MAD 03, 04, 06, and 16) analyzed in this thesis have $\delta^{15}N$ patterns that may suggest evidence of physiological stress. Both MAD 06 and MAD 16 have $\delta^{15}N$ values that increase over time. While a dietary change could cause this, the corresponding decrease in $\delta^{13}C$ values indicates that stress is more likely the cause. Both individuals show skeletal evidence of rickets and have similar $\delta^{15}N$ profiles, but not all individuals with rickets show this pattern, so rickets alone is not the explanation for this apparent increase in nitrogen values. The $\delta^{15}N$ values steadily increase by 0.8‰ from birth until death, at roughly 2.5 and 3.5 years old, respectively. The increase in $\delta^{15}N$ is likely due to chronic stress during the course of each individual's life, stopping, in both cases, with the individual's death. Further studies on the timing of rachitic periods using the study of interglobular dentine, may provide a better insight into the relationship between these two variables.

Individuals MAD 03 and 04 also show increases in $\delta^{15}N$ likely associated with physiological stress; however, these are individuals without skeletal evidence of rickets. Unlike MAD 06 and MAD 16, these individuals show an increase in $\delta^{15}N$ over a roughly 12-month period, between the ages of two and three. It appears as though they were in the process of being weaned off of breastmilk when they experienced a period of stress causing nitrogen isotope values to increase. It is possible that a poor weaning diet and insufficient quantities of breastmilk caused a period of catabolism in these individuals before weaning

was complete. Since MAD 03 lived through this period of stress, nitrogen and carbon isotope data from a permanent tooth, preferably the first molar, would be able to shed light on the duration of this stressful period. As well, since it is possible these individuals experienced episodes of vitamin D deficiency, albeit less severe episodes, a study of interglobular dentine here would be able to tell us about metabolic processes at the time of this stress event.

This Spanish sample had a high prevalence rate of rickets (~60%), affecting individuals regardless of social class (Ríos et al., 2016) and, based on the evidence presented here, with no apparent relationship to breastfeeding and weaning practices. Given this information, the high rate of rickets in this sample is still unexplained. While no longer believed to be a product exclusively of the Industrial Revolution, rickets is certainly linked to factors associated with industrialization. Occupational shifts, urbanization, and industrial pollution are all products of industrialization that have a direct influence on one's susceptibility to vitamin D deficiency and, in turn, rickets (Mays et al., 2006). However, since Spain, even in urban centers such as Madrid, did not experience dramatic industrialization until nearly the turn of the nineteenth century, it is unlikely that factors associated with industrialization are the only cause of increased rates of rickets in this population.

In general, Spain is a country with a relatively high amount of UVB producing sunlight. Madrid, sitting at 40° latitude, experiences high rates of vitamin D producing sunlight for eight months out of the year, with significantly reduced rates during the winter months (Kimlin, 2008). González-Molero and colleagues (2010) show that the prevalence

of vitamin D deficiency in a modern Spanish sample is around 33.9% (assuming deficiency at >20ng/mL), which is considerably lower than rates seen in modern German and British populations. While the prevalence rates seen in the Madrid skeletal assemblage can not be directly compared to the modern study, it does show that, regardless of available sunlight, vitamin D deficiency is common even in modern Spain. It must be recognized, however, that their study diagnosed vitamin D deficiency based on circulating serum levels of 25(OH)D, not the skeletal manifestations that are only seen in severe cases. Hypothetically, it is possible that if circulating 25(OH)D was measured in the infants interred in the Trinitarias Crypt the prevalence rate of clinically-defined vitamin D deficiency would be even higher.

Based on the findings presented here, showing that there is no clear relationship between vitamin D deficiency and breastfeeding and weaning practices, it is most likely socioeconomic and cultural practices that influence exposure to sunlight that caused the high prevalence of rickets in this sample. While not heavily industrialized, Madrid was indeed in a state of population growth throughout the nineteenth century. It is estimated that the population grew from roughly 200,000 in 1804 to 539,835 in 1900 (Fernández García, 2008). Such growth resulted in the creation of multi-storied dwellings in the city by the mid-century; these residences were crowded, dark, and generally unhygienic and would have limited the potential exposure to UVB radiation (Aranzadi, n.d.). This, in addition the fact that mothers were expected to care for infants and children while working within the home, would have greatly reduced the amount of UVB radiation to which these individuals were exposed.

While a lack of sunlight was likely the largest contributor to the prevalence of rickets in this sample, it is possible that the breastfeeding and weaning practices did play a small role. Despite the relatively low concentration of vitamin D in human breastmilk, it does provide a degree of protection from rickets during infancy (see Chapter Four). Similarly, breastmilk provides infants with passive immunity, protecting them from numerous pathogens. However, the time span for which breastmilk can sustain and protect a child is limited. It is generally regarded that breastmilk can exclusively sustain an infant for no longer than six months, after which infants require supplemental foods to meet their nutritional requirements (Jay, 2009; Sellen, 2007). Nearly all the individuals in this sample demonstrate an onset age of weaning between the ages of 10 – 14 months and a complete age of weaning around the age of three. If minimal or nutritionally inadequate supplemental foods were consumed after weaning commenced, the infant or child would have been in an at-risk state. As well, if exposure to sunlight was limited for these infants or children, the consumption of a weaning diet low in vitamin D and calcium would put them at increased risk of rickets, even if there was still some breastmilk in the diet (Thacher et al., 2006). Thus, it is possible that a nutritionally inadequate weaning diet combined with a relatively prolonged weaning period contributed to the high rate of rickets in this sample.

Weaning has long been understood as a dangerous time in any infant's or child's life. During weaning children lose the passive immunity provided by breastmilk and are introduced to a diet containing potentially harmful/contaminated foods. This is seen in the data presented here. While all individuals included in this study show evidence of breastfeeding, few show a full 2 – 3‰ shift in $\delta^{15}N$ values. Most commonly, the reduced

shift in $\delta^{15}N$ is because infants are dying before they are completely weaned off breastmilk. This was clearly demonstrated in 50% of the individuals sampled here, while only three individuals show likely evidence of completed weaning. It is possible that the risks present during weaning caused or exacerbated the rickets seen in these infants and children. If the weaning process caused these individuals to fall ill, it is possible they would be bedridden for prolonged periods, further reducing their exposure to sunlight. However, further information on the timing of rickets in these individuals would be required in order to solidify this idea.

Vitamin D deficiency is a complex metabolic disease that can not be simply explained. While first and foremost it is caused by inadequate sun exposure and a lack of dietary vitamin D, the biocultural variables that influence these factors are numerous. While a likely scenario has been presented, it is limited by a lack of historical and archaeological information on child and infant care in nineteenth century Madrid. Information on the cultural views regarding exposure to sunlight in infants and practices such as swaddling would allow for a better understanding of why these infants and children were experiencing such high rates of rickets. This discussion has shown that whether an individual was breastfed likely did not influence their vitamin D status. While it is possible that a poor weaning diet alongside the prolonged weaning process may have contributed to the rickets seen here, it is more likely other socioeconomic and cultural factors surrounding sun exposure, such as living arrangements and practices of childcare, that influenced one's susceptibility to rickets.

The research presented here can also shed light onto the claims made by Hess (1930), as discussed earlier in section 4.4. Hess (1930) argued that that breastfeeding infants were less likely to develop rickets and those that did showed less severe symptoms. Based on the data presented in this thesis, it appears that breastfeeding did not offer any protection or mitigation of rickets, contrasting the ideas put forth by Hess. All vitamin D deficient individuals were being breastfed in this sample of children buried at the Trinitarias cemetery. Further analysis of more vitamin D deficient children, alongside data on the severity of skeletal lesions, could provide a more detailed analysis of Hess' claims. Recently, Mays (In Press) presented a discussion on the use of historic documentary sources in paleopathology. The paper focuses on the potential issues in using seventeen to nineteenth century written sources in modern palaeopathology, highlighting the lack of consistency in how authors (or physicians) diagnose and report the prevalence of the condition, as well as the incompatibility of comparing historical data and cemetery data. Considering Hess' writing in 1930, it likely that Mays' hesitation may extend to sources of the early twentieth century as well. Echoing another of Mays' (In Press) points, this is not to say that sources such as these are of no use, rather, they are critical tools in creating a biocultural understanding of the past. Most importantly, these sources can provide an excellent basis on which hypotheses can be constructed and tested through skeletal analysis, as this thesis has demonstrated.

7.5 Methodological and historical limitations

Since the introduction of serial dentine microsampling methods, the ability to develop chronologically-specific infant feeding histories has grown dramatically; however,

these methods and the research presented here are not without their limitations. While researchers applying these techniques have been able to collect up to 20 serial samples for adult teeth (Beaumont et al., 2014), and up to 14 in deciduous teeth (King et al., 2018), this research averaged four samples per tooth. In order to ensure enough collagen was collected from each sample, section had to be cut between 2.5 and 3.5mm in length, reducing the number of samples possible. The use of deciduous teeth with incompletely developed roots (and reduced root lengths) also made it difficult to collect more samples. While limiting the amount of data produced, reducing the number of samples taken does have benefits, it ensures adequate carbon and nitrogen in the collagen for isotope analysis.

Another limitation that needs to be acknowledged is that even with the advent of serial sectioning methods, there is no way to determine whether infants were being nursed by their mothers or other females. Regardless of who is providing breastmilk to infants, there will be an increase $\delta^{15}N$ values due to breastfeeding, although if women were consuming isotopically different diets this may impact the nitrogen values of the infants and children. The practice of wet nursing would undoubtedly have been practiced during the nineteenth century in Spain. While the frequency would be decreasing when compared to the previous centuries, wet nursing was likely practiced until around the twentieth century (Ramón and Enrique, 2008; Smith, 2010). Studies, both modern and historical, have noted negative health consequences associated with wet nursing. Ramón and Enrique (2008) discuss that wet nursing was believed to be a causal factor of the high infant mortality rate near the end of the nineteenth century. More recently, Thacher and colleagues (2007), a group of clinicians, discuss the potential of wet nursing leading to

increased rates of vitamin D deficiency. It is possible that since wet nurses often breastfeed multiple children at one time and since the calcium content of breastmilk is reduced with continued breastfeeding, the nutritional quality of a wet nurse's breastmilk is likely lowered (Thacher et al., 2007). This can result in calcium insufficiency and, in turn, vitamin D deficiency. Since maternal breastfeeding and wet nursing are isotopically indistinguishable this is a limitation unlikely to be addressed within the stable isotope literature.

7.6 Conclusions

Through this study of carbon and nitrogen isotopes from serial dentine sections, the breastfeeding and weaning patterns present in a sample of children from nineteenth century Madrid have been discussed. Due to patterns of both $\delta^{13}C$ and $\delta^{15}N$ it is evident that all individuals sampled did consume significant amounts of breastmilk at some point in their lives. Breastmilk likely provided infants with significant amounts of their dietary nutrition for the first 10 to 14 months of life. Some of the individuals in this sample did not survive the weaning process, thus determining the age at which weaning was complete is difficult. In those children who did survive, the weaning process was likely complete by around the age of three. No clear difference was found in the breastfeeding and weaning patterns of individuals who showed evidence of rickets, and while it is possible a poor weaning diet may have contributed to, or exacerbated the rickets seen in this sample, the high rate of rickets is likely caused by socioeconmic variables and childcare practices that affect exposure to sunlight.

Unlike previous studies applying the methods used here, this thesis has shown the potential of using developing deciduous teeth. This has enabled the study of an individual's

history up until their death. Ultimately, this allowed for the determination that many of these individuals died during the weaning process. Even today weaning is recognized as a dangerous time for children and, in the past, this was no different. Future studies will look to incorporate these isotope data with data collected on the timing of interglobular dentine formation to further understand the relationship between weaning, vitamin D deficiency, and infant mortality.

Chapter 8: Conclusions

8.1 Summary of findings

This thesis investigated the breastfeeding and weaning practices in a sample of sub-adults from the Trinitarias infantile cemetery in Madrid, Spain. Through the stable carbon and nitrogen isotope analysis of incremental dentine samples the variability in breastfeeding practices has been highlighted. However, examination of individual isotope profiles did provide information on general trends in breastfeeding and weaning; it is evident that breastfeeding was practiced until 10 – 14 months of age, while weaning was likely complete around the age of three. This is somewhat later than what is written in historical texts, suggesting infant be breastfed exclusively for six to 11 months. These findings do support the possibility that mothers in Madrid were able to breastfeed their infants longer and have a more gradual weaning process due to the comparatively lower involvement of women in factory work in nineteenth century Spain. Comparative isotopic studies of earlier and later Spanish samples are required to determine if this may be linked to the Industrial Revolution and the process of industrialization.

The incorporation of stable isotope and paleopathological data on vitamin D deficiency enabled a discussion on breastfeeding and weaning and their relationship to the development of rickets. Since all individuals sampled here show some evidence of weaning, the stable isotope data do not support the idea that breastfeeding offered any protection from vitamin D deficiency in this sample. As well, the onset age of complementary feeding (10 to 14 months) is consistent in both individuals with and without rickets. Revisiting the hypothesis presented in Chapter One, it appears that the variation

seen in the breastfeeding and weaning histories presented in this thesis is not linked with the presence of vitamin D deficiency. Thus it is most likely socioeconomic and cultural factors that influenced one's exposure to sunlight that caused the high rate of rickets in the larger sample these individuals were taken from. Although it is possible that a poor weaning diet alongside a prolonged weaning process may have been a contributing factor.

Examination of the stable isotope data from these individuals also motivated a discussion on stress, as inferred though $\delta^{15}N$ values. Four individuals (MAD 03, 04, 06, and 16) all demonstrated an increase in $\delta^{15}N$ values at some point in their childhood that is interpreted as nutritional or physiological stress. While interesting, as this provides a unique insight into the lives of these individual, it also demonstrates the value of conducting individual level isotope analyses such as this. In population-level studies of weaning, using bulk samples of bone collagen or dentine means that individual variation in weaning is not captured. Not only does this influence understandings of weaning practices, it also obscures unique patterns in isotope values (e.g. those seen in individual that experienced stress). While some individuals may demonstrate elevated $\delta^{15}N$ values in bulk tissue, without incremental isotope data it is difficult to determine the presence, timing, and duration of these periods of stress. As well, incremental sampling allows for the differentiation of stress from dietary change through the analysis of $\delta^{15}N$ and $\delta^{13}C$ covariation.

8.2 Avenues for future research

While this research has provided new insight into the breastfeeding and weaning practices in nineteenth century Spain, further research on this rachitic sample would be able

to answer questions that remain unanswered. In the older individuals interred at the Trinitarias cemetery it would be beneficial to sample the existing permanent dentition (using the same techniques presented here). Stable isotope data from this source would allow for an expanded individual timeline. Since deciduous molars and incisors finish development at a relatively young age (3.5 and 2.5, respectively), the span of the isotope data is limited. If permanent dentition were included, preferably a canine or first molar, the individual timeline could be extended until either the individual's death, or until the permanent tooth finished development. This would enable a better and more precise understanding of when individuals finished weaning, a question that was difficult to answer with the data presented here.

Since this analysis only requires sampling the tooth dentine, the tooth enamel is reserved for each individual. Recently, researchers have developed a biochemical method to estimate the sex of skeletal remains through the analysis of peptides in tooth enamel (Stewart et al., 2017). Previously, sex estimation of skeletal remains was limited to adults, however, the method developed by Stewart et al. (2017) is able to provide sex estimates to infant and sub-adult remains. Historical sources discuss that breastfeeding and weaning practices were most likely different between boys and girls in nineteenth century Spain, so peptide analysis would add a new variable to examine the stable isotope data, enabling the assessment of sex-based variation in breastfeeding and weaning practice.

Throughout this thesis references have been made to the value of incorporating the study of interglobular dentine (IGD) with the incremental isotope data. During tooth sectioning, a histological slide of each tooth was created in order to examine the presence

and degree of IGD. These data will give an idea of the age in which these individuals were experiencing periods of metabolic disruption, combining this individual isotope profiles will allow for the examination of the relationship between stable isotope ratios and areas of IGD. Further research on non-rachitic children from nineteenth century Spain would also be beneficial, as it would provide some comparative data for this rachitic sample.

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