BIOARCHAEOLOGICAL ASSESSMENT OF DIET AND CHANGES IN FEMORAL AND HUMERAL STABLE ISOTOPIC VALUES AMONG SUBADULTS AT MEDIEVAL ALYTUS, LITHUANIA

by

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ABSTRACT

Establishing a chronology of variation in isotopic values can reveal frailty associated with biological and social age, as well as highlight individuals who vary from typical patterns. Although general dietary characteristics and infant feeding practices were previously unknown for subadults excavated from the cemetery at Alytus, Lithuania (14^{th}-18^{th} centuries), previous research concludes that Alytus' subadults experienced high rates of physiological, metabolic, non-specific stress, in addition to specific diseases like tuberculosis. To investigate nuanced relationships between diet and mortality, nitrogen and carbon stable isotopes from the femoral and humeral midshaft diaphyses of 70 subadults (32 weeks gestation to16 years) were analyzed.

Dietary reconstruction reveals that on average, exclusive breastfeeding continued until around 2 years of age when enriched $\delta^{13}C$ (-19.6‰) and $\delta^{15}N$ values (12.7‰) begin to deplete suggesting introduction of C_3 grain gruels and potential weaning-associated infirmity. Nitrogen values remained slightly elevated in children (3-5 years, 11.2‰) until the beginning of juvenility (5-8 years, 10.3‰) when $\delta^{15}N$ more closely mirrored adult values (16 years, 10.2‰), consistent with predominant consumption of terrestrial animal protein, possibly with riverine influence.

The difference between femoral to humeral $\Delta_{F\text{-}H}{}^{13}C$ (-0.05 ±0.25‰, 1σ) and $\Delta_{F\text{-}H}{}^{15}N$ (-0.01±0.45‰, 1σ) was not significant, though humeral values were on average more enriched. Enrichments in humeral nitrogen and carbon coincided with estimated weaning age. Cohorts experiencing childhood and adolescent growth spurts experienced higher femoral $\delta^{13}C$ and $\delta^{15}N$ values. Examining dietary experience and physiological changes contributes a holistic understanding of subadult morbidity and mortality experiences in Medieval Lithuania.

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CHAPTER 1: INTRODUCTION

Bioarchaeologists aim to meaningfully interpret results from analysis of human skeletal remains within the context of their material and historic cultures. The study of subadult skeletal remains in an archaeological setting is an increasingly important avenue of research intended to highlight the differing experiences of subadults throughout transitional phases in physical and social development within their homes and communities (e.g., Baker et al., 2005; Dupras et al., 2001; Halcrow and Tayes, 2008; Lewis, 2007b; Scheuer and Black, 2000; 2004; Wheeler, 2012). Skeletal and dental aging techniques for subadults provide narrower age ranges to provide cross-sectional observations of physiological changes, and to make more nuanced inferences about life and death in the past (Wright and Yoder, 2003). Skeletal remains of young individuals are no longer seen as mere indicators of fecundity in the archaeological record, but as traces of participants in a culture, who practiced or were subjected to the traditions of their caregivers and, like adults, were certainly affected by socioeconomic status and change.

In stable isotope analyses, bone collagen $\delta^{13}C$ and $\delta^{15}N$ are used to understand dietary practices of specific groups and also individuals, which can then be compared among populations. Understanding the dietary consumption of subadult diets is an integral aspect to understanding those whose diet and protection were at the whim of caregivers for many years. Furthermore, these studies allow investigations into how varying Medieval dietary practices, for example weaning, may have affected a child's ability to survive the initial stresses of infancy followed by growth with a potentially under-developed immune system (e.g., Burt, 2013; Fuller et al., 2006b, Mays et al., 2002; Reitsema, 2013).

Dietary composition and the effects of some disease processes remain unknown for the subadults who are buried at Alytus cemetery. In addition, there is little literature specific to the diet in prehistoric and historic Lithuania (Antanaitis-Jacobs and Girinikas, 2002; Antanaitis-Jacobs et al., 2012), and even fewer related to Medieval Lithuania (Kisieliene, 2012; Stancikaite et al., 2008). Jankauskas and Gerhards (2012) state that there is a need to utilize biological, skeletal data in interpretations of archaeological sites in the Baltic States. The research presented herein contributes to the rarely discussed Medieval Baltic through the reconstruction of diet and consideration of how disease processes affected the subadults in the skeletal sample from a late 14^{th} to early 17^{th} century cemetery at Alytus, Lithuania.

It is essential to contextualize diet and physiological stress with the prevailing religious and secular customs, dietary guidelines, and concepts of childhood during the Medieval period as each of these factors are often inextricably linked to nutrition and general health. Evidence for the interpretation of an archaeological culture taken only from human skeletal remains can often be paradoxical when a researcher is examining only one facet of society.

The premiere aim of this study is to identify the dietary characteristics of subadults from the cemetery of Alytus by comparing bone collagen $\delta^{13}C$ and $\delta^{15}N$ values. Seventy-two femoral diaphyses and 69 humeral diaphyses from a total of 70 individuals aged between 32 weeks gestation and 16 years at death were processed for stable isotopic analysis. None of the individuals chosen for sampling exhibited visible skeletal pathological conditions indicative of specific skeletal malnutrition or specific disease processes (sampled by Eleazer, 2013).

Intra-individual variation in collagen $\delta^{13}C$ and $\delta^{15}N$ between 68 pairs of humeri and femora were also investigated with the objective of highlighting selective mortality in outlying individuals. Examining the same sample of Alytus subadults, Eleazer (2013) concluded that

metabolic processes preferentially maintained the microscopic geometry of the femur over the humerus during periods of growth after the onset of walking. The secondary aim of the study was to compare differences in femoral to humeral $\delta^{13}C$ and $\delta^{15}N$ for each individual under the postulation that the isotopic values might indicate the body's preferential use of protein to maintain growth of the femur over the humerus.

Research Questions and Hypotheses to be Addressed

The relationship between nutrition and the presence of skeletal stress is likely prevalent in the subadult sample from Medieval Alytus (Eleazer, 2013; Jankauskas and Schultz, 1995; 2009; Šereikienė and Jankauskas, 2004). While no individuals from the current sample exhibited evidence of specific nutritional or pathological conditions (e.g. Vitamin C deficiency scurvy or Tuberculosis), there are individuals present who demonstrate non-specific indicators of stress and those who do not exhibit any skeletal stressors but still passed away during childhood. Dietary, pathological, and comparative analyses are collectively effective methods that can be successfully employed to more accurately understand the conditions in which individuals at Alytus experienced life, health, nutrition, and illness. To explore these themes, the following research questions were posited:

1. What are the average $\delta^{15}N$ and $\delta^{13}C$ dietary characteristics for the subadults at Alytus?
    - What types of food could have been consumed?
2. At what age did transitions in feeding and eating occur?
    - Are there individuals who are significantly removed from the sample or cohort means?

- How do the isotopic signatures and timing of dietary change of the subadults at Alytus compare to other Medieval children?

3. Is there significant isotopic variation within humeral and femoral values for each individual?
    - On average is one more enriched or depleted than the other for the sample and within age cohorts?

Thesis Chapter Summaries

Chapter 2 showcases the historical backdrop of Lithuania between the 14th-17th centuries, emphasizing the political and religious atmosphere. Historic data specific to life at Alytus will be detailed, paying special attention to community structure and devastating events. Archaeological data from the cemetery at Alytus will also be provided along with a summary of previous skeletal research conducted on the subadults recovered from the Alytus cemetery. Chapter 3 contains a summary of food recommendations for Medieval Europeans and a catalog of probable food items that could have been available for consumption based on previous research at a contemporaneous costal Lithuanian town, and food items found throughout Lithuania at archaeological sites from the Neolithic to Bronze Age. There is also a discussion of popular infant and childhood feeding trends in Western Europe.

Chapter 4 presents a literature review of anthropology's use of isotopic studies as well as the chemical principles inherent to incorporation of dietary carbon and nitrogen into bone collagen. This chapter also explores current issues pertaining to the difficulties of identifying riverine resources, as well as the interactions between metabolism and nitrogen values.

Chapter 5 presents the materials and methods for the sample used in analysis including a step-by-step documentation of the collagen extraction process. Chapter 6 presents the results of the stable isotopic analysis including summary data for the preservation of the samples, individual $\delta^{13}C$ and $\delta^{15}N$ values, and $\delta^{13}C$ and $\delta^{15}N$ averages for the humerus and femur. The differences in femoral and humeral $\delta^{13}C$ and $\delta^{15}N$ for 68 individuals are also presented.

Chapter 7 discusses infant and child feeding patterns within the context of Medieval society and explores the potential reasons for statistical outliers in $\delta^{13}C$, $\delta^{15}N$, and changes between femoral and humeral $\delta^{13}C$ and $\delta^{15}N$. Finally, Chapter 8 concludes with a summary of the analysis and presents areas of potential future research.

CHAPTER 2: ALUYTUS AND LITHUANIA IN THE 14TH TO17TH CENTURIES A.D.

Medieval Lithuania

Between the years of 1248-1352, the Grand Duchy of Lithuania was an expanding territory, under constant threat of violence and war from the Teutonic Knights (Kozakite, 2011; Rowell, 1994). The Duke Jogalia finally saw the opportunity to adopt Christianity as the country's official religion in 1387, forging new alliances with Christian and Orthodox countries and providing Lithuanians a safer land in which to prosper. This tactical move not only protected Lithuanians from war for several years, but it allowed successive dukes to expand their territory, increase trade, and merge with Poland in 1569 (Rowell, 1994). The Medieval and Modern capitals of the country were both positioned in Vilnius, an urban city northeast of Alytus (Fig. 1).

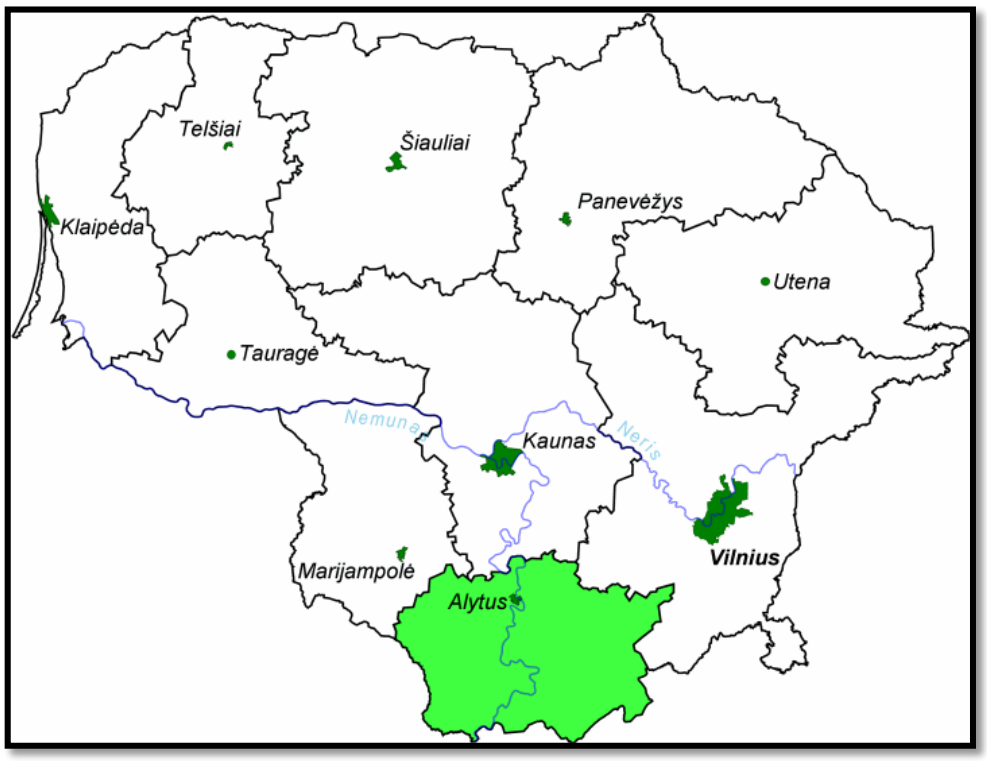


Figure 1. Modern counties in Lithuania. Alytus is highlighted within neon green section. Note Alytus' connection to the Baltic Sea, Kaunas, and the capital of Vilnius by way of the Nemunas and Neris Rivers (Map public domain).

Even after the conversion of the Grand Duchy of Lithuania to Christianity in the mid-1300's, citizens were free to practice religion however they pleased, often times simply adding Christian beliefs on top of older pagan traditions (Jankauskas and Urbvanikus, 2008; Kozakite, 2011; Rowell, 1994). Rowell (1994) described religion in Lithuania as a utilitarian process whereby practitioners would simply add a new god, in this instance Jesus Christ, to their pantheon without fear of retribution for disregarding their pagan gods and traditions. Rulers continued to almost overtly practice pagan customs including cremation of remains, which was in contrast to Christians who practiced austere burials in alignment with the rising sun (Rowell, 1994). Increased trade and pressure from foreign Christians certainly would have influenced citizens to embrace Christian conventions but, as a whole, Lithuania remained more tolerant than most nations (Rowell, 1994). During the Medieval period, persecuted Jews fled the dangerous outer borders of Poland for the safety offered to them in Lithuania affirming the multicultural diversity nurtured in a country determined to keep its people secure (Monter, 1983).

Life in Medieval Alytus

Alytus was initially established as a Hill-Fort in 1365 before growing into a fortified village on the River Nemunas (Fig. 2). Two settlements were established at Alytus on either side of the Nemunas as a defense between Medieval Lithuanians and the Teutonic Knights, and as a village of trade (Kozakite, 2011). The Knights invaded Alytus in 1377 but were quickly driven out and kept at bay by the official adoption of the Christian religion 10 years later (Rowell, 1994). However, in Alytus the first Christian church was not built until 1517 and consequently, farm-centered cults and polytheistic traditions persisted throughout Lithuania well into the 17th century, especially in more rural locals (Kozakite 2011; Rowell 1994).

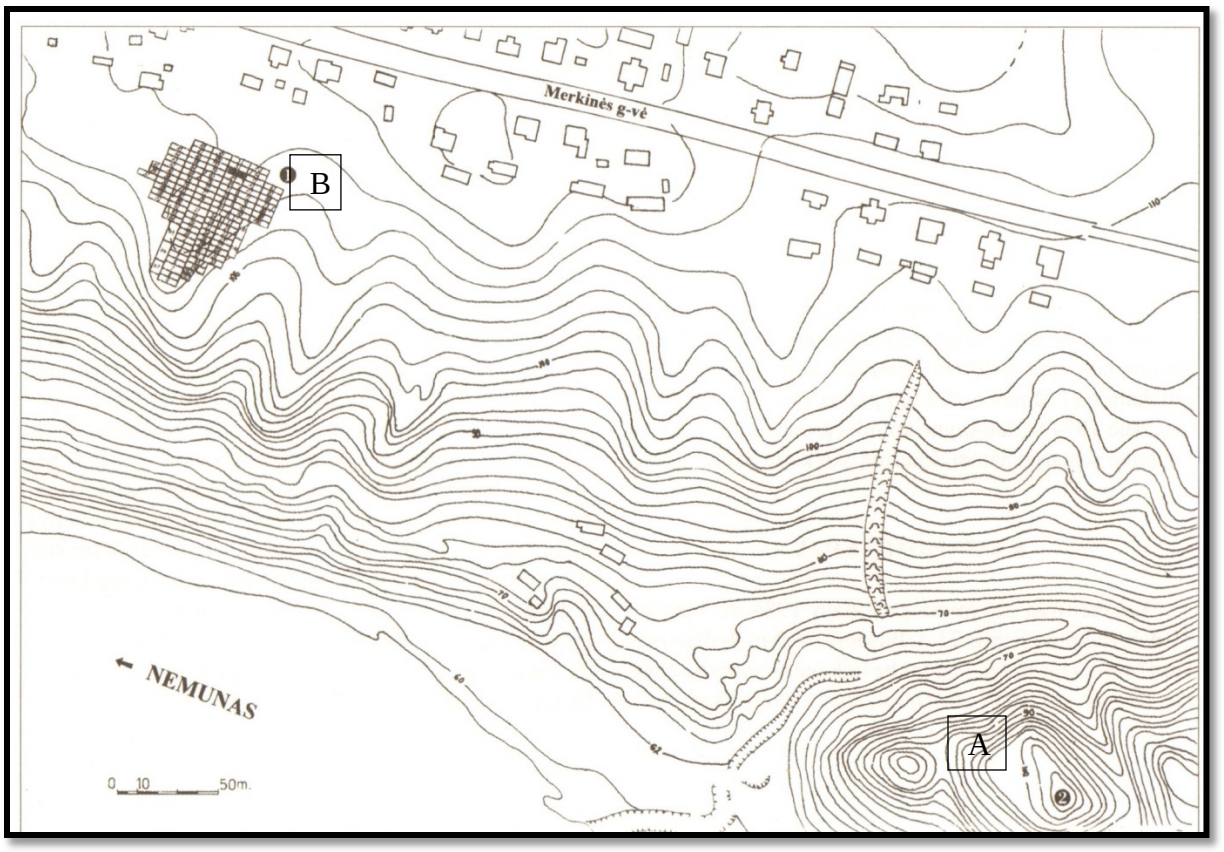


Figure 2. Plan of the Hill Fort (A) and excavated Cemetery (B) on the east bank of Nemunas River at Alytus (Map courtesy of Rimantas Jankauskas).

Most working adults in Alytus participated in animal husbandry, herding, agriculture, trading, hunting, or fishing as feudal peasants for an appointed vassal or lord (Kozakite, 2011). Presence of skilled craftsman such as foresters, smiths, woodworkers, bakers, and millers permitted an expansion of the middle class and provided opportunities of apprenticeships for adolescents (Kozakite, 2011). However, it is accepted that a majority of individuals living and dying in Alytus would have been peasantry who struggled to provide food for their families. While some have argued that Lithuania was not under a strict feudal system, the rural poor were still grossly underprivileged, underpaid, and underfed throughout the Middle Ages (Jankauskas and Urbanvicus, 2008; Rowell, 1994). Subadults at Alytus were most likely the children of these

rural and urban peasants who learned and participated in the blend of pagan and Christian customs while being assimilated into an agricultural and trading village typical of Medieval European lifestyles (Jankauskas and Urbanvicus 2008; Kozakite 2011; Rowell 1994).

The Agrarian laws instituted in 1557 reformed the town of Alytus and brought people from distant farm lands into the center of the village where Alytus' population grew to about 1150-1200 people at its largest (Kozakite, 2011). This height of urbanization brought with it an expanding middle class of craftsman and increased presence of traders for the exportation of barley grain and flax seed (Kozakite, 2011; Rowell, 1994). As a result of the closer quarters, ever-increasing population, and contact with other regions through trade, Alytus was struck by the plague in 1602, 1631, 1668, and 1709-1711 (Kozakite, 2011). The devastation and loss of life was compounded by a series of fires destroying the village (1607, 1619, 1622, 1655, 1661, 1690, 1703, 1707, 1736), crop failure with subsequent famine (1601-1602, 1708), and war with both Russia (1655), and Sweden (1706) (Kozakite, 2011). By 1667 the population of the town had already decreased to only 450 residents, and by 1712 the settlements at Alytus were abandoned for the remainder of the Medieval Period.

The Cemetery at Alytus

Alytus cemetery is the largest excavated cemetery in Lithuania. It was in use beginning in the late 14th century, after the conversion of the Grand Duchy of Lithuania to Catholic Christianity, until the early 18th century, coinciding with the abandonment of the site in 1712 (Kozakite, 2011). The cemetery itself is located only 500 meters northwest of the Hill Fort on the east bank of the River Nemunas (Fig. 2). It is still not known if residents on the eastern or western side of Nemunas were buried in the cemetery or if both populations were interred in the

same grounds. Archaeologists excavated the cemetery at Alytus (Fig. 3) in the 1980's revealing 1,152 intact burials, about 300 disturbed graves, and 2,000 associated grave goods (Kozakite, 2011). Of the burials exhumed approximately 57% contained individuals who were less than 15 years of age when they died (Jankauskas, 1998; Jankauskas and Schultz, 1995).

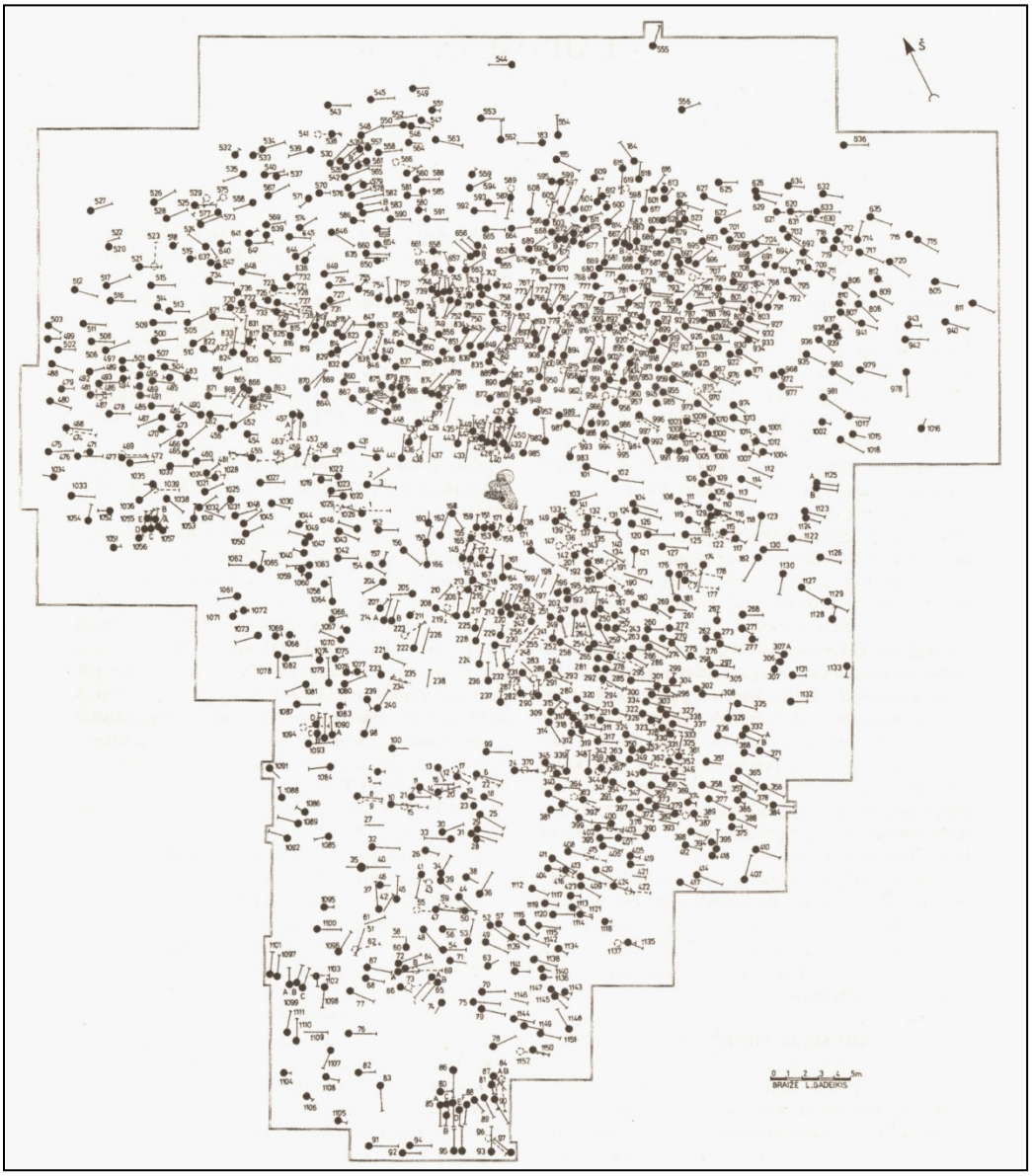


Figure 3. Plan of Alytus cemetery with individual burial numbers (Map courtesy of Rimantas Jankauskas). For cemetery plan with edits by the author demarcating subadults that were used for the current sample please see Appendix A.

The design of the cemetery mirrors the diversity and non-centralized customs of Lithuania during the Medieval period. Mortuary practices vary in terms the presence of burial goods and position of interment, with some individuals having been buried in typical Christian convention with their heads in the west and others buried in a north to south orientation, possibly related to earlier pagan traditions (Kozakite, 2011; Rowell, 1994). Presence of grave goods may also be indicative of retention of pagan traditions to ensure a safe afterlife. There is no concise correlation between type and amount of grave goods with sex, age, position, or location of burials making an understanding of hierarchical burial practices unclear (Kozakite, 2011). Thus, the socioeconomic composition of the cemetery may be reflective of the majority of the Medieval community consisting of peasant townspeople rather than the nobility.

Previous Research of the Subadult Population at Alytus: Pathological Conditions

Increased population density at Alytus village after the 1557 village reforms would have had a significant impact on all members of the agrarian and trading society, especially in terms of their exposure to novel disease and access to food resources. It was previously mentioned that periods of famine and transmittable diseases are historically known to have been prevalent at Alytus during the use of the cemetery. Previous research of subadult skeletal remains at Alytus identified frequencies of non-specific indicators of nutritional stress as well as the presence of tuberculosis identified through aDNA analyses.

Eleazer (2013) found that there were no specific pathological conditions that could be macroscopically identified in the present sample (e.g., tuberculosis, scurvy, or rickets), but recorded non-specific indications of stress in the forms of:

1. Cranial Stress
    - Porotic hyperostosis of coalescing foramina and expansion of diplöe on the ectocranium and/or orbital surfaces
2. Post-Cranial Stress
    - Osteoperiostitis, or abnormal bone deposition on more than one element.

Eleazer (2013) concluded that subadults at Alytus with evidence for chronic stress (the presence of two or more of the lesions outlined above) also had reduced microscopic bone mass in the humeri and ribs, but not in the femora, which would have affected the mechanical loading on midshaft cortical bone, but not significantly.

However, it is difficult to differentiate between lesions formed on the skeleton due to chronic physiological stress and those formed during the healing process of a potential, physiologically robust individual. Wood and colleges (1992) warn that this Osteological Paradox must be taken into consideration prior to concluding individual or population disease experience in the past using human skeletal remains. The skeletal remains of subadults in this study belong to individuals who did not survive childhood and entered the cemetery sample as a result of unknown or unrecorded causes of death. Individuals without visible pathological conditions could indicate a relatively healthy individual or more likely one whom succumbed to disease so rapidly their body was not able to sustain itself and mount a response to fight a disease.

Dietary and Chronic Stress at Alytus

At Alytus, there is overwhelming evidence for the presence of nutritional deficiencies such as vitamin C deficiency scurvy, vitamin D deficiency rickets, as well as iron deficiency, and hereditary anemia (Jankauskas and Schultz, 2009). Šereikiené and Jankauskas (2004) state that

within subadult samples from Lithuania, chronic undernutrition is especially prevalent in the form of protein calorie malnutrition and mineral deficiencies such as iron, copper, and zinc, which are all integral to normal bone development. Famine, crop failure, and unequal access to resources for the poor can all be labeled as external factors contributing to malnourishment of growing subadults. Additionally, subadults who have entered the cemetery sample, and show evidence of chronic stress, likely exhibited lowered immune responses and were subsequently more susceptible to dying due acute illness (Lewis, 2007b).

Nutritional deficiencies often present themselves as non-specific stressors including general subperiosteal depositions on the surface of a bone, dysplasias, dental enamel hypoplasias, or resorptive pits in the eye orbits or on the ectocranial surface. Vitamin D deficiency affects the intestinal absorption of calcium, delaying calcification of deposited osteoid, and leading to bowing or curvature of weight bearing bones due to the proliferation of collagen. Vitamin C deficiency is typically associated with bleeding resulting in resorptive lesions on the eye orbits, mandibular rami, the hard palate, greater wings of the sphenoid, and the infra- or supra-scapular fossae indicating osteoclastic release of nutrients stored in bone into the blood stream (Brickley and Ives, 2008; Ortner, 2003). Once vitamin C is back in the diet, bone may again be deposited in these areas. Anemic conditions typically identified by cranial porotic hyperostosis and cribra orbitalia may be caused by insufficient protein consumption. This may have been due to prolonged breastfeeding, by the absence of meat sources from an individual's diet, or parasitic assaults. Yet, it is often difficult for paleopathologists to disentangle the specific causes of the skeletal lesions. A deficiency in vitamin D may result in malabsorption of vitamin C, which could lead to hemorrhaging, blood loss, and finally acquired anemia where skeletal evidence for all three processes may be present (Brickley and Ives, 2008; Ortner, 2003).

Finally, due to the rapid growth of bones during development, these assaults may even have been corrected for prior to death and thus would not be evident in remains. However, in the archaeological record malnourishment may not always be clear-cut in infancy. Between two and six months after birth a loose apposition of woven bone on the periosteum may appear due to rapid growth with delayed modeling of mature cortical bone rather than being due to an infectious cause (Scheuer and Black, 2004). Eleazer (2013) found that subadults who exhibited cranial and post cranial markers of chronic stress had smaller cortical bone area in bones that were not used for mobility (e.g., the humeri and ribs). The cortical bone areas of femora were not significantly affected by chronic disease, leading Eleazer (2013) to conclude that bone was preferentially removed from non-essential elements to maintain a level of nourishment for continued life.

Šereikiené and Jankauskas (2004) note that in the children (3-5 years) from Alytus stature was shorter in comparison to modern and healthy Lithuanian children. Therefore, it is quite likely that those who died in this social age group likely experienced high nutritional or disease stress, possibly in relation to previous weaning stress or lack of sufficient food calories earlier in development. The researchers also concluded that there was a slight growth spurt during the juvenile stage (5-7 years), which can be interpreted to mean that the juveniles at Alytus had likely survived the malnutrition of childhood and had recovered enough to begin growing (Eleazer 2013; Jankauskas and Schultz 1995; 2009). Šereikiené and Jankauskas (2004) also found that adolescents between 12-15 years at death exhibited a delayed pubertal growth spurt likely indicating that those individuals had lived through many childhood stresses and undernourishment had the potential to physiologically succeed in new social environments.

Infectious and Acute Disease at Alytus

Many acute diseases would have affected the morbidity and mortality of the children at Alytus, but most of these infections cannot be seen on skeletal remains because the individual would have passed away too quickly for the skeleton to show evidence of infection. Šereikienė and Jankauskas (2004) suggest that acute gastrointestinal or respiratory infections likely attributed to the majority of infant deaths in Medieval Lithuania. It is also likely that a greater number of male infants died due to respiratory arrests during the first year (Ulizzi and Zonta, 2002). In Medieval Europe, the threat of the Black Death plague and the Consumption from tuberculosis loomed heavily over the entire continent.

Lithuania, and particularly Alytus, escaped the first waves of the spreading Plague virus but once their territory expanded and barley exportation grew, the town suffered from four major bouts of plague diminishing the population (Kozakite, 2011). Plague is an infectious virus transferred to a human through a flea bite, inhalation of dried rat feces, or aspirated from the lungs of an already infected human (Aufderheide and Rodriguez-Martin, 1998; Roberts and Manchester, 2007). The common bubonic and pneumonic plagues were easily transmitted in newly, densely packed Medieval, urban populations. Due to the lack of treatment and immunity by most Europeans, the virus generally killed individuals within the span of five days allowing no time for the skeleton to exhibit any response that may have been interpretable in the archaeological record (Aufderheide and Rodriguez-Martin, 1998; Roberts and Manchester, 2007). Children already suffering from malnutrition would have been particularly susceptible to mortality from the fevers, headaches, and hemorrhagic inflammation associated with the infection (Roberts and Manchester, 2007).

Tuberculosis is a bacterial infection transferred though aspirated air and water droplets in a cough from an infected individual (Aufderheide and Rodriguez-Martin, 1998; Roberts and Manchester, 2007). It was very likely that each citizen of Alytus was exposed to tuberculosis at some point in their life, many likely contracted varying symptoms, some of which can be seen in the skeletal record. There is a vast literature for the diagnosis of tuberculosis in human skeletal remains, most commonly seen affecting the lower vertebrae of juveniles, including many juveniles at Alytus (Aufderheide and Rodriguez-Martin, 1998; Holloway et al., 2011; Jankauskas 1998; Jankauskas and Schultz 2009; Ortner, 2003). The main symptom of acute infection from tuberculosis is increased basal cranial pressure, which correlates to inflammatory and hemorrhagic meningeal responses (meningitis) in the endocranium (Jankauskas, 1998; Jankauskas and Schultz 1995). Individuals who contract the tuberculosis bacterium can live with meningitis for many weeks, sometimes in a coma allowing bone to rapidly deposit where the blood vessels ruptured in the brain (Lewis, 2007a).

CHAPTER 3: MENU

Dietary Customs for Medieval Christians

Medieval physicians analyzed food properties and advocated dietary regimes based on social position and the ancient Arabic theory of the Humors. Above all else, fresh and unaltered foods were recommended for consumption (Adamson, 2004; Knight, 2002). For the poor, diet would likely have been affected by the availability of garden foods and what little meat could be purchased (Adamson, 2004).

"Delicate" foods and healthy white meats were recommended for the elite and sickly. Veal, wild boar, capon, hen, chicken, partridge, quail, pheasant, woodcock, lark and thrush were considered "delicate" or "dry" humored meats appreciated by the European noble classes (Adamson, 2004). These terrestrial meats may have been desired for both prestige and health reasons by working peasants and craftsman of the rising middle class at Alytus, which may have included working adolescents and their parents. Interestingly, those who may have been sick from various illnesses were also likely drawn to consume more of the "dry," "coarse" meats if available and possibly in addition to barley soups. Foods like fish were considered "wet" humored but were often consumed by Catholic followers during fast days and the season of Lent when four-footed animals were not to be eaten. "Coarse" and hearty foods were recommended for laborers. These included red meats, cheeses, and tougher grained breads like rye and barley (Adamson, 2004). At the moment, there are no direct archaeofaunal or archaeobotanical remains for the individuals buried at Alytus. However, we can hypothesize dietary composition based on recommendations for Christian Europe and edible plants and animals recovered from similar archaeological contexts in Lithuania (Table 1).

Table 1. Some possible food items consumed at Alytus based on European customs (Adamson, 2004) and archaeological preservation of excavated assemblages throughout Lithuania (Antanaitis-Jacobs and Girinikas, 2002; Antanaitis-Jacobs et al., 2012; Kiseliene, 2012; Kozakite, 2011).

Animal Protein	**Field Crops**	**Garden Plants**	**Fruits & Nuts**	**Other**
<u>Wild and Domesticated</u> - Cow/ Auroch (*Bos primigenius)* Cow's milk - Sheep (*Ovis avies*) - Goat (*Capra hircus*) - Pig/ Wild Boar (*Sus suis /Sus scrofa*) - Chicken (*Gallus gallus domesticus*) Chicken's Eggs	<u>Temperate</u> - Barley (*Hordeum vulgare*) - Flax Seed (*Linum usitatissimum*) - Rye (*Secale cereale*) - Buckwheat (*Fagopyrum esculentum*) - Wheat (*Triticum*) - Hemp (*Galeopsis tetrahit*)	- Gourd (*Cucurbita*)	- Wild Blueberries (*Vaccinium myrtillus*) - Wild Raspberries (*Rubus idaeus*) - Wild Strawberries (*Fragaria vesca*)	- Honey - Wine - Sugar - Cheese - Poppy syrup (*Papaver somniferum*)
<u>Forest</u> - Rat (*Rattus rattus*) - Red Deer (*Cervus elaphus*) -Elk (*Alces alces*) -Beaver (*Castor fiber*)	<u>Tropical</u> - Millet (*Panicum miliaceum*)		<u>Trade Goods</u> - Fig (*Ficus carica*) - Hazel (*Corylus avellana*) -Almond (*Prunus amygdalus*)	
<u>Water Foul</u> - Mallard (*Anas platyrhynchos*)				
<u>Freshwater Fish</u> -Pike (*Esox lucius*) -Pikeperch (*Sander lucioperca*)				
<u>Saltwater Fish</u> - Baltic Herring (*Clupea harengus membras*) - Flat Fish (*Pleuronectes platessa*)				

Plant resources

Domesticated barley grains (*Hordeum vulgare*) and flax seed (*Linum usitatissimum*) were cultivated and exported from farms associated with Alytus (Kozakite, 2011). Barley was one such food item that when baked into bread was considered coarser than wheat bread, which was prized by the noble classes (Adamson, 2004). Furthermore, millet (*Panicum miliaceum*) cultivation was intensifying in the Lithuanian capital of Vilnius during the 6th century and again in the 13th- 14th century (Stancikaite et al., 2008). Farming of millet was also seen early in the Neolithic Baltic at archaeological sites in Lithuania (Antanaitis-Jacobs and Girinikas, 2002; Antanaitis-Jacobs et al., 2012).

Klaipeda is a Medieval archaeological town situated on the Baltic Sea in costal lowlands (Fig. 1). It lies 280 kilometers to the northwest of Alytus and was likewise a center of trade in Medieval Lithuania (Kisieliene, 2012). The temporal and geographic setting of Klaipeda makes the botanical remains found at the site comparable to Alytus. Archaeologists excavated botanical remains of:

1. Grains: *S. cereale* (Rye), *Fagopyrum* (buckwheat)
2. Weeds: *Galeopsis tetrahit* (common hemp-nettle weed), *Ch. Album* (goosefoot weed), *Sp. Arvensis* (corn spurry weed), *P. lapathifolia* (willow weed)
3. Garden plants: *Cucurbita* (gourd),
4. Forest plants *V. myrtillus* (European, wild blueberry), *R. idaeus* (European, wild raspberry), *F. vesca* (European, wild strawberry), *C. avellana* (common hazel), and
5. Trade foods fig and walnut, all of which were exploited by the townspeople (Kisieliene, 2012).

Kisieliene (2012) suggested that most of the grains would have been brought into the town from outside farms but the garden plants and forest vegetation would have been cultivated locally. These botanical food items may have been similarly consumed by the townspeople of Alytus who had access to the forest, their own surrounding cropland, and were also located on a trade route.

Protein resources

For the peasantry beef was the cheapest and coarsest meat available while pork was the easiest to prepare for storage over the winter in addition to being available in the Alytus Forest (Table 1) (Adamson, 2004). Cheese was also considered an appropriate "rough, white meat" but as a source of protein, this food lacks the essential nutrients found in animal meat such as iron. Adamson (2004) also notes that Medieval physicians accorded the meat of any animal caught by someone of the lower class to have been an acceptable and healthy food for a laborer. This meant that in times of extreme famine people could likely have been consuming the poorest cuts of meat and even rats, the harbingers of Plague.

Previous isotopic research on human and faunal assemblages from Lithuanian archaeological sites dating to the Mesolithic through Bronze Age reveal that over time, the consumption of wild forest species (e.g., elk (*Alces alces*), deer (*Cerbus elaphus*), boar (*Sus scrofa*), auroch (*Bos primigenius*), beaver (*Castor fiber*), bear (*Ursus arctos*)) transitioned to predominant consumption of domesticated cattle (*Bos taurus*), sheep (*Ovis avies*), goats (*Capra hircus*), and pig (*Sus suis*) during the middle Neolithic (Antanaitis-Jacobs et al., 2012). Many of these wild forest species were contained in a menagerie at Alytus for breeding (Kozakite, 2011). Furthermore, the late Neolithic to early Bronze Age site of Turlojiske (located on the river

Kirsna in southwestern Lithuania) is a close locational proxy for Alytus and the types of faunal remains (mallard, red deer, and cattle) that might have been present during the Middle Ages. Thus, we may expect Alytus' population to exhibit isotopic signatures close to Bronze Age human values when domesticated protein was chiefly exploited with some use of forest and fresh-water fowl protein.

Freshwater fish also would have been immediately available from the river Nemunas and very likely consumed as a protein during Catholic observances of fasting (Adamson, 2004). Antanaitis-Jacobs and colleagues (2012) report that after the Neolithic, fish assemblages in Lithuania consist almost entirely of pike (*Esox lucius*), perch (*Perca fluviatilis*), and pikeperch (*Sander lucioperca*) species. Trade ships coming from the Baltic Sea may well have traded in salted herring (*Clupea harengus membras*) to the town of Alytus as well.

Cultural Customs for Medieval Infants and Children

No literature pertaining specifically to the culture of childhood diet in Lithuania is obtainable. Therefore a discussion of dietary customs for Alytus' subadults must be hypothesized based on writings in Western European Medieval texts and cookbooks. Medieval dietary practices throughout Europe recommended newborn infants to be fed their own mother's breast milk, or that of a well-tempered wet nurse if the mother had to return to work or died while giving birth (Adamson, 2004). Weaning was recommended to begin upon teething (eruption of deciduous incisors occurs around the first year), but may have occurred closer to the age of two when most of the deciduous dentition had already erupted (Adamson, 2004). Weaning foods were supposed to be easily digestible and delicate tempered foods; things like chewed breads

dipped in sugar, honey, wine, or water and boiled meats or almonds were suggested (Adamson, 2004).

The prescribed food for infants may have had deleterious effects on their life expectancy. The breast-milk produced within the first few days after birth, the colostrum, was not recommended for ingestion, which regrettably is composed of desirable antibodies and proteins indispensable for the sustained survival of the neonate (Dettwyler and Fishman, 1992; Ulijaszek, 1990; Lewis, 2007b). The foremilk (breast milk produced first in the morning) was also not recommended to give to breastfeeding infants, but this prohibition permitted infants greater access to the hind milk, which is higher in fat content and calories providing adequate nutrition for the growing newborn (Fujita et al., 2012). Upon weaning, animal milk sweetened with honey or sugar was recommended (Lewis, 2007b). However, the digestion of cow's milk can overload an infant's kidneys, concentrating urine, and causing bleeding in the gut (Lewis, 2007b). Unfortunately, honey was also a dangerous additive as in allows the natural microflora of botulism to grow in undeveloped and small intestines of infants (Dupras et al., 2001; Lewis, 2007b). For babies who would not sleep or stay calm, narcotic plants such as *Datura stramonium* or poppy syrup may also have been implemented to subdue the infants (Knight 2002; Šeškauskaite and Gliwa, 2006). Furthermore supplementation of breast milk with animal milk during weaning possibly led to protein deficiency anemia, which likely inhibited the infant's natural production of zinc. Zinc is an element necessary to develop immunity in infants after the age of eight months when passive immunity received from the mother in utero no longer exists (Dettwyler and Fishman, 1992; Ulijaszek, 1990; Lewis, 2007b).

Early childhood foods in Medieval European cookbooks remained similar to those foods that the individual was weaned onto with the addition of boiled meats and nuts, which could

have potentially lead to salmonella poisoning (Adamson, 2004). Between the ages of six and ten a juvenile was thought to be ready to eat the more course foods of adults, which would have benefited the nutrition of the individual as they were preparing to enter the adult workforce. Therefore, if Lithuanians were following western European customs, a subadult older than the age of six was more likely to receive adequate customary nutrition. Juvenile and adolescent individuals are thus hypothesized to have consumed the same types of food as the adult peasantry; temperate grains supplemented by terrestrial and freshwater proteins from the nearby forest, herds, and rivers. However, it is possible that inhabitants of Medieval Alytus did not practice the exact dietary customs as their western counterparts.

CHAPTER 4: STABLE ISOTOPE ANALYSIS OF BONE COLLAGEN

Isotopic Analyses in Anthropology

Anthropologists first discovered that geochemical analyses could be used to reconstruct diet while employing ^{14}C radiocarbon dating methods to archaeological bone excavated in the Americas (Ambrose and Krigbaum, 2003; Katzenberg 2008). It was discovered that the carbon ratio of $^{14}C/^{13}C$ in maize was elevated over typical $^{14}C/^{13}C$ of temperate grains, rendering radiocarbon dates more chronologically recent than artifacts from the same horizon. Following this discovery, it was proposed that the ratio of $^{13}C/^{12}C$ would also be enriched in individuals consuming maize over their counterparts whose diet consisted mainly of temperate plants. The first use of this method was by Vogel and van der Merwe (1977) who demonstrated that ^{13}C values could be utilized to cross-sectionally track the introduction and increased importance of maize through time in archaeological communities. Vogel and van der Merwe (1977) compared carbon isotopic signatures from the non-maize consuming hunter-gatherers of the Archaic and Early Woodland to their maize eating Late Woodland predecessors.

What followed was a focus on providing evidence to demonstrate that carbon and nitrogen isolated from bone collagen were reflective of actual dietary proteins consumed from plant and animal resources (DeNiro and Epstein, 1978; 1981). Next, the fractionation process was investigated allowing for the creation of models to demonstrate where a consumer fell in a food-web. Collagen demonstrates about a +5‰ increase in carbon over the comparative plant food in small animals like rats (Kreuger and Sullivan, 1984; Vogel and van der Merwe, 1977; van der Merwe, 1982). Schoeninger and DeNiro (1984) also utilized controlled studies on mice to discover the +3‰ trophic level elevation of an organism's nitrogen isotopic value over the

nitrogen value of its protein source. The introduction of nitrogen to anthropological isotopic studies allowed researchers to utilize both carbon and nitrogen elements to interpret exploitation of either marine or terrestrial protein (Schoeninger and DeNiro, 1984). Nitrogen and carbon analysis was utilized to reconstruct the type and timing of changes in subadult diet for this study. Oxygen and hydrogen isotopes may also be considered to trace the introduction of water into infant diet but will not be discussed here (see Katzenberg, 2008 for a review).

Chemical principles used in isotopic analyses

Human bone is comprised of about 70% inorganic hydroxyapatite crystals and 30% organic materials in the form of collagens, proteins, proteoglycans, and lipids (Katzenberg, 2008; Myllyharju, 2004). Osteoblasts secrete type I collagen, a flexible fibril forming collagen composed of an α chain; a triple-helix of repeated glycine, proline, and hydroxyproline amino acids with nitrogen and carbon telopeptides at bone ends of the helices (Myllyharju, 2004). Bone collagen is an excellent source with which to investigate an individual's average dietary protein because collagen is comprised of about 35% dietary carbon and 11-16% dietary nitrogen received directly from protein consumption (van Klinken, 1999). Thus, formation of the osteoid matrix from osteoblastic activity relies upon adequate protein nutritional intake for anabolic construction.

All isotopes of an element have the same number of protons and electrons but are quantifiably unique based on their mass, or total neutrons (Hoefs, 1997; Katzenberg, 2008; Pollard, 2007). Stable isotopes, as opposed to radioactive isotopes, of an element do not decay over time and their preservation in collagen allows researchers to measure the ratios of abundances of one stable isotope to another (Pollard, 2007). Researchers measure the atomic

ratio of ^{15}N to ^{14}N in a sample of collagen to determine the trophic level of a consumer over its food source. Likewise, the ratio of stable ^{13}C present in a sample compared to ^{12}C is measured for carbon analyses. However, initial carbon values are related to the three distinct photosynthetic pathways in which plants fixate and react to carbon dioxide from the atmosphere or biocarbonate in water. Plants are adapted to utilize $C_{3,}$ or Calvin pathways in temperate regions, $C_{4,}$ or Hatch-Slack pathways in tropical regions, or CAM intermediate pathways (van der Merwe, 1982; Vogel and van der Merwe, 1977; Katzenberg, 2008; Pollard, 2007).

The difference in isotopic ratios brought about by anabolic transfer of fixated nitrogen and carbon isotopes from food source to consumer is referred to as the "fractionation" of an isotope (Hoefs, 1997; Katzenberg, 2008). The fractionation of ^{15}N and ^{13}C relative to the depleted ^{14}N and ^{12}C are the values measured in isotopic studies. For example, the stable forms of nitrogen are ^{14}N and ^{15}N where ^{15}N has one more neutron than ^{14}N. Therefore ^{15}N is atomically heavier and more abundantly found in bone collagen because ^{14}N can be used more readily in metabolic or chemical reactions with common, atmospheric N_2 (Pollard, 2007).

To solve for the amount of fractionation an isotope has undergone, the weight of a heavier isotope of an element is compared to its lighter isotope and subsequently compared to a chosen standard (DeNiro, 1985; Katzenberg, 2008; Schwarcz and Schoeninger, 1991). The following equations solve for the value of the fractionation of any isotope (carbon or nitrogen) from comparing the sample and a known standard:

$$\delta^{13}C‰ = \left[\frac{(^{13}C/^{12}C)_{\text{Sample}}}{(^{13}C/^{12}C)_{\text{PDB Standard}}} - 1\right] \times 1000 \quad \textbf{(1)}$$

$$\delta^{15}\mathrm{N}‰ = \left[\frac{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\mathrm{Sample}}}{(^{15}\mathrm{N}/^{14}\mathrm{N})_{\ \mathrm{AIR\ Standard}}} - 1\right] \times 1000$$

(2)

The delta value (δ) of an isotope is reported in permil (‰) because there are small and meaningful differences between the ratio of the sample to the standard, and it is this value that is used to analyze the effect of anabolic fractionation from diet to collagen (Hoefs, 1997; Katzenberg, 2008; Pollard, 2007). The standard comparison available for nitrogen is AIR, or the ambient inhalable reservoir, and the PeeDee Belemnnite (PDB) standard for carbon (Schwarcz and Schoeninger, 1991).

Carbon isotopes and the reconstruction of majority plant type consumption

Carbon isotopes are effective in differentiating between the consumption profiles of staple plant types, which differentially metabolize atmospheric carbon dioxide (CO_2) and ground water through their distinctive photosynthetic pathways. Carbon is fractionated from plant to consumer by about +5‰. Furthermore, the enzyme used in photosynthesis and the arrangement of cells during photosynthesis determines the final fractionation of $\delta^{13}C$ in plant material (Hoefs, 2009).

Temperate plants (terrestrial grasses, wheat, barley, rice, fruits, vegetables, beans, shrubs and trees) use the C_3 or Calvin photosynthetic pathway. The Calvin pathway depletes more carbon in a plant relative to the atmosphere by utilizing the enzyme ribulose biophosphate for photosynthesis. In using this enzyme in conjunction with permeable mesophyll cells, C_3 plant $\delta^{13}C$ values concentrate between -36‰ and -19‰ (global mean -26.5‰) (Hoefs, 2009; Pollard, 2007; Katzenberg, 2008; Smith and Epstein, 1971; van der Merwe, 1982). The majority of plant

types in Lithuania are classified as C_3 plants (with the only known exception of millet) and therefore, isotopic signatures in human remains for $\delta^{13}C$ values are expected to be around 21.5‰.

Tropical C_4 plants, adapted for hot and dry environments (maize, sorghum, millet, sugar cane, and tropical grasses) utilize the Hatch-Slack mode of photosynthesizing atmospheric carbon. Hatch-Slack photosynthesis depletes much less carbon during photosynthesis through the presence of less permeable cellular sheaths and the use of the phosphoenolpyruvate carboxylate enzyme. This process results in enriched, non-overlapping $\delta^{13}C$ values between -17‰ and -9‰ (global mean -12.5‰) (Hoefs, 2009; Pollard, 2007; Katzenberg, 2008; van der Merwe, 1982). Therefore, it could be expected that an animal that habitually consumes C_4 plants would have $\delta^{13}C$ values around 7.5‰.

A third photosynthetic pathway for CAM plants produces $\delta^{13}C$ values that are intermediate to C_3 and C_4 plants. These plants are adapted for environments where water may sometimes be more difficult to come by. Therefore cacti, succulents, and pineapples will likely have intermediate $\delta^{13}C$ signatures (Katzenberg, 2008). It is unlikely that the Alytus samples will exhibit $\delta^{13}C$ values consistent with either C_4 or CAM plant ranges as Lithuanian vegetation would not be adapted to tropical environments. However, the presence of millet in the archaeobotanical record at the capital of Vilnius during the 13th to 15th centuries suggests that analysis of isotopic signatures should consider enriched $\delta^{13}C$ values as possibly being reflective of the consumption of some non-C_3 plant types (Antanaitis-Jacobs and Girinikas, 2002; Antanaitis-Jacobs et al., 2012; Stancikaite et al., 2008).

Nitrogen to account for trophic level position

Bioarchaeological isotopic studies most often utilize nitrogen $\delta^{15}N$ to describe the type of plant or meat protein consumed by an individual or group based on the principle nitrogen enrichment through successive trophic feeding levels (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984). Nitrogen is fractionated in the body during metabolic transfer of one amino acid to another by the enzyme glutamate-oxaloacetate transaminase (van Klinken et al., 2000). However, nitrogen can also be recycled and brought back into the blood stream through reabsorption in the kidneys. When nitrogen is recycled, the isotopic values enrich as the heavier ^{15}N isotope is retained while ^{14}N is depleted in urea output (van Klinken et al., 2000).

Controlled feeding experiments on mice by DeNiro and Epstein (1981) first established the occurrence of +3‰ enrichment in $\delta^{15}N$ in the bone collagen of a consumer over the bone collagen $\delta^{15}N$ of their protein source when nitrogen is metabolized from food and utilized in the construction of bone collagen. For instance a grass may have a $\delta^{15}N$ of 3‰, which may then be eaten by a rabbit whose bone collagen will be enriched 3‰ over the grass to 6‰. Therefore, an herbivore will be on a second trophic level, and any organism that also receives the majority of its protein from plant sources will have $\delta^{15}N$ values around 6‰. It follows that a carnivore, say a human eating the same rabbit, would demonstrate a $\delta^{15}N$ value closer to 9‰ (DeNiro and Epstein 1981; Schoeninger et al., 1983). The process continues on throughout the food chain especially in marine environments where humans may ultimately consume a carnivorous fish or larger mammals yielding $\delta^{15}N$ values anywhere between 10‰ and 18‰ (Pollard, 2007; Lee-Thorp, 2008; Schoeninger and DeNiro, 1984). Thus it stands that $\delta^{15}N$ values will be more

enriched in individuals whose protein consumption is primarily from terrestrial animal protein and even more so if the individual is consuming marine animal protein.

The initial ‰ of $\delta^{15}N$ nitrogen in plants is fixed by two manners. Nitrogen-fixing plants like legumes (beans, soy beans, peas, lentils) are able to receive nitrogen through bacterial activity at the plant's roots. The bacteria Rhizobium aids in fixing the nitrogen directly with oxygen and hydrogen allowing the plant to take in all atmospheric nitrogen (N_2) without fractionation, yielding a plant with a $\delta^{15}N$ value of 0‰ - the atmospheric standard (Katzenberg, 2008; van Klinken et al., 2000).

However, many plants are non-nitrogen fixing and absorb the element through the decomposition of surrounding organic material into either ammonia (NH_3) or nitrate (NO_3) leaving the plant more enriched in nitrogen than their leguminous counterparts (van Klinken et al., 2000). It should follow that nitrogen values reported for a human sample will reflect the importance of either plant protein (depleted $\delta^{15}N$) or meat protein (more enriched $\delta^{15}N$) based on trophic levels within a regional food web (DeNiro and Epstein, 1981; Hedges and Reynard, 2007; Katzenberg, 2008).

No study has reliably quantified or classified the $\delta^{15}N$ signatures of specific plants due to the volatile nature of their nitrogen fixing mechanisms, which greatly change nitrogen values. Factors such as available nitrogen in the soil, salt content of the soil, microbial activity, aridity of the environment, and presence of fertilizers will all result in different base nitrogen levels for plant proteins (Hedges and Reynard, 2007; van Klinken et al., 2000). For example, if $\delta^{15}N$ values are near 0‰, this could indicate a legume or another nitrogen fixing plant but may also be indicative of a non-nitrogen fixing plant (expected to have slightly more enriched $\delta^{15}N$) from a

dense forest area (Koerner et al., 1999; van Klinken et al., 2000). Nitrogen is scarce in the soil of dense forests and therefore also in animals eating those resources (DeNiro and Epstein, 1981). Conversely arid environments, like deserts, promote water retention in the cycling of nitrogen as a survival mechanism for both plants and animals to hold onto water (Ambrose and DeNiro, 1986; Heaton et al., 1986). Water-stressed organisms in these conditions have an increase in the base amount of $\delta^{15}N$ not only because of differential nitrogen plant in-take but also because animals will release more ^{14}N through urea production regardless of protein consumption (Ambrose and DeNiro, 1986; Hedges and Reynard, 2007; Sillen, 1989).

The introduction of nitrate heavy fertilizer to the agricultural landscape through additives such as manure or even of the churning of soil will increase the foundational value of nitrogen in non-nitrogen fixing plants (van Klinken, 2000). Increased use of fertilizer on crops would show substantially higher nitrogen values per trophic level, whereby first level carnivores could exhibit isotopic collagen values greater than 8-10‰. Thus, nitrogen isotopic values cannot be used to distinguish between specific plant types as $\delta^{13}C$ can because they vary greatly by habitat and modes of production, which must always be considered in interpretations of isotopic results.

Issues in analyzing nitrogen isotopes

Nitrogen fractionation is still widely understudied in humans and thus an area of controversy. There exists both a lack of knowledge and faulty interpretations of how various metabolic processes ultimately affect $\delta^{15}N$ values. For example, the amount of protein eaten may influence nitrogen levels, growth and starvation will enrich or deplete nitrogen levels, the exact isotopic values for food components in diets are largely unknown in ancient populations, and milk and meat from the same animal are indistinguishable (therefore subadults drinking animal

milk after weaning may appear to maintain enriched nitrogen levels even if they are not consuming meat protein) (Hedges and Reynard, 2007).

DeNiro and Epstein (1981) found a significant difference in nitrogen values for animals of the same species that were given the same diet. Within species variation of $\delta^{15}N$ composition could therefore lead to a misunderstanding of nitrogen values when comparing individuals in a bioarchaeological sample. Hedges and Reynard (2007:1241) also consider this a "practical issue" to the current interpretations of nitrogen values. They note that within species variation may be due to physiological stress, which is common in archaeological populations and probably causes differences in nitrogen values that cannot accurately be explained by dietary intake alone.

In times of stress as well as growth, the body is no longer functioning at homeostasis, or its proper balance, and attempts to maintain life through either increasing or decreasing stores of elements. Nitrogen isotopes are known to change to right any functional imbalance (Hedges and Reynard, 2007; Katzenberg and Lovell, 1999). The amount of nitrogen in protein eaten must be equal to the amount excreted as ammonia in urea, a factor known as nitrogen balance. Nitrogen equilibrium occurs during normal remodeling and thus reflects the homeostatic state of bone (Marieb and Hoehn, 2003; Orwoll, 1992). During growth, the body is in positive nitrogen balance, maintaining a larger proportion of lighter isotopes in the nitrogen pool for tissue formation, yielding more depleted $\delta^{15}N$ values than expected for dietary reconstructions in stable isotope analyses (Hedges and Reynard, 2007; Reitsema, 2013).

Disease stress is especially important to consider due to the nature of the skeletal assemblages we study. In considering the effects of disease on nitrogen isotope values, a researcher must be wary that isotopic values may denote a chronic disease process that affected the elemental content of the bone collagen. Through their examination of pathological bone

collagen isotopic values, Katzenberg and Lovell (1999) have shown that protein stress, malnutrition, and prolonged infection will also increase levels of $\delta^{15}N$. During negative nitrogen balance (periods of protein calorie malnutrition, starvation, or injury) the element is not utilized to form or reform tissues but is catabolized for energy. The body can recycle stores of elements by anabolizing ammonia formed during urea production in the kidneys or through breaking down bone structure in order to maintain life thereby repeating the fractionation process and elevating nitrogen values when nitrogen is ultimately utilized again to maintain deposition of bone for longitudinal growth or to fight off infectious disease (Marieb and Hoehn, 2010; Orwoll, 1992; Reitsema, 2013). Even when an individual is malnourished, the body will utilize varying mechanisms to maintain growth and deposition of bone (Reitsema, 2013). In times of famine or water stress the body will increase its stores of ^{15}N relative to ^{14}N through the increased concentration of urea output (Hedges and Reynard, 2007; Katzenberg and Lovell, 1999; Mekota et al., 2006). This allows an organism to adapt to an arid climate and fluid dehydration (Hedges and Reynard, 2007).

Wright and Yoder (2003) believe that dietary reconstructions have the ability of illuminating causes for mortality, especially in studies of archaeological subadults. In Hedges and Reynard's (2007) evaluation of the complexities and unexplained variables of how metabolism affects protein synthesis in bone collagen, the authors suggest that the quality of diet, at this point, is not easily inferred utilizing nitrogen values without considering other isotopic values and the archaeological or inferred cultural context.

Nitrogen and carbon isotopes to differentiate between marine and freshwater diet

Alytus' location on the River Nemunas and their presence in trade with the Baltic Sea suggest that individuals living there might have had access to aquatic protein and therefore may also have exploited these resources when others were scarce. Furthermore, Christian fasting and feasts may have promoted the consumption of fish proteins over four-legged animals. Assessing potential consumption marine or freshwater protein is done so through interpreting both $\delta^{15}N$ and $\delta^{13}C$ signatures. Isotopic carbon values can be used to differentiate between coastal and freshwater protein and nitrogen values can establish greater trophic levels caused by the human consumption of carnivorous animal protein. Furthermore, there are biological causes for the discrepancies of isotopic values in bone collagen of consumers of marine meat, which are produced by the same chemical processes that occur on land but through differing mechanisms.

Protein derived from marine based diets will reflect more enriched $\delta^{13}C$ values in human bone collagen than collagen values from individuals who consumed majority terrestrial based diets due to carbon sources available during photosynthesis. Aquatic carbon derives from 0‰ carbonate (CO_3) whereas the atmospheric carbon dioxide (CO_2) utilized by terrestrial plants is already depleted by about -7‰ (Hoefs, 2009; Katzenberg, 2008). Aquatic plants like phytoplankton and algae correlate to an average global $\delta^{13}C$ around -20‰ (Lee-Thorp, 2008; Smith and Epstein, 1971). Marine resources may be more enriched in $\delta^{13}C$ than freshwater resources. However, depending upon the plant or animal's habitat within the water and their trophic position, values can substantially vary (Katzenberg, 2008). Many prehistoric studies of Mesolithic and Neolithic Europe focus on detecting the transition from hunter-gatherer diets to agricultural introductions of grains based on the depletion of $\delta^{13}C$ values and the decrease in the

consumption of marine protein throughout time (Richards et al., 2002; Tauber, 1981). Thus, most researchers have correlated enriched carbon signatures to marine diets, especially when considered with high trophic levels demonstrated by nitrogen isotopic signatures.

A further reason marine protein resources may be further enriched in nitrogen than their terrestrial counterparts (not solely based on trophic level enrichment) is because the most common form of nitrogen in the oceans is nitrate (NO_3), which is about 2-3‰ more enriched than soil nitrate (Lee-Thorp, 2008; van Klinken et al., 2000). Therefore, by the time a human eats marine protein, their nitrogen isotopic content is greatly enriched over what is commonly seen for a terrestrial meat consumer due to higher initial fractionation (DeNiro and Epstein, 1981; Lee-Thorp, 2008). Freshwater and coastal marine resources may show a similarly elevated effect of nitrogen levels over terrestrial values.

Examining Weaning Practices

Stable isotope analyses are now a well-established method for examining changes in infant feeding practices from exclusive consumption of breast milk to the addition of soft foods or gruels to supplement breast milk until adult diet is reached (e.g., Burt, 2013; Dupras et al., 2001; Fuller et al., 2006a,b; Fuller et al., 2003; Herring et al., 1998; Jay et al., 2008; Katzenberg et al, 1996; Pearson et al., 2010; Schurr, 1997; Turner et al., 2007; Waters-Rist et al., 2011; Wright and Schwarcz, 1998; Richards et al., 2002). Bioarchaeologists strive to understand the practices of weaning in the past for a number of reasons. A change in diet indicates the biological and developmental time period in which an infant may have been increasingly more susceptible to malnutrition due to weanling diarrhea upon introduction of adult foods (Dettwyler and Fishman, 1992; Ulijaszek, 1990; Wright and Yoder, 2003). Extended exclusive breastfeeding or

weaning may also have left an infant exposed to disease without having built up its own immunity (Dettwyler and Fishman, 1992; Ulijaszek, 1990). Therefore, dietary reconstructions through stable isotopic analysis can further illuminate inferences about food culture in the past in addition to providing another line of evidence for analysis of juvenile mortality.

A trophic level effect can be observed in the carbon (~ 1‰) and nitrogen (~3‰) of exclusively breastfeeding infants over their mothers. Fogel and colleagues (1989) were the first to begin tracking the consumption patterns of infants through nail sampling at various ages finding about +2.4‰ enrichment of nitrogen over maternal values. Preceding birth, the expected nitrogen isotope values are closer to the maternal means, if slightly higher. Pregnancy, however, tends to leave adult females slightly depleted in nitrogen even when consuming terrestrial meat (Fuller et al., 2006a). Thus, neonatal nitrogen values, while remaining a trophic level higher, will mirror the depletion of maternal values. Fuller and colleagues (2006a) suggested that the concurrent depletion might point to the greater use of ^{15}N to maintain the fetus' growth. After birth, many researchers (e.g., Dupras et al., 2001; Herring et al., 1998; Fuller et al., 2006a, b; Katzenberg et al., 1996; Schurr, 2007) conclude that ^{15}N is enriched about one trophic level over the maternal values, but can vary slightly from about 2-3‰. Because infants still receive protein directly from breast milk during the weaning process, $\delta^{15}N$ values are hypothesized to take longer to return to the maternal mean than carbon values (Fuller et al., 2006a). Thus nitrogen is likely indicative of the duration of the entire weaning process. The sudden rise of $\delta^{15}N$ after birth is followed by the gradual depletion of $\delta^{15}N$ toward mean sample population values after breastfeeding is supplemented by weaning foods.

In a modern sample, Fuller and colleagues (2006a) also concluded that the 1‰ enrichment of $\delta^{13}C$ could be utilized to demonstrate the age at which the introduction of weaning

foods occurred (Dupras et al., 2001; Wright and Schwarcz, 1998). The small trophic level enrichment of carbon however was followed by a quick decline in values toward maternal averages. Fuller and colleagues (2006a) concluded that the decline was consistent with the introduction of carbohydrate-heavy soft weaning foods to supplement breast milk. Therefore, in a cross-sectional analysis of dietary $\delta^{13}C$ values at a given age, a newborn infant's $\delta^{13}C$ will begin around the mother's mean, will increase about 1‰ higher than the maternal value during breastfeeding, and quickly return to maternal values when weaning begins.

Unfortunately we do not know exactly what foods were recommended to have been consumed in Lithuania, specifically. As discussed in Chapter 3, it may be hypothesized that parents in Lithuania were inspired by physicians writing in Christian Europe, influenced by Classic physicians like Galen and Soranus, who recommended the cessation of breastfeeding by two years of age (Adamson, 2004; Mays et al., 2002). Mays and colleagues' (2002) found that depletion in $\delta^{13}C$ and $\delta^{15}N$ between 1 to 2 years in a sample from Medieval Wharram Percy, England coincided with these historic practices. Infants fed during the Medieval period in Western Europe were recommended to be weaned off of breast milk onto a soft food diet of gruels made of flour or cereal in water or milk and sweetened with sugar or honey (Adamson, 2004; Lewis, 2007b). Depending upon cultural customs and parental preference, weaning could have occurred sooner or later in the infant's life, which may have had effects on the subadult's health and nutrition throughout growth.

Therefore, it is necessary to analyze subadult diet in comparison to estimated age at death under the assumption that the isotopic values for the age in which the bone sample was modeled is also reflective of diet at or around the time of death. In subadults, this assumption is easier to make as bone collagen turns over much more quickly than in adults (Hedges et al., 2007). While

discussions of juvenile diet generally focus on the changes during infancy and early childhood, Lewis (2007b) suggests that older children and possibly juveniles are consuming a diet unique to adults. Thus identification of variation among all age cohorts should be examined for distinctive dietary signatures.

CHAPTER 5: MATERIALS AND METHODS

Alytus Subadult Bone Collagen Samples

Samples consisting primarily of cortical bone were taken from the midshaft diaphysis of right humeri and femora for 70 subadults (there are two individuals both with two humeral and two femoral samples each) ranging in age from 38 weeks gestation to 16 years at death from the Alytus Cemetery collection housed at Vilnius University in Lithuania (Table 2). In total, 72 femoral and 69 humeral diaphyseal samples were processed for collagen extraction. Individuals in the sample account for about 11% of the subadult population recovered from Alytus cemetery based on individuals reported in Šereikienė and Jankauskas (2004).

Table 2. Age distribution of subadult sample from Alytus used in stable isotope analysis

Cohort	Age Range (Years)	Age Average (Years)	n=	Sample Size /Reported Number Excavated
Fetus	25wk– 36.9wk	-0.20	2*	**
Neonate	37wk– 0.09	0.00	5	**
Infant 1	0.10 – 1.00	0.40	6	5.61%
Infant 2	1.01 – 2.99	2.06	13	10.08%
Child	3.00 – 4.99	3.84	7	5.79%
Juvenile 1	5.00 – 8.59	6.33	15*	10.22%
Juvenile 2	8.60 – 11.99	10.05	13	14.77%
Adolescent	12.00 – 15.99	13.02	9	11.69%
Young Adult	16.00 +	16.00	2	**
Total	**32wk -16 y**	**5.98**	**70**	10.93%

*2 Samples same individual
** Šereikienė and Jankauskas (2004) do not include subadults less than 3 months or greater than 16 years at death in stature estimation table.

The resected diaphyseal samples, presence of cranial and postcranial lesions, and estimation of age at death were collected or determined for all subadults by Eleazer (2013).

Utilizing Eleazer's records of observed pathological conditions, one of four stress groups was applied to the individuals in this study, which included:

1. Postcranial Stress Only (n=17)
2. Cranial Stress Only (n=15)
3. Both Postcranial and Cranial Stress (n=9)
4. Neither Postcranial nor Cranial Stress (n=31)

A Biocultural Approach to Creating "Childhood" Cohorts

The notion of adulthood and childhood during the Medieval period differs greatly from modern constructs. Bioarchaeologists were once guilty of making interpretations of past cultural experiences of subadults through analyzing all individuals in a cemetery sample whose chronological age, based on skeletal development, was under 18 years at the time of death and subsequently projecting an interpretation of extended childhood and play that is experienced in industrialized societies (Baker et al., 2005; Lewis, 2007b; Perry, 2006; Scheuer and Black, 2004). In late Medieval Europe (after 1500 AD), adolescents as young as 8-12 years were beginning to participate in occupational apprenticeships where they were likely treated as adults in the home and working in the same arduous environments as what we would consider skeletally mature, biological adults (Lewis, 2007b; Orme, 2003).

Assessment of immature skeletal remains and dietary practices associated with those individuals require a detailed perspective of ontogeny in childhood. Often, Bogin's (1997) evolutionary classificatory model is used to construct cohorts as it breaks down subadulthood into periods of biological constraints such as eating and protection through considering human growth as social mammals (used by Eleazer, 2013). However, standardized or accepted divisions

of age cohorts are still absent within studies subadult skeletal remains (Halcrow and Tayles, 2008; Lewis, 2007b; Perry, 2006). A primary goal of this study was to determine if bone growth or health status had affected isotopic values in the humerus and femur. Therefore, cohorts were designed to reflect concurrent physiological changes of the bone, growth of the brain, disease exposure, mobility, social interaction, and hypothesized diet (Bogin, 1997; Goldman et al., 2009; Lewis, 2007b; Orme, 2003; Scheuer and Black, 2004; Wheeler, 2012). These factors, especially contraction of illness or experience of malnutrition, are quintessential to understanding the most likely causes of death in a given environment at a certain age.

A total of nine subadult cohorts were formed through careful consideration of the biological age, developmental growth, and Medieval cultural practices (Fig. 4). The term "subadult" is used when referencing individuals under the age of 16 years at death whereas specific cohort terms will be used when discussing specific ages. For the sample, cohorts include: (1) Fetus: 25-36.9 weeks gestation, (2) Neonate: 37 weeks gestation- 1 month (3) Infant 1: 0.1-1.0 years, (4) Infant 2: 1.0- 2.9 years, (5) Child: 3.0-4.9 years, (6) Juvenile 1: 5.0-8.5 years, (7) Juvenile 2: 8.6-11.9 years, (8) Adolescent: 12.0-15.9 years, and (9) Young Adult: 16.0-19.9 years.

Caveats to Cohort Grouping

While cohort age ranges are systematically defined and applied to biological data based on estimated skeletal age and probable social changes throughout life, cohorts still may not reflect the exact ages at which important cultural transitions occurred for every individual within the sample. This is especially true of the dietary experiences of infants, as weaning practices are known to vary throughout the world and within communities based on caregiver choice

(Dettwyler and Fishman, 1992). The absence of adequate sex estimation for subadults is a substantial barrier to understanding differences between male and female treatment and diet in the past (Lewis, 2007). The absence of these methods also widens later age cohorts to account for both male and female growth during and after puberty.

Through contributions of biological methodology and specifically isotopic analyses, the changes in diet during infancy can be assessed and subsequent variation in childhood diet utilized to illuminate social and biological age differences within a cemetery sample. Diet may also have been one of the main factors contributing to stress and disease experience for young individuals during the Medieval period, which is often influenced by the prevailing economic and political affairs. Access to nutrition and exposure to disease are inextricably linked with the probability of successful attainment of skeletal maturity and survival into adulthood (Dettwyler and Fishman, 1992). Therefore, dietary reconstructions and analyses of separate age cohorts and anomalous individuals within cohorts have the ability to strengthen inferences made about life experiences in the past.

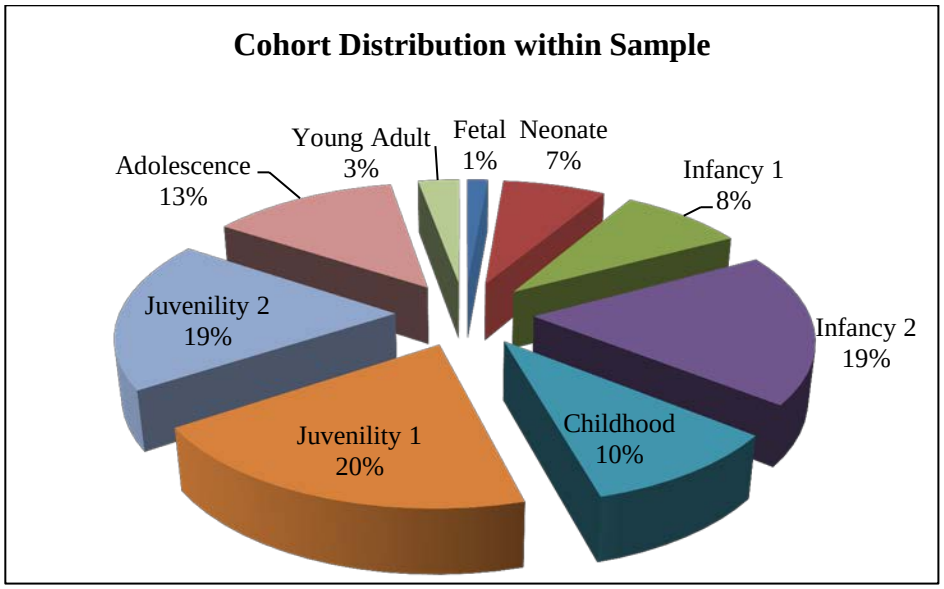


Figure 4. Percentage of individuals in sample by cohorts

Collagen Extraction Protocol

The University of Central Florida Bioarchaeology Laboratory's collagen extraction protocol is a modified Longin (1971), Nelson (1969), and Brown et al. (1988) method revised by the Laboratory for Stable Isotope Science (LSIS) at Western University, Canada with the goal of attaining the maximum possible yield of collagen with the removal of post-depositional, organic contaminants. To prepare bone samples for collagen extraction, the whole bone was first washed in de-ionized water and dried in an oven for 24 hours at 60º Celsius. Approximately 3-5g of bone was crushed when available. However, the mass for many samples had less than one gram available for sample processing.

Following rinsing with water and recording the sample weight, the bone was rinsed in a 2:1 chloroform-methanol solution three times over 90 minutes to remove any remaining lipids and left to dry for about 24 hours. Next, the sample was de-mineralized in a 0.5 molar hydrochloric acid solution until all of the inorganic material was removed.

After complete de-mineralization, the sample was rinsed in de-ionized water at least three times until a pH of 2.5-3.0 was reached. Next, it was rinsed in a 0.1 molar solution of sodium hydroxide to remove any remaining organic content, specifically humic and fluvic acids that would skew carbon isotopic values (Katzenberg et al., 1995). Once the sodium hydroxide solution remained clear, the humic acids were considered removed and the bone was rinsed a minimum of eight times in de-ionized water until the pH of the water solution was neutral. The sample was rinsed with a weak, 0.25 molar solution of hydrochloric acid, which was subsequently removed and replaced by water so that the organic bone was in a solution of slightly acidic water with a pH between 2.5-3.0. The sample was placed in an oven for about 16 hours at 90ºC to solubilize the collagen in the water solution. Finally, the solution was placed in

a dram vial and left to dry in a 90ºC oven for about two to three days yielding the bone's preserved collagen. The vials of collagen were weighed and the percentage of collagen yield calculated from the following equation:

$$\frac{(\text{Weight of Collagen in Vial (mg)} - \text{Weight of Vial (mg)})}{\text{Weight of Sample (mg)}} \times 100 \quad \textbf{(3)}$$

A sample weight of 0.60 mg was then measured into tin capsules. The samples were sent to the Colorado Plateau Stable Isotope Laboratory for continuous flow combustion- isotope ratio mass spectrometry using a Thermo- Electron DELTA V Advantage Mass spectrometer configured through CONFLO III using a Carlo Erba NC2100 Elemental Analyzer. The laboratory used eight NIST peach leaves as sample standards for every 86 bone collagen samples, no duplicates were run. The industry standard typically reported as analytical error is ±0.1‰ for carbon and ±0.2‰ for nitrogen. The Alytus Subadult samples were run in three separate batches where the known values of NIST peach leaves demonstrated analytical errors more precise than industry standards (Carbon = ± 0.05‰, 0.02‰, and 0.07‰; Nitrogen = ± 0.06‰, 0.07‰, and 0.06‰) yielding error values of one standard deviation for the current study at a maximum of ±0.07‰ (±0.14‰, 2σ) for both carbon and nitrogen.

Statistical Method

The large sample size for this study (n=72) provides sufficient power for statistical analysis. Nonparametric Spearman's tests were employed in order to test for correlations between rankings of $\delta^{13}C$ and $\delta^{15}N$ isotopic values for the humerus and femur, estimated age, and ordinal stress grouping of all individuals. Spearman's statistics are reported as rho ($\rho_{\text{degrees of freedom, } p \text{ value}}$) and were preferred over parametric Pearson's correlations so as not to violate the

following assumptions: ratio/interval variable type, outlier sensitivity, and homoscedacity. The only two assumptions of Spearman's correlation are that the variables are relatively normally distributed and that there is a monotonic relationship, which does not have to be linear, between the two variables being tested. Neither assumption is violated by the present data enabling its use for investigation of correlations.

CHAPTER 6: RESULTS

Sample Preservation

Preservation summary data for humeral and femoral samples are listed in Tables 3 and 4 below with individual values located in Appendix B. Most samples were considered well preserved and were judged on an individual basis established by the percentage weights of collagen, atomic carbon to nitrogen ratios, and percentage of carbon and nitrogen in the sample.

Modern or well-preserved human bone collagen makes up about 22% of the 30% organic content of bone (Baker, 1946; Waters-Rist et al., 2011). Therefore, a sample of archaeological bone might yield a percentage of preserved collagen between 3% and 22%, which may be considered well preserved, though values have been accepted as low as 1% (Ambrose, 1990; van Klinken, 1999). Percentage weight of collagen yields for both the humeral and femoral samples of subadults at Alytus ranged between 1.41% and 35.29%.

Interestingly, because juveniles are in an almost constant state of growth, the amount of collagen found in a sample may possibly be as high as 26% due to the rapidly deposited osteoid and extra collagen during growth and modeling (Baker, 1946; Waters-Rist et al., 2011). Similarly, rapidly deposited woven bone from a periosteal reaction would have a higher percentage of collagen. These facts were supported using the non-parametric Spearman's rho to test for correlation between the two continuous variables. It was found that there was a relationship between decreasing percentage weight of collagen with increased age for the present sample (ρ_{66} = -0.498 [humeral], p = <0.05; ρ_{70} = -0.550 [femoral], p =< 0.05). In 52 samples, the percent weight of collagen was greater than 22%. Only two samples from burials 813 (35.29% [humerus]) and 186 (28.31% [femur]) were higher than 26% collagen yield. Neither of these

individuals have outlying values in the other digenetic variables considered below (e.g., C:N, %C, %N, $\delta^{13}C$ and $\delta^{15}N$) and were therefore kept for analysis.

Table 3. Summary results for all humeral data used in analysis.

Humerus	% Weight Collagen	Atomic C:N	%C	%N
Mean	17.85	3.24	42.37	15.28
± 1SD	7.43	0.03	2.95	1.09
Maximum	35.29	3.31	46.20	16.71
Minimum	1.41	3.16	31.97	11.53
Range	33.87	0.15	14.23	5.18

Table 4. Summary results for all femoral data used in analysis.

Femur	% Weight Collagen	Atomic C:N	%C	%N
Mean	16.63	3.23	43.44	15.71
± 1SD	7.61	0.03	2.26	0.82
Maximum	28.31	3.30	46.03	16.78
Minimum	3.42	3.15	35.35	12.68
Range	24.89	0.15	10.68	4.10

To be certain that the high collagen yields extracted were actually bone protein collagen, the atomic C:N ratio is reported and well preserved samples between 2.9 and 3.6 were considered largely absent of diagenetic contaminants (DeNiro, 1985; Ambrose, 1990; van Klinkin, 1999). All samples (with the exception of Burial 573's humeral sample which will be detailed below) exhibited C:N ratios between 3.15 and 3.31 and are thus considered well preserved. To further test for viability of the samples, percentage weight of carbon and nitrogen was also considered (Van Klinken, 1999). Bone collagen is comprised of about 26 - 44% carbon and 11-16% nitrogen. Samples with lower percentages of the elements were shown by van

Klinken (1999) to more greatly affect resultant isotopic values than samples with higher carbon or nitrogen percentages.

For all samples, %C ranges from 31.97% - 46.20% and %N ranges from 11.53% - 16.78%. Samples that fall outside of the expected range all exhibit greater %C or %N possibly indicating a slight addition of organic carbon and nitrogen (van Klinken, 1999). However, almost all %C and %N values are within two standard deviations from sample means and were therefore kept for the final analysis. Burial 573 was removed from analysis due to a high C:N ratio (3.69) in addition to low carbon (17.27%) and low nitrogen (5.46%) percentages.

The young age at death for the sample, high preservation, and near-perfect, average C:N ratios allowed for the inclusion of individuals with high percentage of collagen for analysis. In summary, it is unlikely that the majority of collagen extracted was diagenetically contaminated. Three individuals (757, 886, and 994) did not have humeral samples to compare to femoral, two individuals (882 and 604) had duplicate samples, and one humeral sample was removed from the analysis (573). Therefore, isotopic values of 72 femoral and 68 humeral samples from 70 individuals were examined.

General Dietary Characteristics

Appendix B contains all of the isotopic values for each humeral and femoral collagen sample by burial identification number. The general dietary characteristics for individuals at Alytus reflect the hypothesized values expected based on probable available food items and those typical of a Medieval European population. Subadults in this sample were predominantly consuming C_3 temperate plants and grains reflected by both humeral and femoral isotopic values

(Table 5). Additionally, there is a strong linear relationship between the increase in $\delta^{15}N$, trophic level protein and a simultaneous enrichment in $\delta^{13}C$ (Fig. 5).

Table 5. Sample average and ranges of $\delta^{13}C$ and $\delta^{15}N$for humeral and femoral samples

	Humeral $\delta^{13}C$‰	Femoral $\delta^{13}C$‰	Humeral $\delta^{15}N$‰	Femoral $\delta^{15}N$‰
Mean	-20.02	-20.06	11.08	11.11
± 1SD	0.43	0.40	1.22	1.13
Maximum	-18.80	-18.85	14.28	14.17
Minimum	-21.03	-20.87	8.57	8.74
Range	2.23	2.02	5.71	5.43

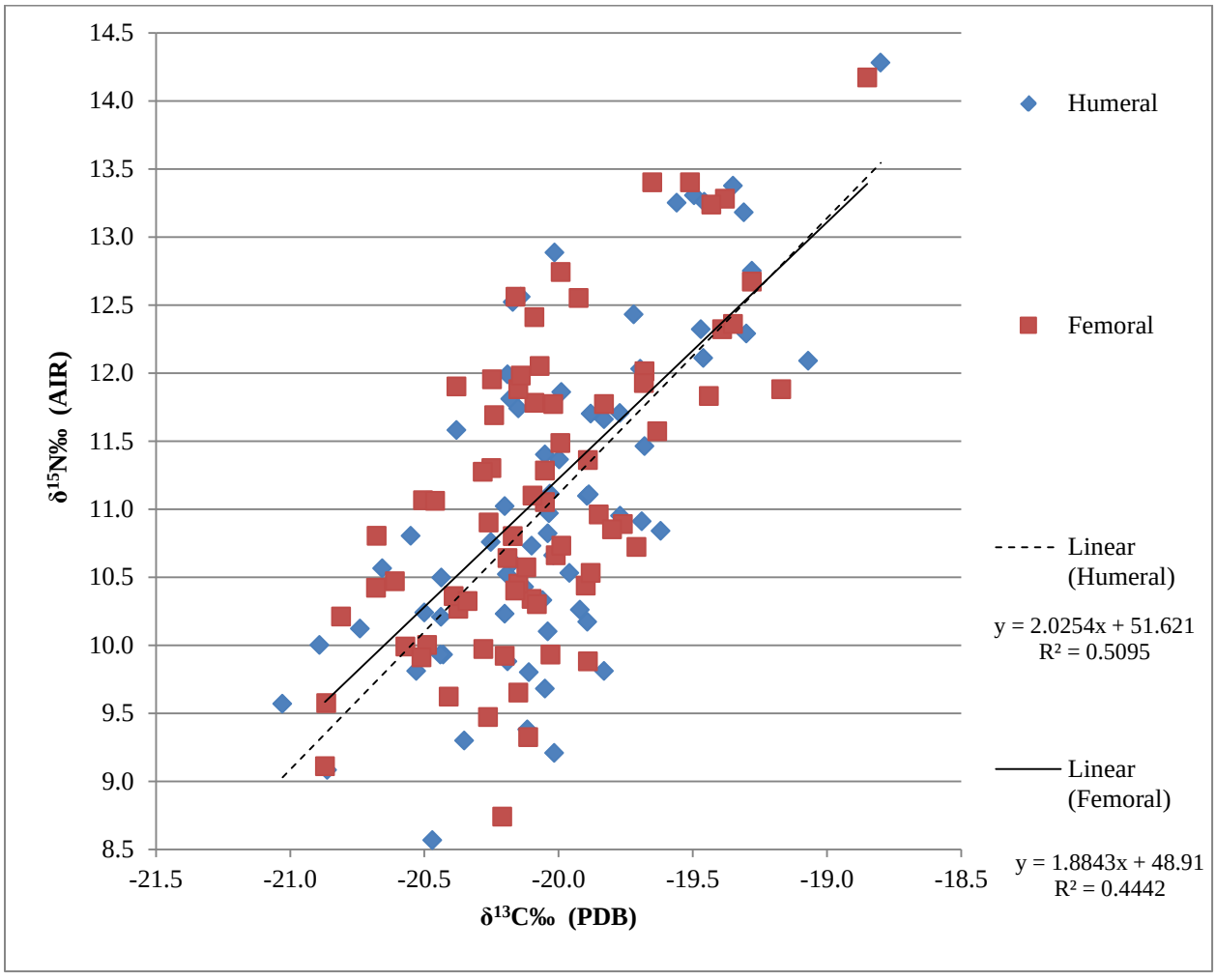


Figure 5. $\delta^{13}C$ by $\delta^{15}N$ humeral and femoral values for each individual with linear equation lines.

While the linear relationship between isotopic values is augmented by trophic level enrichments in breastfeeding infants, the positive correlation holds true for the older cohorts as well (Fig. 6 and 7). The average humeral $\delta^{13}C$ and $\delta^{15}N$ for the whole sample was -20.01 ± 0.43‰ and 11.09 ± 1.21‰. The average femoral $\delta^{13}C$ and $\delta^{15}N$ for the whole sample was -20.06 ± 0.40‰ and 11.11 ± 1.13‰. The consumption of marine resources is not likely as $\delta^{15}N$ values among non-breastfeeding cohorts do not typically exceed 11.5‰ and are below 10.5‰ on average.

$\delta^{13}C$ Results

Among all samples, $\delta^{13}C$ ranged from -20.88‰ to -19.16‰ with a mean humeral value and one standard deviation of -20.02 ± 0.43‰ and a mean femoral value and one standard deviation of -20.06 ± 0.40‰ indicating a predominant consumption of C_3 plant resources. However, the level of enrichment may also signify influence of riverine resources, which will be detailed further in the discussion section. All cohort averages, and standard deviations are provided in Table 6 below. Overall, there is little variation in $\delta^{13}C$ of which 95% is only 0.80‰ from the average.

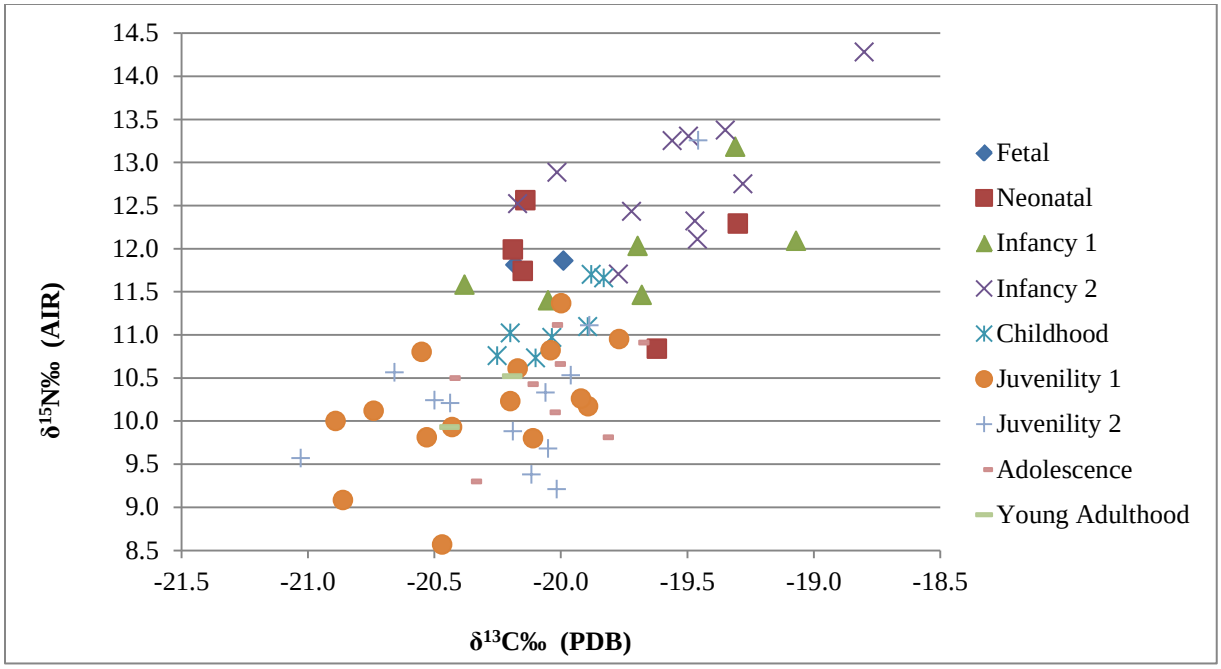


Figure 6. $\delta^{13}C$ by $\delta^{15}N$ humeral values for each individual labeled by cohort.

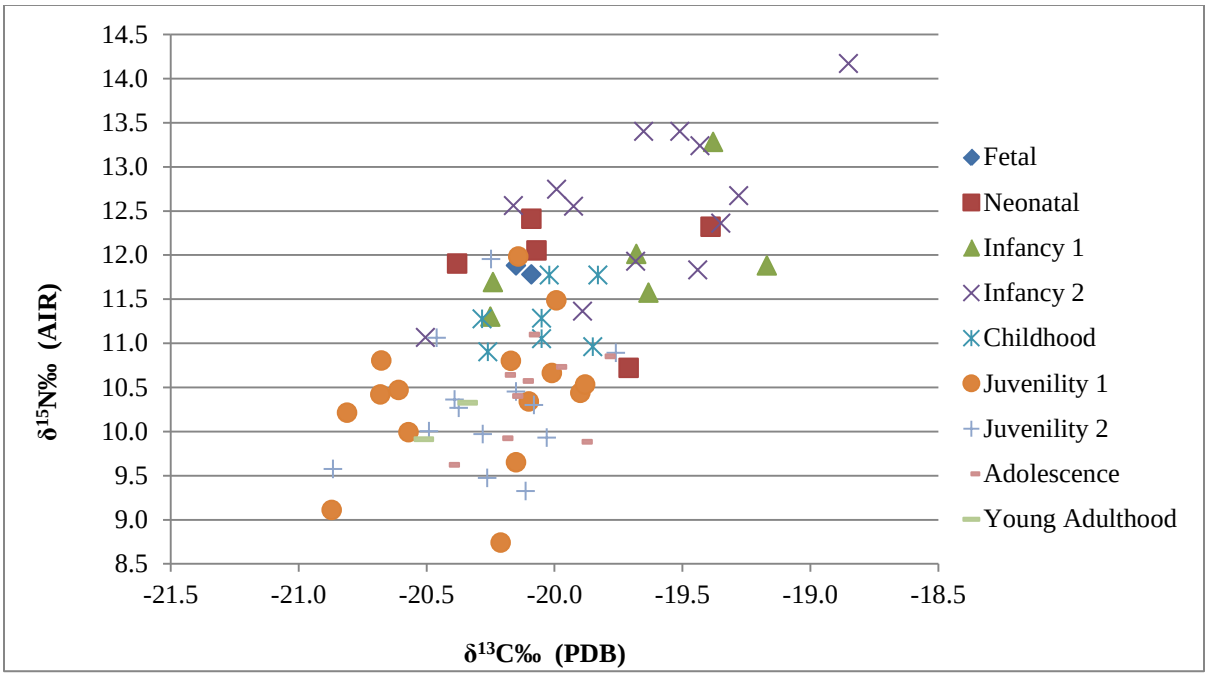


Figure 7. $\delta^{13}C$ by $\delta^{15}N$ femoral values for each individual labeled by cohort.

Table 6. $\delta^{13}C$ averages and standard deviations for sample and cohorts.

Cohort	Average Age (Years)	Humeral $\delta^{13}C$‰	±1 SD	Femoral δ13C‰	±1 SD
Fetus	-0.20	-20.09	0.13	-20.12	0.04
Neonate	0.00	-19.88	0.40	-19.93	0.38
Infant 1	0.40	-19.70	0.48	-19.73	0.44
Infant 2	2.06	-19.55	0.37	-19.67	0.43
Child	3.84	-20.03	0.17	-20.05	0.18
Juvenile 1	6.33	-20.30	0.36	-20.32	0.34
Juvenile 2	10.05	-20.20	0.41	-20.27	0.27
Adolescent	13.02	-20.07	0.25	-20.10	0.18
Young Adult	16.00	-20.32	0.18	-20.42	0.12
Total	**5.98**	**-20.02**	**0.43**	**-20.06**	**0.40**

There is a strong statistical correlation between age and $\delta^{13}C$ values (ρ_{66} = -0.372 [humeral], p = <0.05; ρ_{70} = -0.417 [femoral], p = <0.05) in the form of a negative, linear relationship for both humeral and femoral samples (Figs. 8 and 9). In general $\delta^{13}C$ values deplete as age and consumption of C_3 grains increase. Yet this relationship is more complex due to the effects of breastfeeding trophic level enrichments. The mean cohort values demonstrate enrichment in $\delta^{13}C$ between the Fetus and Infant 2 cohorts by 0.54‰ (humeral) and 0.45‰ (femoral) indicative of peak trophic level enrichment before the age of two years at death. After two years of age, $\delta^{13}C$ levels deplete by 0.48‰ (humeral) and 0.38‰ (femoral), to approach the sample mean by five years at death. Thus, individuals from this sample were likely being weaned off of exclusive breast milk protein beginning around two years at death and individuals who lived to five years would no longer show evidence of $\delta^{13}C$ from the weaning process. Mean carbon values vary slightly through Juvenile and Adolescent cohorts but by Young Adulthood are ultimately depleted below Infant 2 values by 0.77‰ (humeral) and 0.75‰ (femoral).

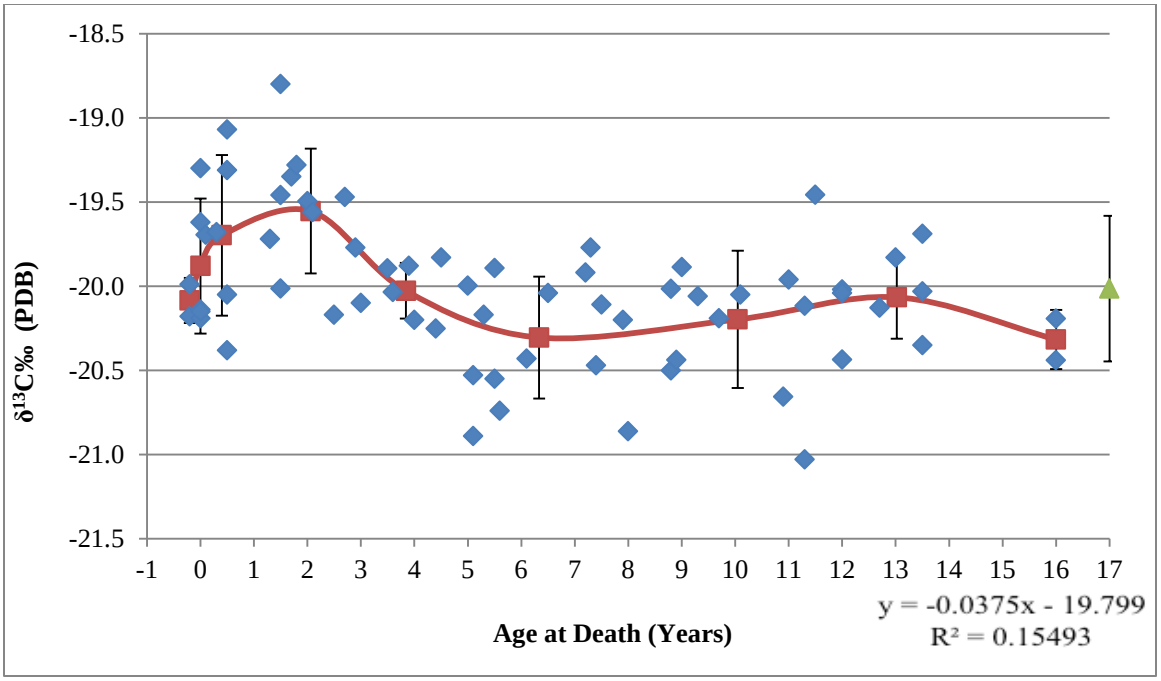


Figure 8. Humeral $\delta^{13}C$ by age at death. Cohort means are plotted as squares with 1 standard deviation error bars. Sample mean and 1 standard deviation are denoted by triangle to the far right of the graph.

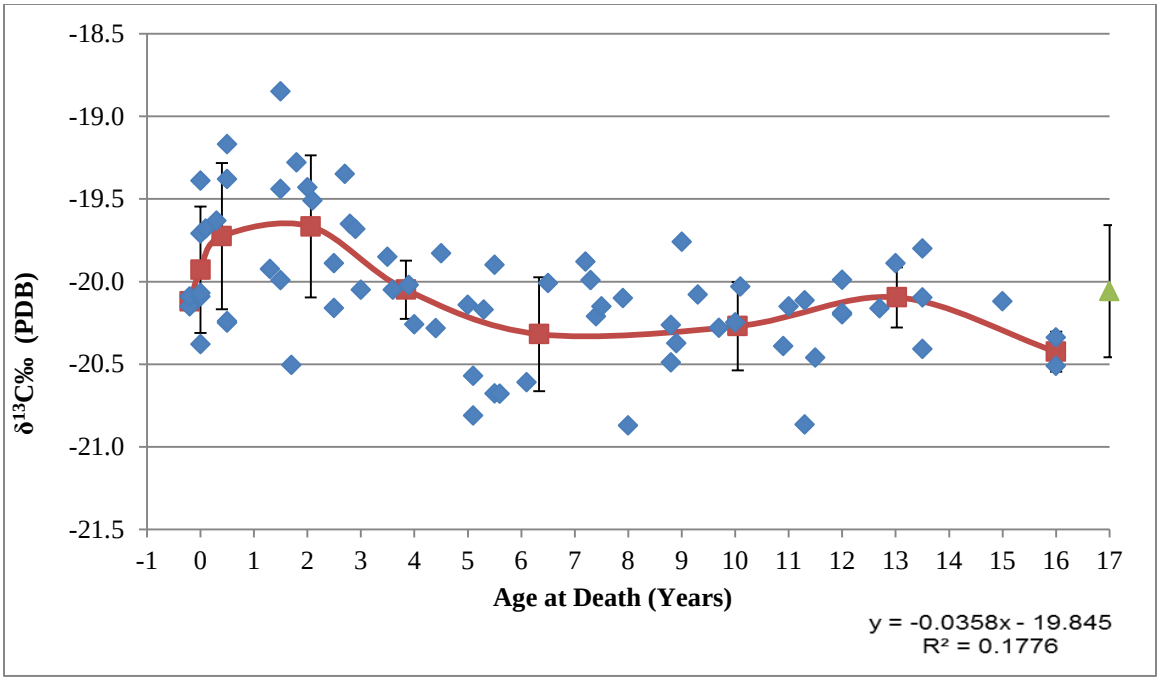


Figure 9. Femoral $\delta^{13}C$ by age at death. Cohort means are plotted as squares with 1 standard deviation error bars. Sample mean and 1 standard deviation are denoted by triangle to the far right of the graph.

$\delta^{15}N$ Results

Among all samples, $\delta^{15}N$ ranged from 8.64‰ to 13.52‰ with a mean humeral value and one standard deviation of 11.09 ± 1.21‰ and a mean femoral value and one standard deviation of 11.11 ± 1.13‰, likely indicating a predominant consumption of terrestrial, animal protein. All cohort averages, and standard deviations are provided in Table 7 below. Once again, these values of enrichment might also be associated with some incorporation of low trophic level, riverine protein resources to be discussed in the next chapter.

Nitrogen isotopic values reveal a strong statistical correlation between age and $\delta^{15}N$ values (ρ_{66} = -0.690 [humeral], p = <0.05; ρ_{70} = -0.724 [femoral], p = <0.05) in the form of a negative, linear relationship for both humeral and femoral samples (Figs. 10 and 11). Nitrogen values deplete more noticeably than $\delta^{13}C$ as age increases due to the more dramatic effects of breastfeeding trophic level enrichments detected by nitrogen isotopes. The mean cohort values demonstrate a difference in enrichment of $\delta^{15}N$ between the Fetus and Infant 2 cohorts by 0.97‰ (humeral) and 0.73‰ (femoral) indicative of peak trophic level enrichment before the age of two years at death. Therefore, nitrogen enrichment during breastfeeding follows the same trend as detected in carbon. After two years of age, $\delta^{15}N$ levels deplete by 1.68‰ (humeral) and 1.27‰ (femoral), to approach the sample mean by five years at death. Thus, individuals from this sample were likely being weaned off of exclusive breast milk protein beginning around two years at death and individuals who lived to five years would no longer show evidence of $\delta^{15}N$ from the weaning process. By Young Adulthood nitrogen is almost one trophic level depleted below Infant 2 values by 2.58‰ (humeral) and 2.44‰ (femoral).

Table 7. $\delta^{15}N$ averages and standard deviations for sample and cohorts.

Cohort	Average Age (Years)	Humeral $\delta^{15}N$‰	±1 SD	Femoral δ15N‰	±1 SD
Fetus	-0.20	11.84	0.04	11.83	0.07
Neonate	0.00	11.88	0.66	11.88	0.68
Infant 1	0.40	11.96	0.67	11.96	0.69
Infant 2	2.06	12.81	0.71	12.56	0.87
Child	3.84	11.13	0.40	11.29	0.36
Juvenile 1	6.33	10.17	0.72	10.37	0.81
Juvenile 2	10.05	10.33	1.07	10.27	0.72
Adolescent	13.02	10.35	0.59	10.41	0.50
Young Adult	16.00	10.23	0.42	10.12	0.29
Total	**5.98**	**11.09**	**1.21**	**11.11**	**1.13**

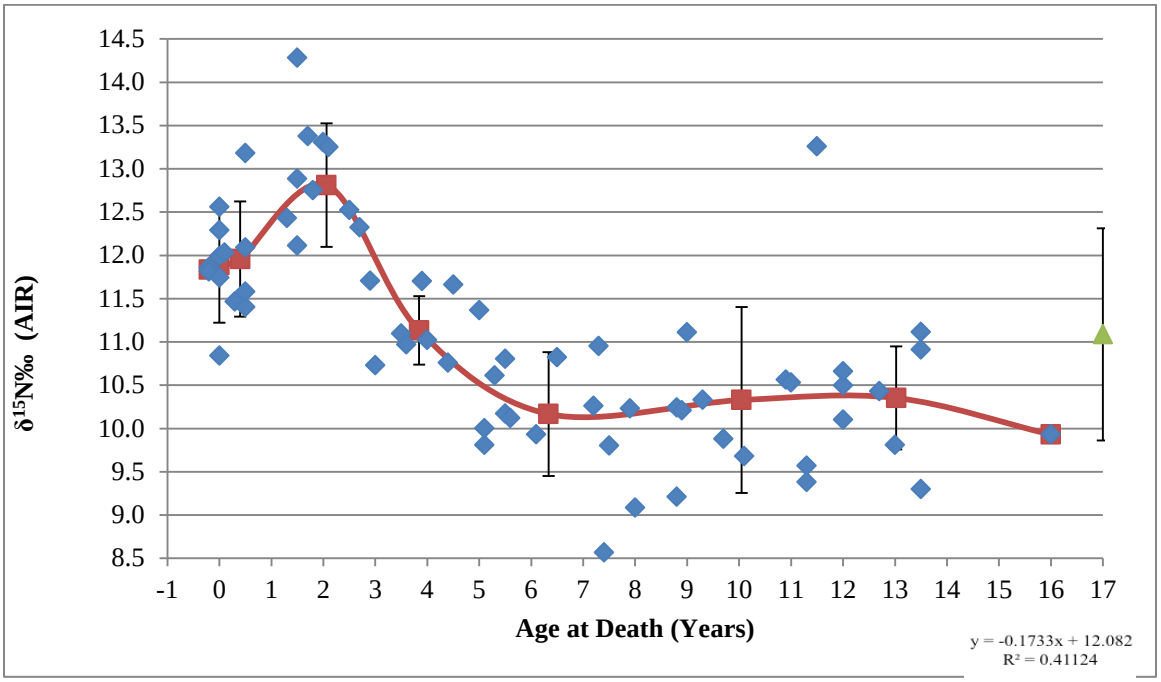


Figure 10. Humeral $\delta^{15}N$ by age at death. Cohort means are plotted as squares with 1 standard deviation error bars. Sample mean and 1 standard deviation are denoted by triangle to the far right of the graph.

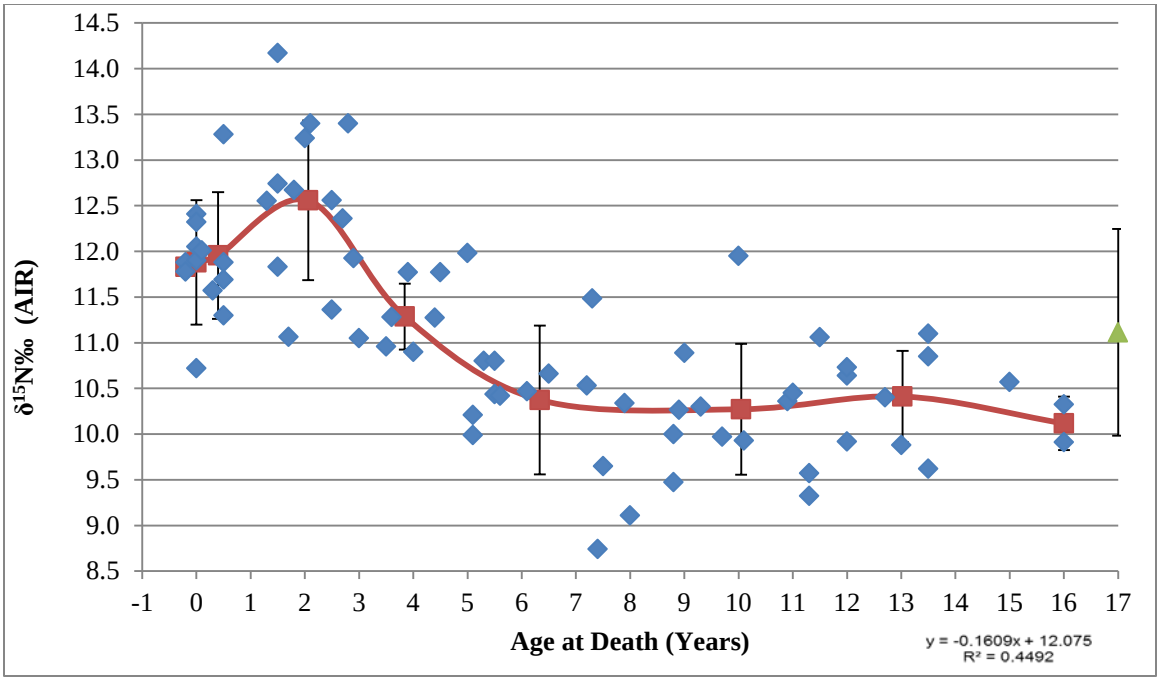


Figure 11. Femoral $\delta^{15}N$ by age at death. Cohort means are plotted as squares with 1 standard deviation error bars. Sample mean and 1 standard deviation are denoted by triangle to the far right of the graph.

Summary

Both humeral and femoral values indicate that subadults at Alytus were most likely consuming terrestrial meat and C_3 plant resources. Furthermore, both humeral and femoral data indicate the same timing of changes in diet. Infants were likely being breastfed exclusively before the age of two years after which protein from breast milk was supplemented by other foods depleting the nitrogen and carbon pools toward the sample means. The process of weaning is detectable in subadult bone samples after the ages of two years and before five years signifying a late as well as prolonged weaning practice by adults in Alytus.

A cross-sectional examination of combined humeral and femoral cohort averages reveals that individuals in the first year of life (Neonate and Infant 1 cohorts) were being exclusively breastfed, which is marked by increases in of $\delta^{13}C$ and $\delta^{15}N$ by approximately 0.2‰ and 0.1‰,

respectively (Table 8). Between one and two years (within the Infant 2 cohort) there is another average enrichment on the scale of 0.1‰ of $\delta^{13}C$ and 0.7‰ of $\delta^{15}N$ resulting in an overall breastfeeding enrichment of by approximately 0.4‰ and 0.8‰ in carbon and nitrogen, respectively, between one month to two years at death.

Table 8. Averages of combined humeral and femoral $\delta^{13}C$ and $\delta^{15}N$ values for each cohort.

Cohort	All $\delta^{13}C$‰	±1 SD	All $\delta^{15}N$‰	±1 SD
Fetus	-20.10	0.08	11.83	0.05
Neonate	-19.90	0.37	11.88	0.63
Infant 1	-19.71	0.44	11.96	0.65
Infant 2	-19.61	0.4	12.67	0.80
Child	-20.04	0.16	11.21	0.37
Juvenile 1	-20.31	0.35	10.27	0.76
Juvenile 2	-20.23	0.34	10.30	0.89
Adolescent	-20.08	0.21	10.38	0.53
Young Adult	-20.37	0.14	10.17	0.30

Differences in Femur - Humerus $\Delta^{13}C$ and $\Delta^{15}N$

There is on average a -0.05 ± 0.25‰ difference between femoral and humeral $\delta^{13}C$ values and -0.01 ± 0.45‰ difference between femoral and humeral $\delta^{15}N$ values (Table 9). The mean values indicate that for the entire sample, the humerus was on average slightly more enriched than the femur in both $\delta^{13}C$ and $\delta^{15}N$ yielding less negative carbon and higher nitrogen values from humeral bone collagen samples. Separately, $\delta^{13}C$ and $\delta^{15}N$ values of the humerus are correlated to $\delta^{13}C$ and $\delta^{15}N$ values of the femur, respectively, indicating that the differences between isotopic values of the two different cortical bone samples are not statistically discernable (^{13}C‰ $\rho_{66} = 0.763$, $p = <0.05$; ^{15}N‰ $\rho_{66} = 0.942$, $p = <0.05$). Differences in femoral

and humeral values for most individuals are within a 95% confidence interval range ($\Delta^{13}C‰ \pm 2\sigma$ = -0.55‰ and 0.45‰; $\Delta^{15}N‰ \pm 2\sigma$ = -0.91‰ and 0.89‰).

Table 9. Average $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$ for entire sample.

	$\Delta_{F\text{-}H}{}^{13}C‰$	$\Delta_{F\text{-}H}{}^{15}N‰$
Mean	-0.05	-0.01
± 1SD	0.25	0.45
Maximum	0.65	0.63
Minimum	-1.16	-2.31
Range	1.81	2.94

In general, there is no significant difference between the humeral and femoral isotopic values taken from midshaft for the majority of individuals sampled. Therefore, individuals who exhibit differences in femoral minus humeral ($\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$) values outside $\pm 2\sigma$ calculated from the sample mean will demonstrate a unique difference in the elements used to form collagen during a stage of growth (Fig. 12). Three individuals have a significant change in $\delta^{13}C$ and two of the same individuals are also outliers in $\Delta^{15}N‰$. Differences between humeral and femoral values become more interesting when viewed as cross-sectional changes throughout developmental age cohorts.

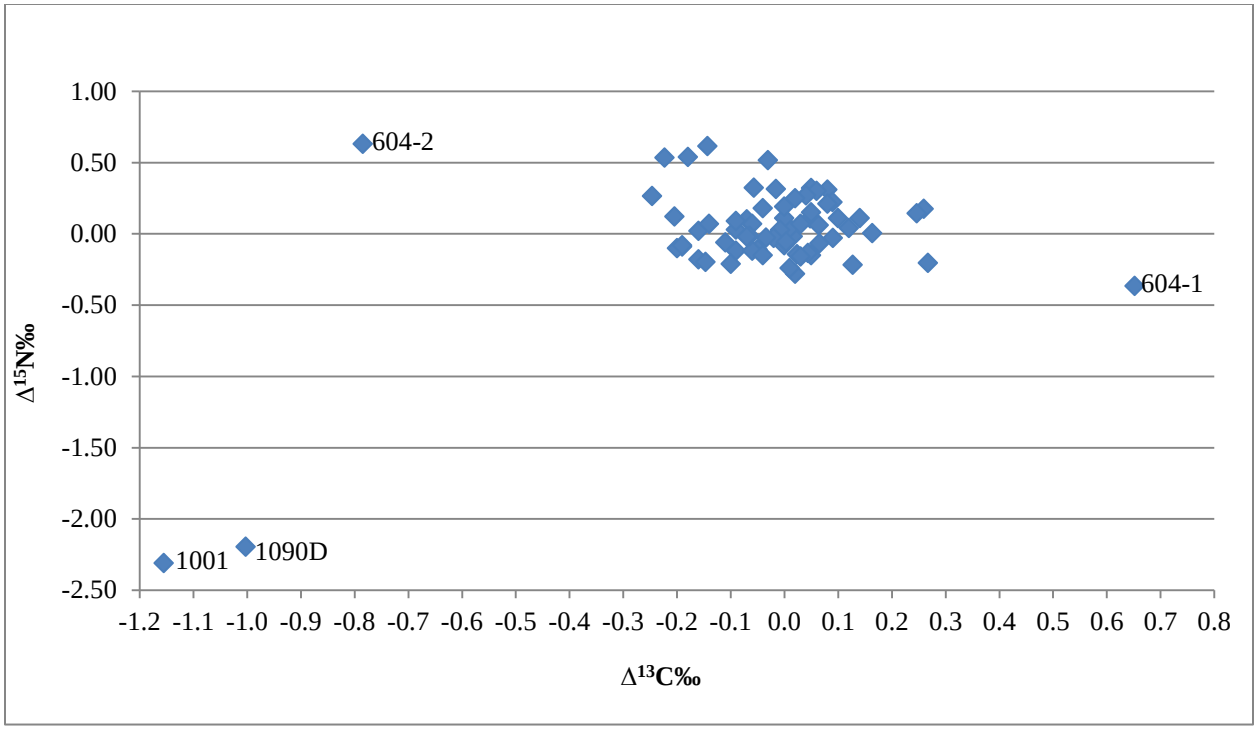


Figure 12. Change in $\Delta_{F\text{-}H}{}^{15}N‰$ plotted against $\Delta_{F\text{-}H}{}^{13}C‰$. Burial numbers of outliers are marked.

Femur - Humerus $\Delta^{13}C$ Cohort Results

Change in femoral to humeral $\delta^{13}C$, within each of the nine cohorts, is negative indicating greater enrichment of the carbon element within the collagen used to form the humerus (Table 10). This trend is also evident by plotting the cohort averages of $\delta^{13}C$ against age at death for both the femoral and humeral data within the same graph (Fig. 13).

A scatter plot of $\Delta_{F\text{-}H}{}^{13}C‰$ against age at death highlights three individuals outside ± 1σ (64% from mean) and three individuals ± 2σ (95% from mean) (Fig. 14). Thus while there is little difference between bone collagen sample sites at midshaft, the outliers can be explored within the context of biological development to further understand why these individuals might have died and why they might demonstrate abnormally enriched $\delta^{13}C$ values.

Table 10. Average and 95% confidence interval ranges for $\Delta_{F\text{-}H}{}^{13}C‰$

Cohort	$\Delta_{F\text{-}H}{}^{13}C‰$	± 1SD	Range 95% Confidence Interval	
Fetus	-0.0350	0.18	-0.40	0.33
Neonate	-0.0480	0.11	-0.27	0.17
Infant 1	-0.0277	0.12	-0.27	0.21
Infant 2	-0.0938	0.36	-0.81	0.63
Child	-0.0217	0.07	-0.16	0.12
Juvenile 1	-0.0131	0.30	-0.61	0.59
Juvenile 2	-0.0745	0.33	-0.73	0.59
Adolescent	-0.0262	0.12	-0.27	0.21
Young Adult	-0.1084	0.05	-0.21	-0.01
Total	-0.05	0.25	-0.55	0.45

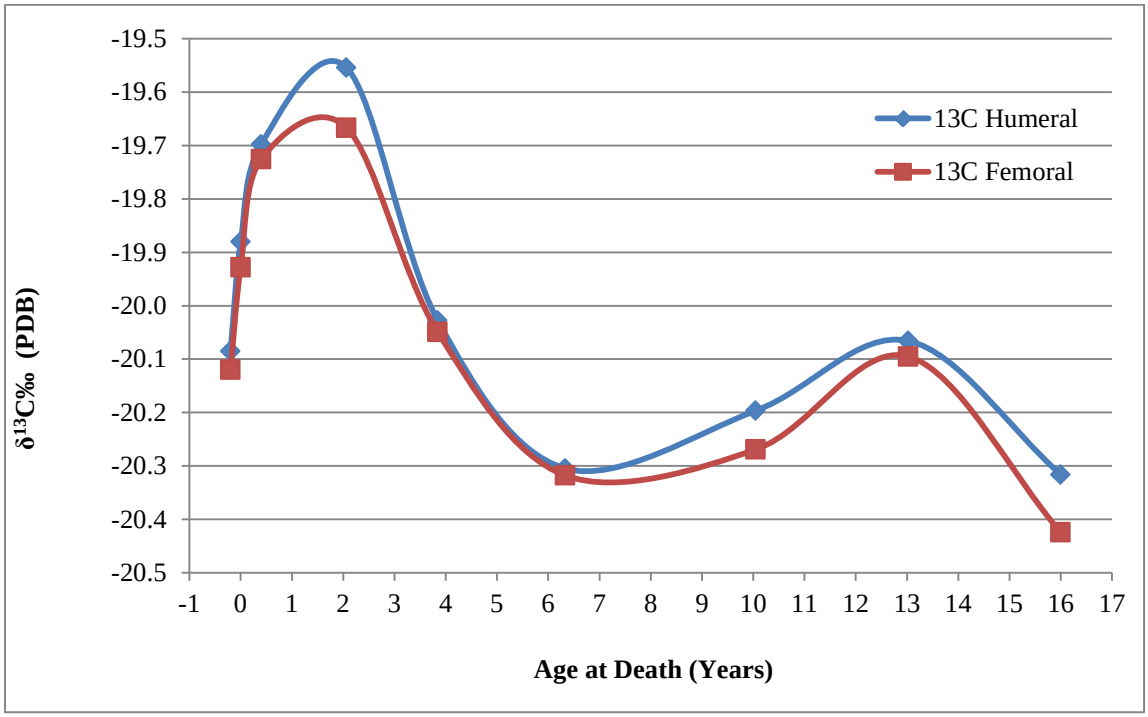


Figure 13. Plot of cohort average $\delta^{13}C$ against age at death for all cohorts.

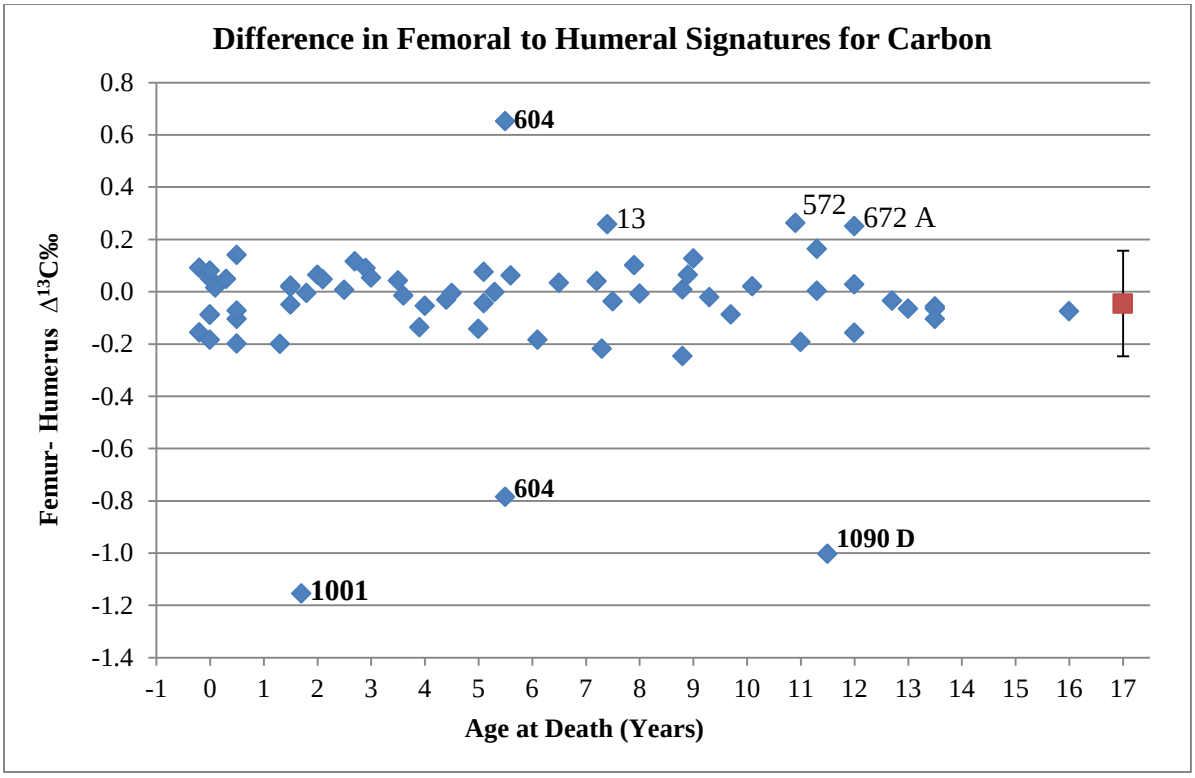


Figure 14. Change in $\Delta_{F\text{-}H}$ ^{13}C‰ plotted against age at death.

A bar graph presenting the number of individuals in each cohort with an enrichment of δ^{13}C in either their humeral or femoral samples shows that in most cohorts there is not a predominant bone that is more enriched than the other associated with a cohort (Fig. 15). The individual enrichments again demonstrate that in general there is no significant difference between humeral or femoral δ^{13}C in most samples. However, within Infant 2 there are five samples that have enriched femoral δ^{13}C and four samples from the Adolescent cohort that have enriched δ^{13}C in their humeral sample.

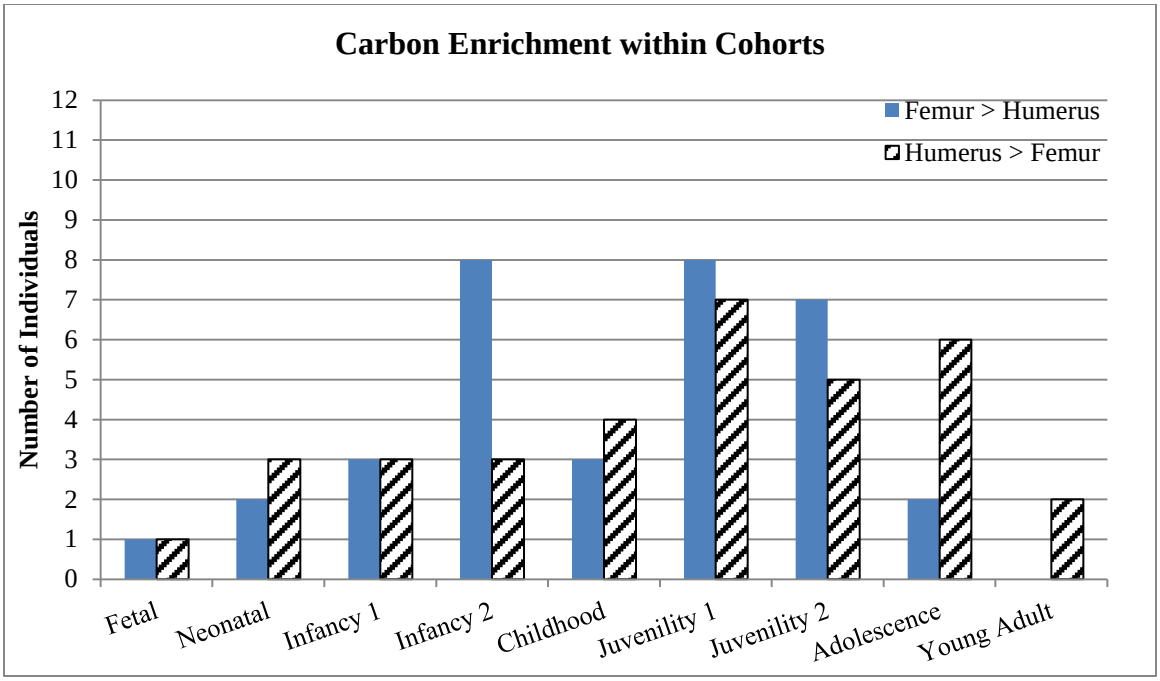


Figure 15. Number of individuals in each cohort with $\Delta_{F\text{-}H}{}^{13}C‰$ having been greater in the femur (solid fill) and humerus (line fill).

Femur - Humerus $\Delta^{15}N$ Cohort Results

Change in femoral to humeral $\delta^{15}N$ within the Fetal, Neonatal, Infancy 1 and 2, Juvenility 1 and 2, and Young Adult cohorts, is negative indicating greater enrichment of the nitrogen element within the collagen used to form the humerus. Only during the Child, Juvenile 1, and Adolescent cohorts is the femur more enriched than the humerus in nitrogen (Table 11). This difference can be seen by plotting the cohort averages of $\delta^{15}N$ against age at death for both the femoral and humeral data within the same graph (Fig 16).

The scatter plot of $\Delta_{F\text{-}H}{}^{15}N‰$ against age highlights individuals outside ±1σ (64%) from $\Delta_{F\text{-}H}{}^{15}N$ ‰ mean and, in bold, the same three individuals ±2σ (95%) from both $\Delta_{F\text{-}H}{}^{15}N‰$ and $\Delta_{F\text{-}H}{}^{13}C‰$ means (Fig. 17) There is little difference between isotopic values of bone collagen

sampled at midshaft. However, it is evident that the variation within $\Delta_{F\text{-}H}{}^{15}N$ ‰ is much greater than $\Delta_{F\text{-}H}{}^{13}C$ ‰ at every age.

Table 11. Average and 95% confidence interval ranges for $\Delta_{F\text{-}H}{}^{15}N$‰

Cohort	$\Delta_{F\text{-}H}{}^{15}N$‰	± 1SD	Range 95% Confidence Interval	
Fetus	-0.0050	0.04	-0.09	0.08
Neonate	-0.0040	0.19	-0.38	0.38
Infant 1	-0.0019	0.13	-0.26	0.26
Infant 2	-0.2199	0.71	-1.64	1.20
Child	0.1534	0.24	-0.33	0.63
Juvenile 1	0.2069	0.29	-0.37	0.79
Juvenile 2	-0.1964	0.65	-1.50	1.10
Adolescent	0.0704	0.15	-0.23	0.37
Young Adult	-0.1100	0.12	-0.35	0.13
Total	-0.01	0.45	-0.91	0.89

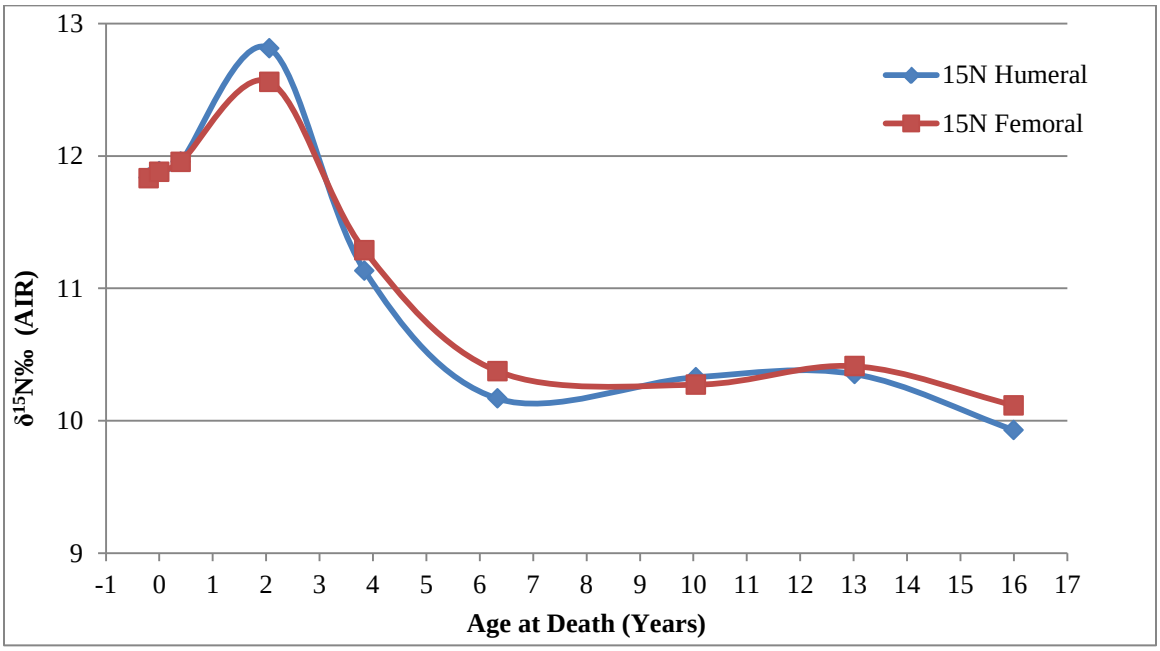


Figure 16. Plot of cohort average $\delta^{15}N$ against age at death for all cohorts.

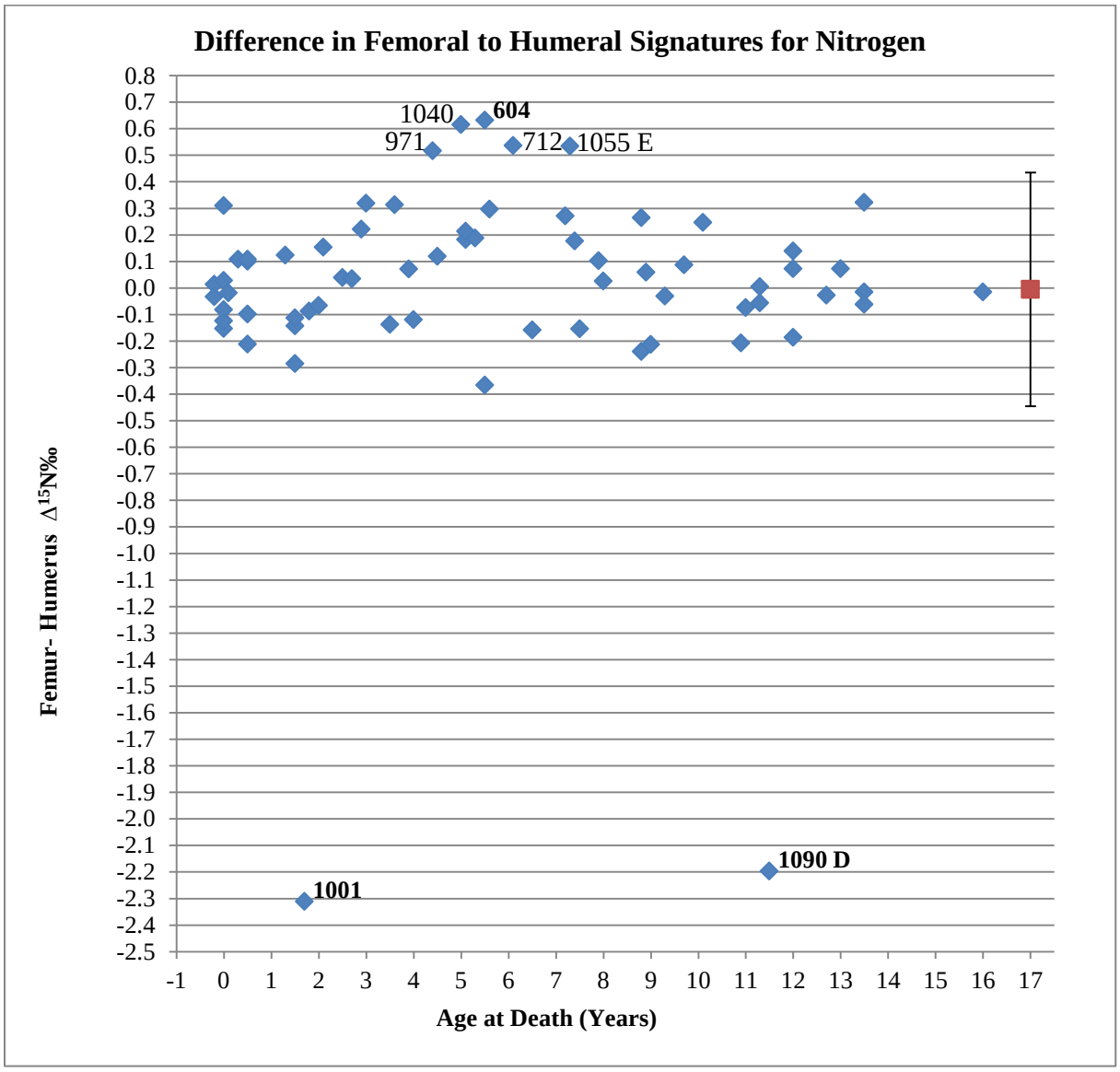


Figure 17. Change in $\Delta_{F\text{-}H}$ ^{15}N‰ plotted against age at death.

The bar graph below represents the number of individuals in each cohort who demonstrate an enrichment of δ^{15}N in either their humeral or femoral samples (Fig 18). Most cohorts are relatively equal in the number of individuals who are enriched in one bone over the other. However, there are three individuals within the Child cohort and nine from the Juvenile 1

cohort that have greater femoral $\delta^{15}N$ than humeral $\delta^{15}N$. The individual enrichments again demonstrate that aside from during the Juvenile 1 cohort, in general there is no strong difference between humeral or femoral $\delta^{15}N$ for most individuals.

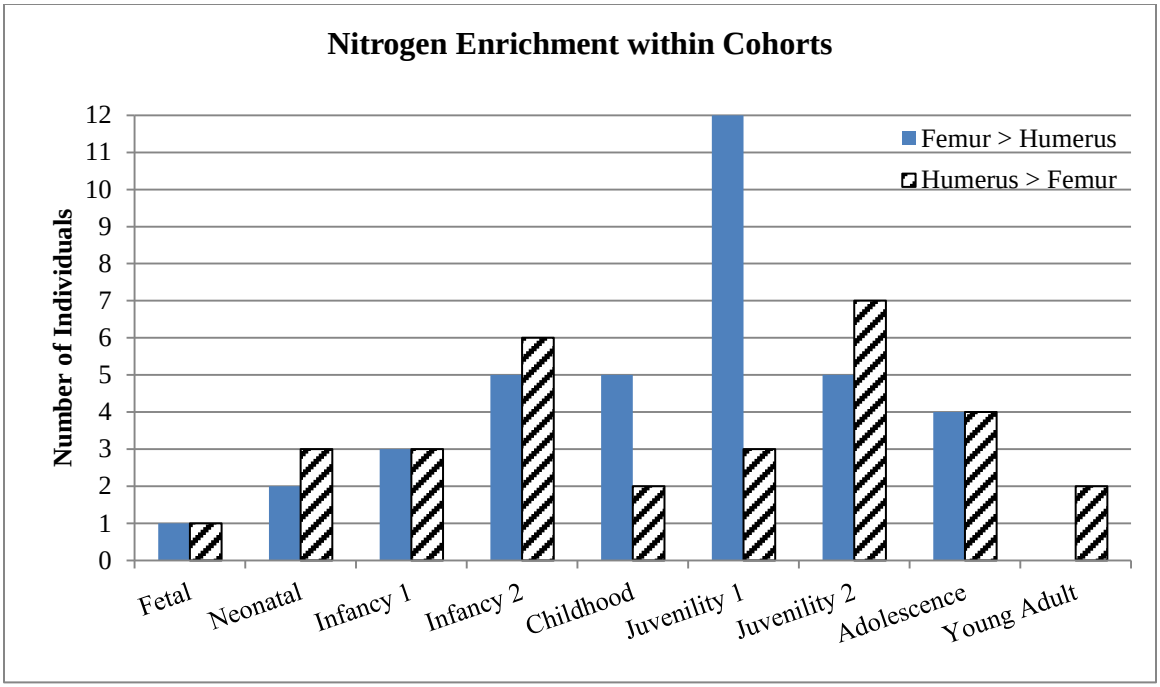


Figure 18. Number of individuals in each cohort with $\Delta_{F\text{-}H}{}^{15}N‰$ having been greater in the femur (solid fill) and humerus (line fill).

Correlations between stress grouping, age, and isotopic variables

Spearman's statistical tests revealed that there were no statistically discernable correlations between ranked age or isotopic values for individuals within one of the four stress groups (Table 12). Therefore, the presence or absence of stress was not correlated with age at death or isotopic values.

Table 12. Test statistics for correlation between stress group with age and isotopic value.

Spearman's ρ Correlation for Stress Group	Age	$\delta^{13}C‰$ Humerus	$\delta^{15}N‰$ Humerus	$\delta^{13}C‰$ Femur	$\delta^{15}N‰$ Femur	Δ_{F-H} $^{13}C‰$	Δ_{F-H} $^{15}N‰$
ρ	-0.065	0.124	0.126	0.228	0.191	0.181	0.037
p-value	0.586	0.312	0.306	0.054	0.108	0.140	0.764

However, the test statistics for femoral $\delta^{13}C‰$ ($\rho_{70} = 0.228$, $p = 0.054$) demonstrated a p-value close to significance, a correlation between stress group and femoral $\delta^{13}C‰$ might be evident. In order to test which of the stress categories were significantly different from one another, a one-way ANOVA of femoral $\delta^{13}C‰$ ($F(3,68) = 2.92$, $p = 0.040$) with a Tukey post-hoc test revealed that individuals with cranial stress (-19.84 ± 0.41) had femoral $\delta^{13}C‰$ values more enriched over individuals without any skeletal stress (-20.19 ± 0.27), a 0.35‰ difference (Table 13).

Table 13. Summary statistics for correlation between stress group with age and isotopic value.

		Age	$\delta^{13}C‰$ Humerus	$\delta^{13}C‰$ Femur	$\delta^{15}N‰$ Humerus	$\delta^{15}N‰$ Femur	Δ_{F-H} $^{13}C‰$	Δ_{F-H} $^{15}N‰$
Postcranial Stress	Average	7.58	-19.96	-20.01	10.96	11.07	-0.02	0.09
	± 1SD	5.28	0.44	1.00	0.40	0.97	0.09	0.26
Cranial Stress	Average	4.06	-19.83	-19.84	11.56	11.71	-0.02	0.03
	± 1SD	4.25	0.43	1.22	0.41	1.24	0.14	0.19
Postcranial and Cranial Stress	Average	6.13	-20.12	-20.07	11.08	11.15	0.04	0.07
	± 1SD	4.54	0.63	1.59	0.60	1.57	0.07	0.19
Neither Postcranial nor Cranial Stress	Average	5.98	-20.10	-20.19	10.92	10.84	-0.10	-0.10
	± 1SD	0.33	0.33	1.20	0.27	0.95	0.35	0.63

CHAPTER 7: DISCUSSION

Comparative $\delta^{15}N$ and $\delta^{13}C$ food-web for non-breastfeeding subadults at Alytus

To examine juvenile and adolescent diets, average values were obtained from all individuals over five years at death who would have no longer shown any evidence for enrichment due to breastfeeding. Thus between 5-16 years at death, the average humeral $\delta^{13}C$ was -20.22‰ ± 0.35‰ and $\delta^{15}N$ was 10.26‰ ± 0.79‰. The average femoral $\delta^{13}C$ was -20.06‰ ± 0.29‰ and $\delta^{15}N$ was 10.34‰ ± 0.68‰. It is unlikely that subadults at Alytus were habitually exploiting marine (ocean) or C_4 resources based on depleted $\delta^{15}N$ and $\delta^{13}C$ values, respectively. While millet (*Panicum miliaceum*) was grown in Lithuania beginning in the Bronze Age and at the capital of Vilnius during the Medieval period, these data do not support habitual consumption of the carbon enriched (-9‰) plant. Although it is unlikely that those at Alytus routinely exploited oceanic resources, the accessibility of residents to both forest and riverine protein sources must be explored.

Antanaitis-Jacobs and colleagues (2012) report $\delta^{15}N$ and $\delta^{13}C$ values of food resources available to early inhabitants of the Baltic region between the Neolithic and Bronze Ages. Forty-two faunal remains were processed from prehistoric, archaeological sites throughout Lithuania, Latvia, Estonia, and the island of Gotland. Currently, these are the only published isotopic faunal data for Lithuania and they are at the very least 2,000 years older than the earliest use of the cemetery at Alytus in the late 14th century. Some remains originate from the more recent subatlantic climatic period, which is a period of ongoing warming and increased ocean salinity. It is also important to note that these ancient samples will not reflect any increased base-nitrogen content of soil due to agricultural activities like churning of the soil and adding manure as

fertilizer. Therefore, caution must be taken in making interpretations about diet based on these data alone.

One site, Turlojiškė/ Kirsna, is barely 50km from Alytus and is similarly identified as an inland river site making it increasingly comparable to Alytus and contains three faunal samples from a red deer, mallard, and cow. While the Antanaitis-Jacobs et al. (2012) data are not ideal for comparison to individuals at Alytus, it is important to place the Alytus data within the current foodweb for the Baltic countries. Doing so will allow for a general comparison of what those at Alytus may have consumed.

Figure 19 exhibits the faunal data from Antanaitis-Jacobs et al. (2012) with the average humeral and femoral values for the non-breastfeeding subadults in the Alytus sample within a box denoting two standard deviations from the mean. As expected, the subadults do not appear to be eating oceanic protein, which is enriched in both carbon and nitrogen. The sample of seals and perches (average -17.08‰; 12.34‰) from Antanaitis-Jacobs and colleague's (2012) research are enriched in carbon and nitrogen by around 5‰ over the freshwater ducks (average -22.79‰; 7.3‰) and forest mammals (average -21.53‰; 6.61‰).

Reitsema and colleagues (2013) also recently published isotopic data on the Pre-Medieval and Medieval faunal remains from the archaeological site of Kaldus in Torun, Poland, which is about 450 km southwest of Alytus. Once again, this data comes from another geographically (and possibly culturally) distant site but is a Medieval town situated on a river. Overall, Reitsema et al.'s (2013) data are similar to and add further depth to the faunal data in Antanaitis-Jacobs et al. (2012) (Fig. 19).

Interestingly, non-breastfeeding subadults from Alytus fall within their own niche, but are closest to the values reported for the Baltic Late Neolithic badger and marten specimen,

animals which tend to eat small mammals and other foods commonly found in the forest. A dog sample from Reitsema's study in Poland also falls within the boundary of subadult diet. This is interesting because, typically domesticated dogs will be fed scraps from their owners. To determine what individuals at Alytus were most likely eating based on the current data, 5‰ was subtracted from the $\delta^{13}C$ subadult averages and 3‰ from the $\delta^{15}N$ subadult averages, which solves for the typical fractionation amount between food and consumer. Two animals, a Mid-Neolithic mallard and a Medieval pike are about 5‰ more depleted in carbon and 3‰ more depleted in nitrogen than the subadult sample. Therefore, it can be concluded that non-breastfeeding subadults at Alytus were likely consuming C_3 plant types in addition to terrestrial animal and riverine protein resources.

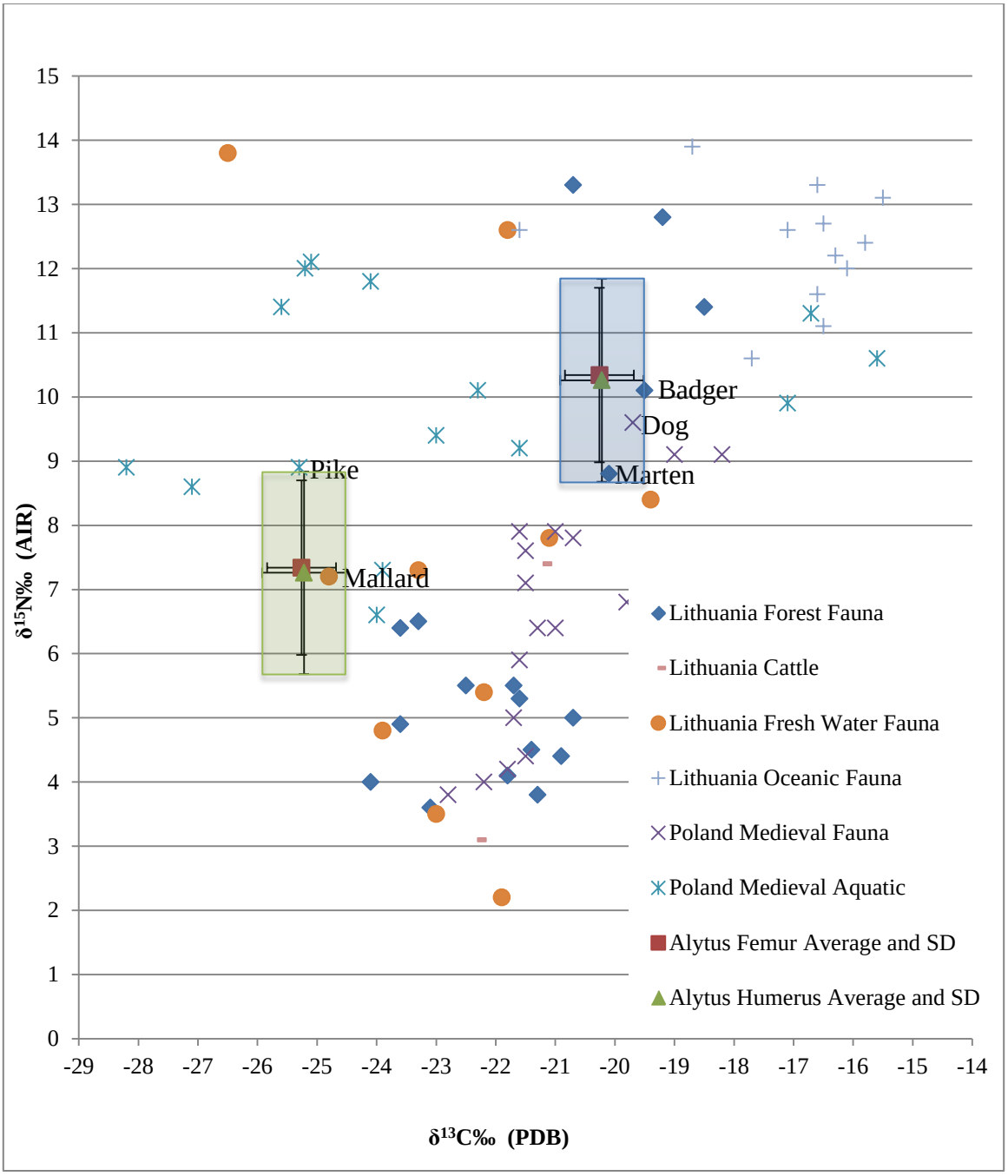


Figure 19. Possible Alytus food-web (after Antanaitis-Jacobs et al., 2012; Reitsema et al., 2013). Femoral and Humeral averages for individuals who no longer show evidence for breastfeeding are plotted with ± 2σ. Blue box is proper placement in graph; green box is placed to indicate values of possible food sources based on changes in fractionation from food to consumer.

Comparative breastfeeding and weaning practices in 11th-16th CE England, 6th-15th CE Greece, and 4th-6th CE England

It is important to compare and contrast infant and childhood feeding practices to chronologically and geographically similar cultures. However, as this research is the first to look at Medieval subadult diet in Eastern Europe, more culturally disparate populations were used for comparison. Three populations were chosen to graphically compare isotopic values of individuals less than 16 years at death. The publications were chosen on the basis that the study provided acceptable aging methods, utilized bone collagen, reported ages, preservation quality, and isotopic values for each individual. Transposing the same age at death cohorts, which were constructed for this thesis to encompass individuals experiencing similar biological changes in growth, individual isotopic values were utilized to calculate and compare cohort average isotopic values (Figs. 20 and 21). The studies presented below are in chronologically descending order from the Late Medieval in Britain (Burt, 2013), to Byzantine Greece (Bourbou et al., 2013), and finally to Sub-Roman Britain (Fuller et al., 2006b).

Utilizing rib collagen carbon and nitrogen isotopes, Burt (2013) found that individuals from Medieval Fishergate (11th-16th centuries, York, England) were breastfed exclusively until around one year when weaning foods were introduced. Breastfeeding enrichment was no longer detectable in individuals who died after two years for Fishergate House infants, which was consistent with the Wharram Percy infants (Fuller et al., 2003 [dentine]; Mays et al., 2002 [ribs]). Not only were these contemporaneous Fishergate House infants being weaned off of breast milk almost a year before their counterparts at Alytus, but the entire process was no longer evident by the age of two years at death.

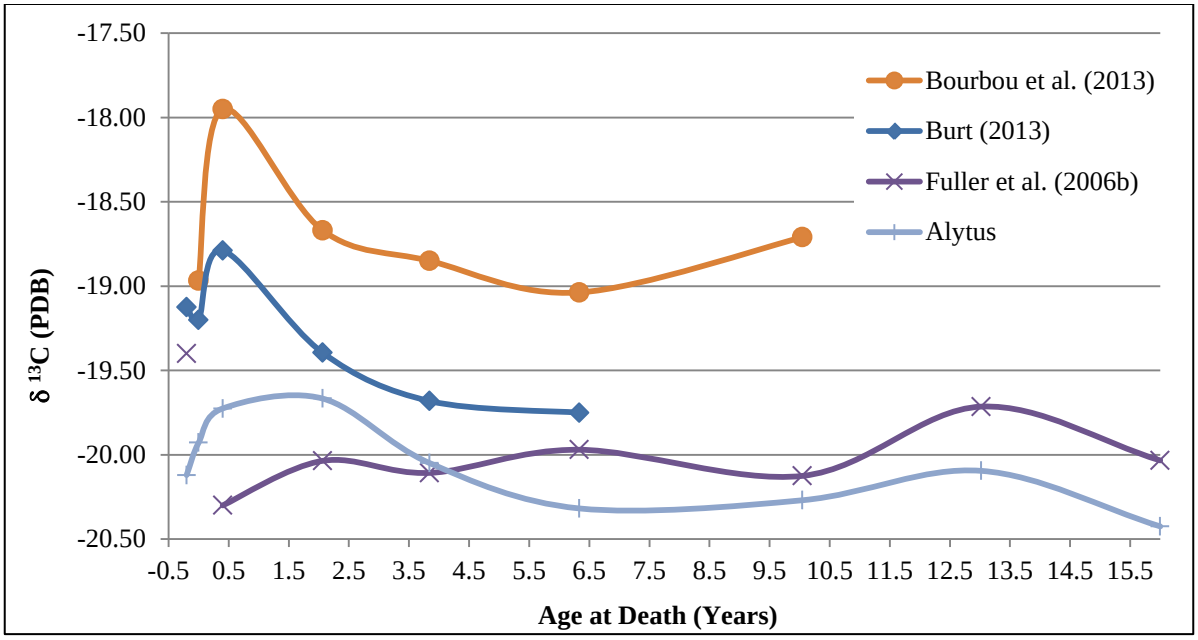


Figure 20. Average cohort $\delta^{13}C$ from Medieval Samples (Bourbou et al., 2013; Burt, 2013; Fuller et al., 2006b).

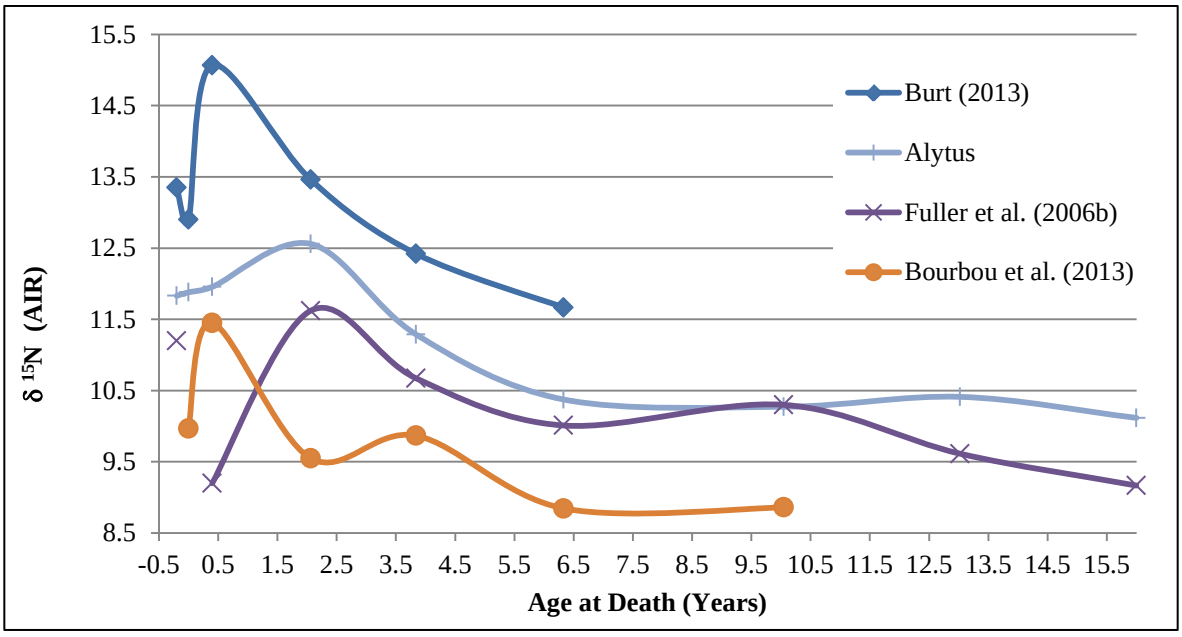


Figure 21. Average cohort $\delta^{15}N$ from Medieval Samples (Bourbou et al., 2013; Burt, 2013; Fuller et al., 2006b).

Ribs are thought to exhibit greater rate of turnover in both subadults and adults due to their largely trabecular composition. It follows that the Fishergate House data should be more reflective of isotopic values closer to the time of death for infants relative to humeral or femoral data. However, the high rate of turnover in growing humeri and femora should similarly detect swift changes in dietary practices, if at a minimally delayed point in time through presenting a more gradual return to sample averages. Therefore, cautious estimations of the timing of the weaning process at Alytus must be made as these could have occurred slightly earlier than expected due to bone modeling rates. Interestingly, the Fishergate House sample maintained enriched $\delta^{13}C$ and $\delta^{15}N$ between 2-4 years, which was interpreted as a specialized childhood diet containing higher trophic level protein content (Burt, 2013). The Alytus sample too preserves enrichment throughout early childhood, but due to the delayed breastfeeding peak and steady decline, the results of this study indicate that the specialized diet for individuals at Alytus was specific to weaning foods and extended breastfeeding.

Most writings about the process of weaning come from historic, Greco-Roman suggestions (e.g. Soranus and Galen) and fashions popular in Western Europe. Thus, average cohort isotope signatures for the data at Alytus were compared to eight contemporaneous samples in Byzantine Greece analyzed by Bourbou and colleagues (2013) from rib and long bone collagen. The researchers found that pinpointing an exact age for the commencement of weaning was difficult due to geographically disparate samples and small sample sizes. The ribs used for the analysis likely have a greater rate of turnover, and would reflect isotopic values more recent to the time of death than long bones used for the study likely would. As will be discussed later, the different bones used for isotopic analysis may have hindered the conclusions for understanding the timing of the introduction of weaning foods at the Greek sites. Upon

examining cohort averages it does appear that the peak trophic level enrichment for individuals buried at the Greek sites occurred early, before one year as was seen in the Medieval British sample (Burt, 2013). However they concluded that by four years of age, the process was no longer isotopically detectable and that this prolonged and gradual process, also seen in Alytus, was likely related to Roman customs (Bourbou et al., 2013).

Fuller and coworkers (2006b) looked at predominantly rib (and some femoral) $\delta^{13}C$ and $\delta^{15}N$ in a 4^{th}-6^{th} Century subadult sample (Queensford Farm, Oxfordshire). The authors concluded that exclusive breastfeeding had peaked at two years at death and subsequently declined due to weaning, which lasted until about three or four years at death. By transposing current cohort definitions onto Fuller and colleagues (2006b) age at death estimations and isotopic data, cohort means and smooth marked scatter plots demonstrate that Sub-Roman British breastfeeding and weaning durations are remarkably similar to feeding practices and duration found at Alytus in the current study.

Age, stress, and diet variability in $\delta^{15}N$ and $\delta^{13}C$ for subadults at Alytus

Individuals who vary greatly from sample and cohort averages are exclusively from the Infant (0.10-2.9 years) and Juvenile (5.0-11.9 years) cohorts. Almost every individual with a significantly enriched isotopic value, relative to the sample or his or her specified cohort, is an infant (Figs. 22-25). This of course is likely related to factors associated with breastfeeding enrichment, especially in those who are only variable from the sample mean (Burial numbers: Humeral- 879, 212, and 944; Femoral- 350). In contrast, significantly depleted individuals are all juveniles. These individuals likely reflect subadults in a period of growth and possibly their introduction into agricultural, trading, or apprenticing jobs like the adults at Alytus.

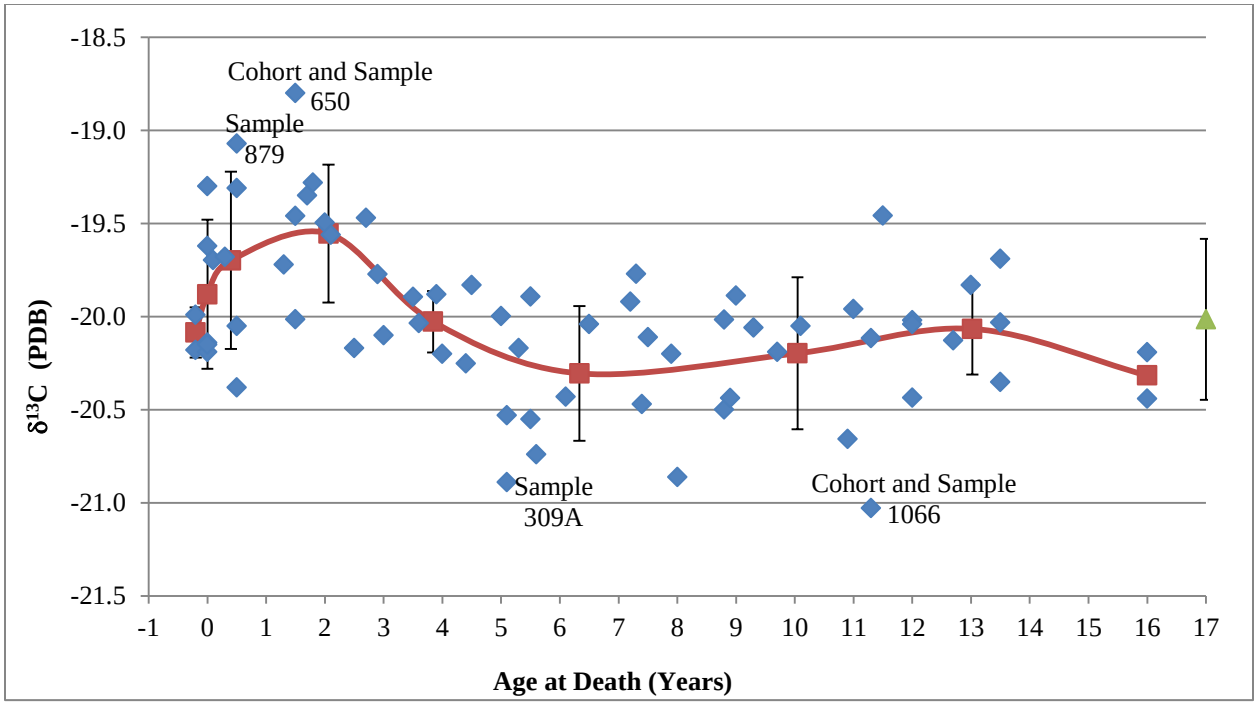


Figure 22. Humeral $\delta^{13}C$ with labeled outliers (± 2σ)

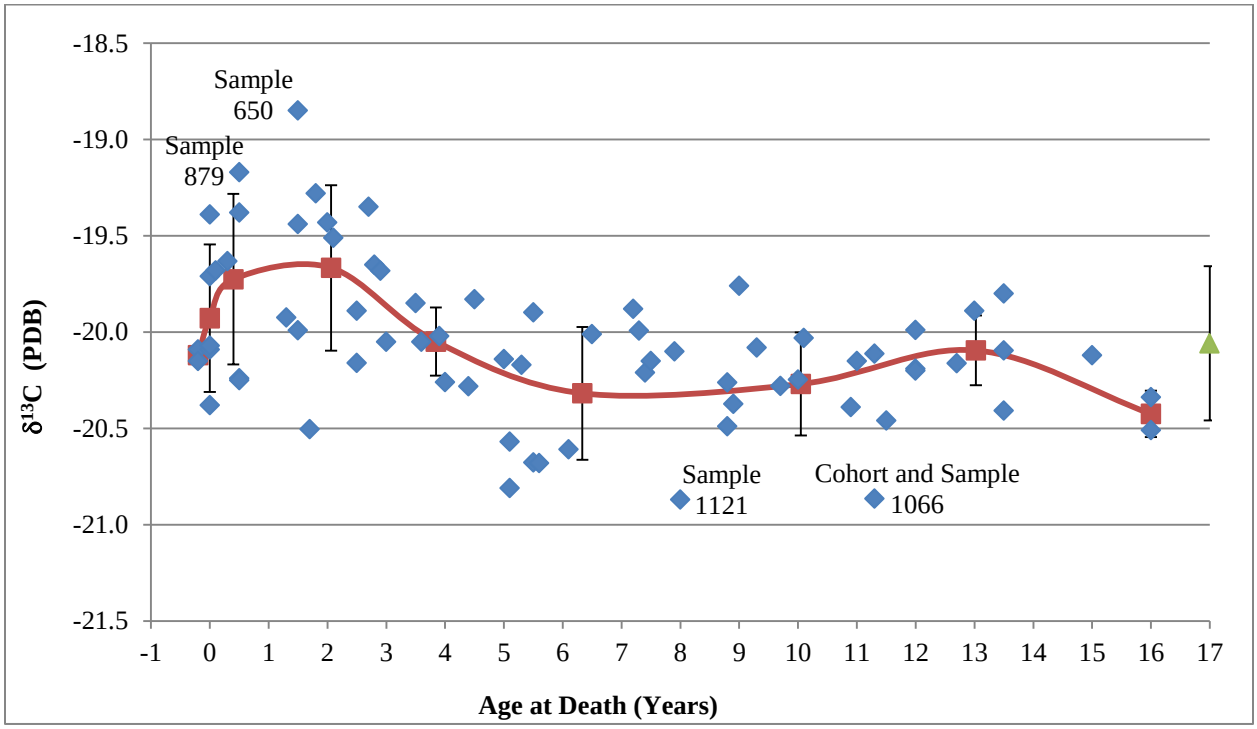


Figure 23. Femoral $\delta^{13}C$ with labeled outliers (± 2σ)

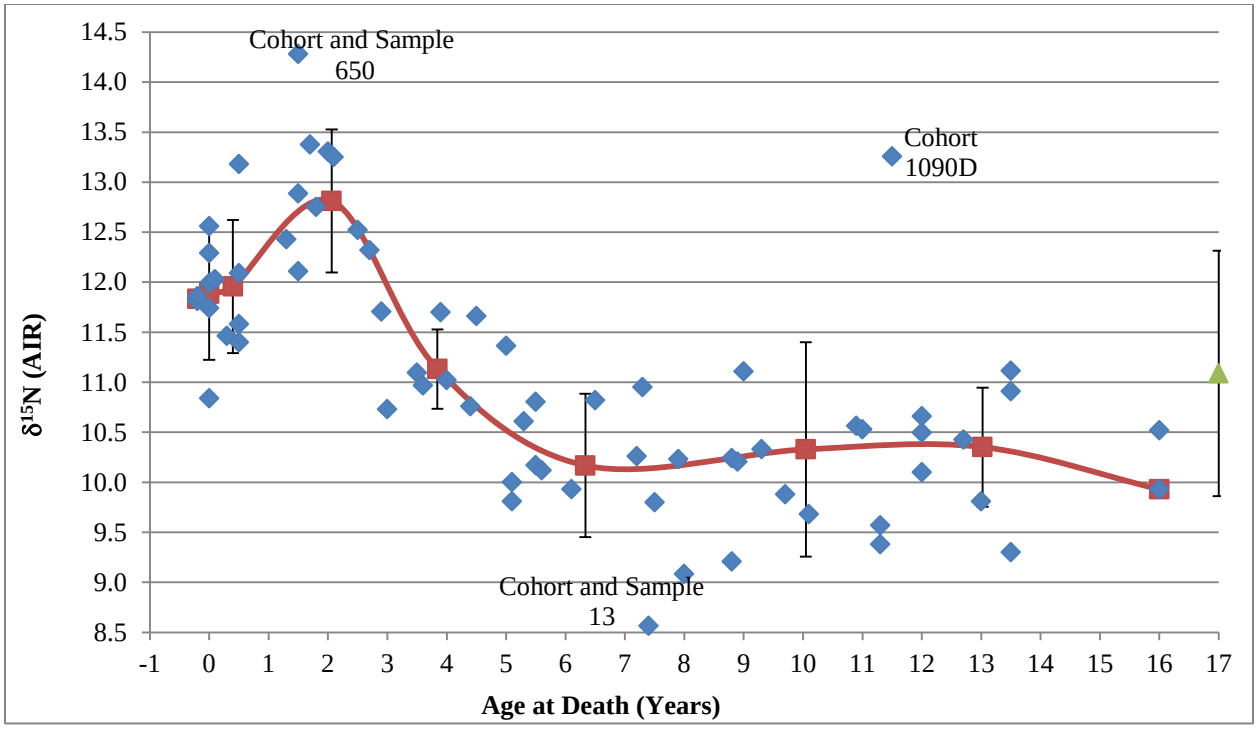


Figure 24. Humeral $\delta^{15}N$ with labeled outliers (± 2σ)

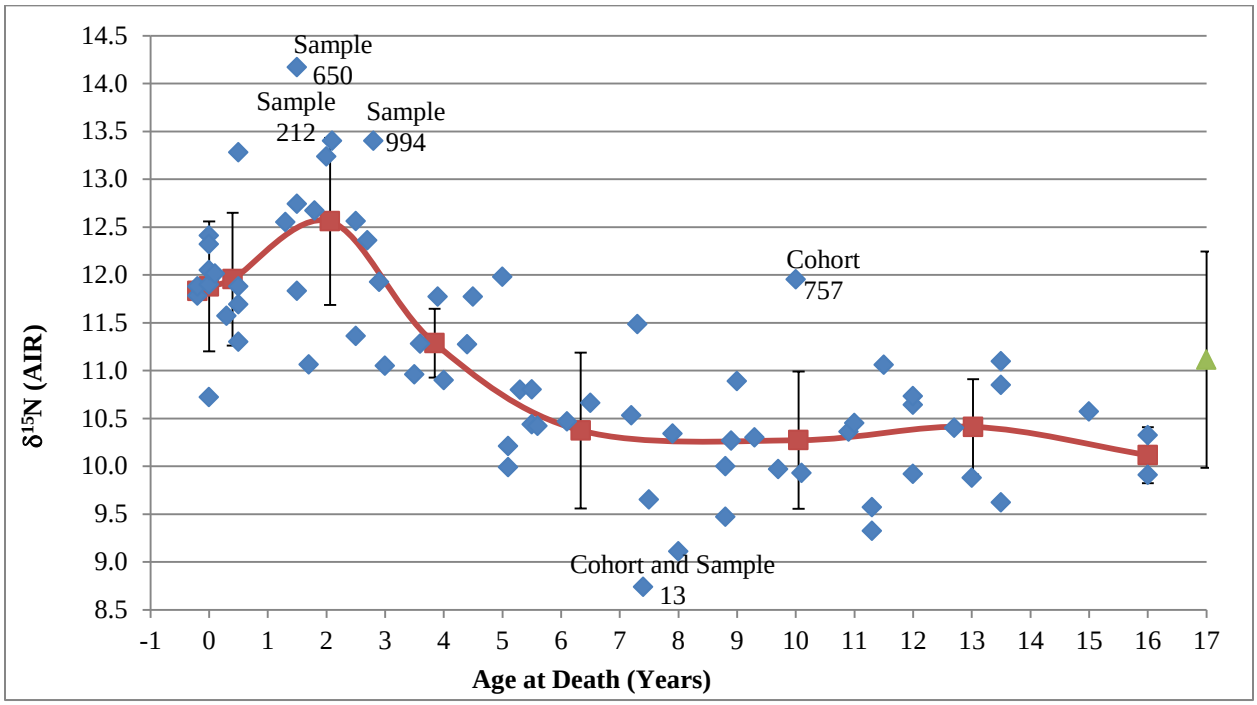


Figure 25. Femoral $\delta^{15}N$ with labeled outliers (± 2σ)

Cohorts were constructed with the goal of grouping individuals who would likely have been facing similar physiological and dietary experiences at given ages (e.g. breastfeeding enrichments, weaning illnesses, growth spurts). Consequently, individuals with isotopic values greater than two standard deviations from the mean of their cohort grouping are further analyzed to investigate mortality and disease experience.

Prolonged exclusive breastfeeding and weaning of Infants and Children

Around two years of age the breastfeeding peak enrichment, of $\delta^{13}C$ and $\delta^{15}N$ values, gradually deplete by approximately 0.4‰ ($\delta^{13}C$) and 1.5‰ ($\delta^{15}N$) until five years, indicating that the weaning process at Alytus likely took place over the course of about three years during the Infancy 2 and Childhood cohorts. Carbon and nitrogen deplete in unison highlighting the retention of trophic level enrichments for both elements. This result is contrary to Fuller and colleagues (2006a) finding that carbon will deplete faster than nitrogen (although their sample was more likely to pick up on small changes in carbon over a shorter period of time using incremental nail and hair samples).

The only outlier present between one month and one year at death was Infant 879 who was six months old at death (Fig. 22-25). This individual was more enriched in carbon for both its humeral and femoral sample but only over the sample average, which is most likely due to the known trophic level enrichment of breastfeeding infants (Tables 14 and 15). Likewise, Infants 212 and 994 (2.1 and 2.8 years, respectively) are significantly enriched in femoral nitrogen but only over sample means, which serves to highlight their trophic level enrichment due to breastfeeding, as they are not distinct from others in their cohort. In contrast Infant 650, who was 1.5 years at death, was significantly enriched in carbon and nitrogen over humeral and femoral

sample and cohort averages. While it is very likely that Infant 650 was also still being breastfed (possibly even exclusively), the amount of enrichment may be indicative of negative nitrogen balance wherein the body had begun to recycle nutrients to maintain life as not enough protein was being consumed. Both cranial and postcranial stressors are associated with burial 650, which further supports the claim that this infant was physiologically stressed.

Table 14. Presence of postcranial and cranial stress in individuals with humeral outliers (± 2σ). Bolded values are outside of 95% confidence interval.

Burial ID	Age	Cohort	$\delta^{13}C‰$	$\delta^{15}N‰$	Sample or Cohort Outlier	Postcranial Stress?	Cranial Stress?
879	0.5	Infant 1	**-19.07**	12.09	Sample	Yes	
650	1.5	Infant 2	**-18.80**	**14.28**	Cohort and Sample	Yes	Yes

Table 15. Presence of postcranial and cranial stress in individuals with femoral outliers (± 2σ). Bolded values are outside of 95% confidence interval.

Burial ID	Age	Cohort	$\delta^{13}C‰$	$\delta^{15}N‰$	Sample or Cohort Outlier	Postcranial Stress?	Cranial Stress?
879	0.5	Infant 1	**-19.17**	11.8	Sample	Yes	
650	1.5	Infant 2	**-18.85**	**14.17**	Cohort and Sample	Yes	Yes
212	2.1	Infant 2	-19.51	**13.4**	Sample	Yes	
994	2.8	Infant 2	-19.65	**13.4**	Sample		Yes

Prolonged exclusive breastfeeding (likely experienced by burial 650) and delayed cessation of weaning may have been a necessity for peasant families living in poverty or through famines. It would not be surprising that a weanling diet would contain no other protein than breast milk or possibly cows' milk, as meat might be tougher to chew for children with deciduous dentition and challenging to acquire for the poor (Lewis, 2007b). Paired with

additional foods, breast milk could have remained a minor source of protein and passive immunity for the infant (is even recommended as nutrition until two years of age [WHO, 1991]).

However, by six months of age more vital nutrients, like calcium, iron, and zinc, are required than human milk alone can provide thereby leaving infants at Alytus in a state of nutrient deficiency (Dettwyler and Fishman, 1992; Ulijaszek, 1990; WHO 1991). While extended weaning practices would have given infants additional protein in times of need, it also put them at an increased risk of malnutrition after one year (Dettwyler and Fishman, 1992; WHO, 1991). Adequate protein consumption is integral to catalyzing osteoid production and mineralization of bone for growth. Furthermore, the rapid and metabolically expensive surge in brain growth between 3-7 years requires children to consume substantially more nutrients than at previous ages (Bogin, 1997; Lewis, 2007b). Children under five years of age utilize 24-60% more metabolic energy then adults do to maintain brain function (Bogin, 1997). Therefore, it is essential for individuals between three and five years to receive greater caloric intake, then is available in breast milk alone, to maintain life.

Once weaning began for infants and children, they would have been introduced to novel pathogens in water, foods, or on serving tools. The process of weaning was likely physiologically stressful resulting in diarrhea, followed by malnourishment and a heightened susceptibility to diseases and parasitic attacks due to a compromised immune system (Ulijaszek, 1990; Lewis, 2007b). Furthermore, weaning foods such as animal milks or honey sweetener could also have negatively impacted the individual's ability to digest foods. Cow's milk could have caused kidney failure by concentrating urine and causing bleeding in the gut whereas honey could have resulted in botulism for the weaning infants (Dupras et al., 2001; Lewis, 2007b).

The depletion in both nitrogen and carbon also signifies that infants were being weaned onto carbon depleted, temperate grain paps rather than gruels made of millet. The only millet isotopically analyzed comes from the Bronze Age in Lithuania but was reported to have been -9‰ (Antanaitis-Jacobs et al., 2012). Therefore, we might expect infants that habitually consumed millet in the form of paps to exhibit substantially more enriched carbon around -14‰. The introduction of C_3 heavy grain diets too would have left these infants further malnourished as cereals are notably lacking in vitamin C and inhibit the absorption of calcium therefore interfering with vitamin D synthesis (Brickley and Ives, 2008).

Life and death after weaning: The juveniles

As the Juvenile cohort age range is quite long and merges seven years of growth and development, the cohort was divided at 5-8.5 years and 8.6-12 years to both equalize cohort sample sizes and to obtain a better perspective on isotopic and physiological changes that occur during the seven years of skeletal development. Individuals between 5-12 years may have been considered contributing members of their family aiding in house or field labor through imitation of their parents and eventually joining the workforce (Lewis, 2007b; Orme, 2003). Interestingly, most of the individual outliers for the juvenile cohort are further depleted relative to cohort or sample means (Tables 16 and 17).

Individual 13 demonstrates depleted nitrogen levels, which are usually indicative of a lower protein diet then others in the sample. However, this juvenile was estimated to have been 5.1 years at death, which could signify malnutrition due to extended weaning period, after which the body had to route nitrogen to the brain in order to maintain life and maintain cognitive development. The extended weaning could have left this individual more prone to disease and

illness carried in newly introduced adult foods. An acute bout of illness would certainly account for the lack of skeletal lesions. Šereikienė and Jankauskas (2004) also found that between 5-7 years, individuals at Alytus were going through a growth spurt. Growing would account for a positive nitrogen balance in the body wherein more $^{14}N/^{15}N$ was being used to grow than was excreted through urea depleting the overall pool of nitrogen.

Table 16. Presence of postcranial and cranial stress in individuals with humeral outliers (±2σ). Bolded values are outside of 95% confidence interval.

Burial ID	Age	Cohort	$\delta^{13}C‰$	$\delta^{15}N‰$	Sample or Cohort Outlier	Postcranial Stress?	Cranial Stress?
309A	5.1	Juvenile 1	**-20.89**	10.00	Sample		
13	7.4	Juvenile 1	-20.47	**8.57**	Cohort and Sample		
1066	11.3	Juvenile 2	**-21.03**	9.57	Cohort and Sample	Yes	Yes
1090D	11.5	Juvenile 2	-19.47	**13.26**	Cohort		

Table 17. Presence of postcranial and cranial stress in individuals with femoral outliers (±2σ). Bolded values are outside of 95% confidence interval.

Burial ID	Age	Cohort	$\delta^{13}C‰$	$\delta^{15}N‰$	Sample or Cohort Outlier	Postcranial Stress?	Cranial Stress?
13	7.4	Juvenile 1	-20.21	**8.74**	Cohort and Sample		
1121	8	Juvenile 1	**-20.87**	9.11	Sample	Yes	Yes
757	10	Juvenile 2	-20.25	**11.95**	Cohort	Yes	
1066	11.3	Juvenile 2	**-20.87**	9.57	Cohort and Sample	Yes	Yes

Juvenile 1066 is estimated to have been 11.3 years old at their time of death and presented postcranial and cranial stress lesions. The depleted $\delta^{13}C$ and $\delta^{15}N$ values signify a low

protein and high C_3 diet, which potentially could indicate a specialized diet. Typically when one is too sick to eat, porridges or other soft foods made of bread are eaten. Also, this individual may not have been able to afford animal proteins or did not consume them, which resulted in their depleted values. A more thorough analysis of the specific skeletal stressors that appear on this individual would greatly aid future discussions of these isotopic results.

In contrast, older Juveniles 1090D (11.5 years, humeral cohort outlier) and 757 (10 years, femoral cohort outlier) were substantially enriched in $\delta^{15}N$ when compared with their cohort. This enrichment is likely due to negative nitrogen balance, possibly due to malnourishment and subsequent re-fractionation of the element as there is no current evidence for enriched marine food consumption. Even when an individual is malnourished, the body will do what it can to maintain growth and deposition of bone (Reitsema, 2013). This might be important if these individuals were beginning puberty.

Twelve years at death was used as the cessation of this cohort as it most likely occurs just before the onset of puberty in males (10 for females) (Bogin, 1997; Lewis, 2007b). Unfortunately, there are still no reliable ways to estimate the sex of subadults and therefore, the later age was used to include all likely pre-pubertal individuals in the sample and more accurately attempt to place pubertal individuals into the adolescent cohort. However, it is recognized that some of the deceased in this cohort may have been females who had already begun puberty and therefore were undergoing a state of growth.

Positive Nitrogen Balance in Fetuses and Young Adults

The young adult burials in this sample (688 and 702) are not located near the fetal remains (882) in the Alytus cemetery and are therefore not hypothesized to have been the fetus' mother

(Fig. 26). However, as there were no estimations of sex provided for the 16 year-olds, it is possible that at this age, pregnancy could have been a factor contributing to death if those individuals were female thus meriting comparison between the two cohorts.

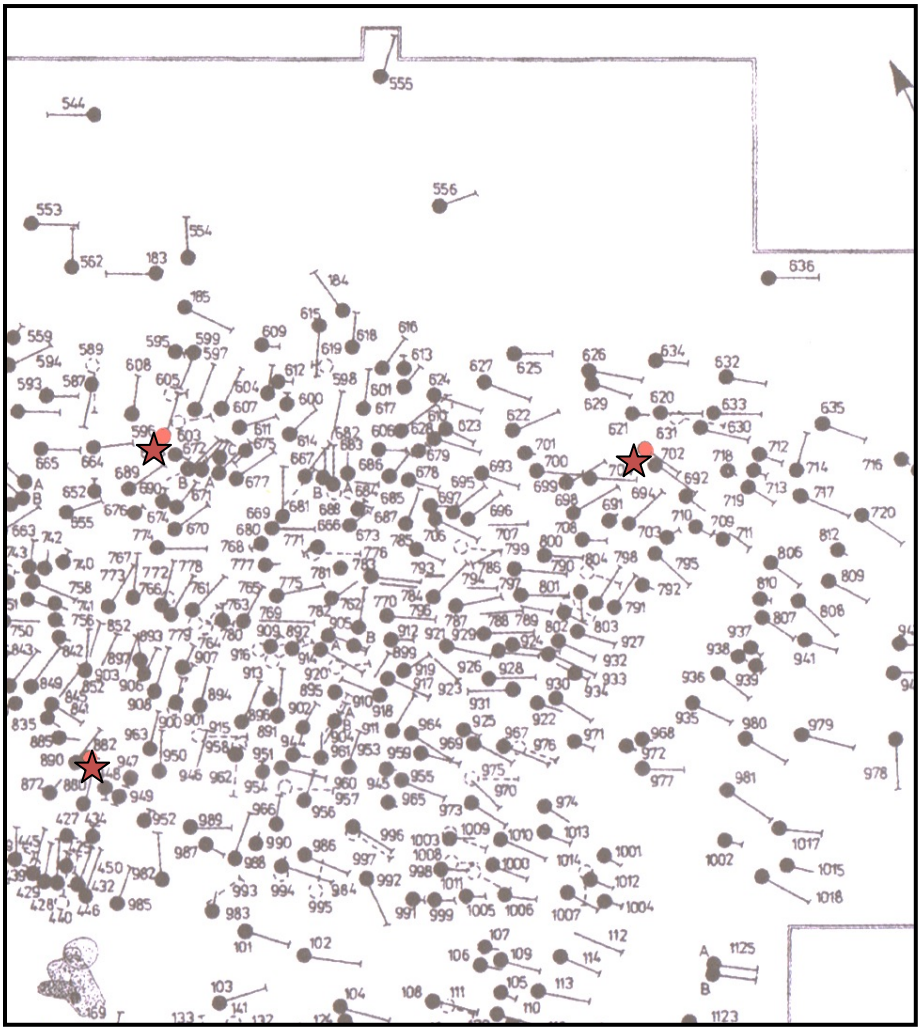


Figure 26. Close up of northeastern portion of Alytus cemetery plan. Burials for Fetus (882) and two Young Adults (688 and 702) are marked in red. Note that neither are located near the fetus. Arrow points north.

The average of the two fetal samples (Burial 882), demonstrate enrichment over the average of the two young adults in the sample in $\delta^{15}N$ (1.61‰ [humeral]; 1.71‰ [femoral]) and a slight enrichment in $\delta^{13}C$ (0.23‰ [humeral]; 0.30‰ [femoral]). Enrichment in isotopic values for fetuses is of course directly related nutrients received via placenta in the fetal environment and a period of growth during which the metabolism is high, bones are rapidly forming, and all deciduous dentition begins to mineralize. Thus the body of a fetus will likely be in positive nitrogen balance.

The young adults were also depleted relative to the sample mean in $\delta^{15}N$ (0.86‰ [humeral]; 0.89‰ [femoral]) and $\delta^{13}C$ (0.30‰ [humeral]; 0.36‰ [femoral]). Furthermore, the young adults in this sample only vary from adult female mean at Alytus in $\delta^{15}N$ by at most 0.15‰ (Katie Whitmore, pers. comm.). Fuller and colleagues (2004; 2006a) found that 20 weeks before the birth of an infant the hair and nail $\delta^{15}N$ in pregnant women deplete by about 0.5-1.0‰ likely reflecting positive nitrogen balance directed at fetal growth. As is the case with pregnancy in an adult, we might also hypothesize that individuals 688 and 702 were in positive nitrogen balance. Yet, the positive nitrogen balance of the young adults may also reflect a growth spurt in their own limbs, which would account for their relatively depleted isotopic values. Šereikiené and Jankauskas (2004) found a delayed pubertal growth spurt likely took place between individuals in the cemetery sample who died between 12 -15 years.

While subadult bone turnover is high, as individuals age the time it takes for bone to turn over completely will also increase meaning that a sample of bulk collagen will reflect older values. Hedges and colleagues (2007) demonstrated that bone turnover in 10-15 year-olds was only about 10-30% per year. In a modern sample, Goldman and colleagues (2009) found that

midshaft cortical bone at this age is mostly resting or is only being added to below the periosteum. Therefore, it is possible then that the depletion in nitrogen is reflective of this period of pubertal growth that took place during adolescence. If this is true, it also supports the hypothesis that these individuals were most likely reproductively mature. Certainly this argument could be strengthened by larger cohort sample sizes, sex estimation, or incremental isotopic analysis of hair.

Disparities in Cohort $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$

Individuals who died between fetal age and before the first year had humeral and femoral isotopic values that are very similar in composition but isotopic averages demonstrate that the humerus is more enriched throughout the first year of life (Figs. 27 and 28). Infants at Alytus who died between 1 to 12 months would not have been highly mobile but going through the processes of learning to roll over, crawl, stand, and finally clumsily walk. During this time of rapid growth, humeral diaphyses would have microscopically been preferentially developed over femoral cortices by mobility and use, as appositional modeling is largely dependent upon activity levels through muscle use and mechanical stress. At first glance, it appears that more enriched $\delta^{13}C$ and $\delta^{15}N$ elements are being routed to the humerus over the femur within the first year of life. However, it seems more likely that bulk $\delta^{13}C$ and $\delta^{15}N$ values from humeral samples are in actuality reflective of more recently deposited isotopes enriched from trophic level effects of breastfeeding. In other words, the rate of turnover is likely higher for the humerus then the femur during this substantial period of growth and arm-led mobility resulting in deposition of more enriched $\delta^{13}C$ and $\delta^{15}N$ values.

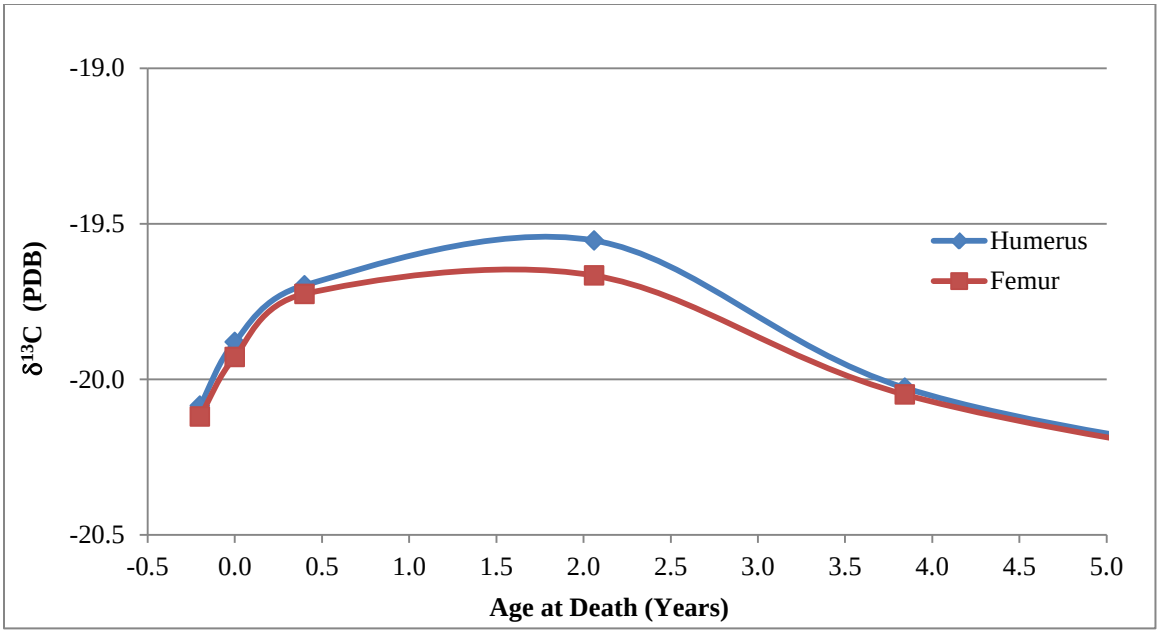


Figure 27. Detail of smooth scatterplot of average cohort $\delta^{13}C$ against age at death every 0.5 years for Fetus through Infant 2 cohorts spanning duration of exclusive breastfeeding and weaning.

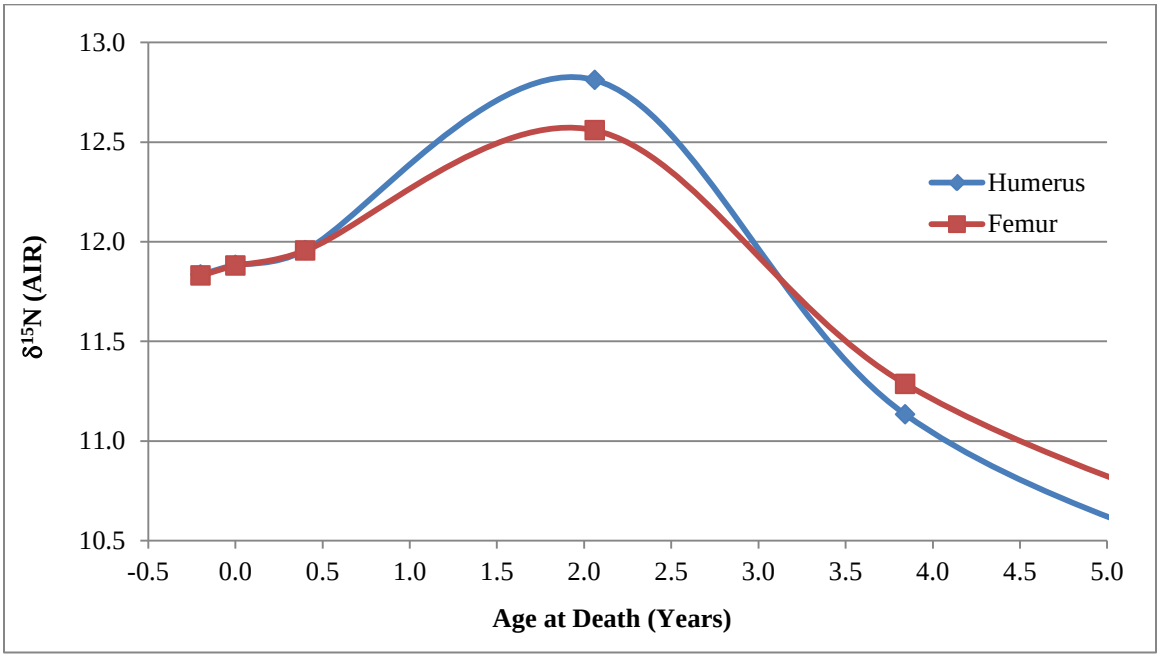


Figure 28. Detail of smooth scatterplot of average cohort $\delta^{15}N$ against age at death every 0.5 years for Fetus through Infant 2 cohorts spanning duration of exclusive breastfeeding and weaning.

Between one to two years the infant is almost certainly walking and continuing to grow. During this second infancy cohort, there is an increasing larger difference between the average humeral to femoral carbon and nitrogen isotopic values. Once again, it is possible that humeral values remain more enriched due to prior breastfeeding trophic level augmentation. But between one and two years, the rate of deposition for femoral osteons should be higher as it is now used more frequently for mobility. Most importantly, bulk averages achieved from analyzing femoral isotopes will more quickly reflect the supplementation of breast milk protein with weaning foods.

After all evidence for weaning is absent from bone collagen at 5 years of age, carbon and nitrogen are relatively stable. For $\delta^{13}C$, the humerus remains enriched throughout juvenility (Fig. 29). However, during the first juvenile cohort nitrogen remains more enriched in the femur then the humerus and the relationship flips, but only slightly, during the second juvenile cohort (Fig. 30). Modeling of bone in the femora and humeri will undergo major transitions during juvenility in cortical area, which might be better reflected in the nitrogen isotopes replicating a period of femoral development in the form of increased height gain in 5-7 year-olds followed by humeral development. Goldman and colleagues (2009) demonstrated that femoral geometries tended to show signs of mid-shaft cortical bone drift in shape and size in their “older child” cohort (9-11 years) when bone morphologies also began to approach adult shape, including those associated with biological sex.

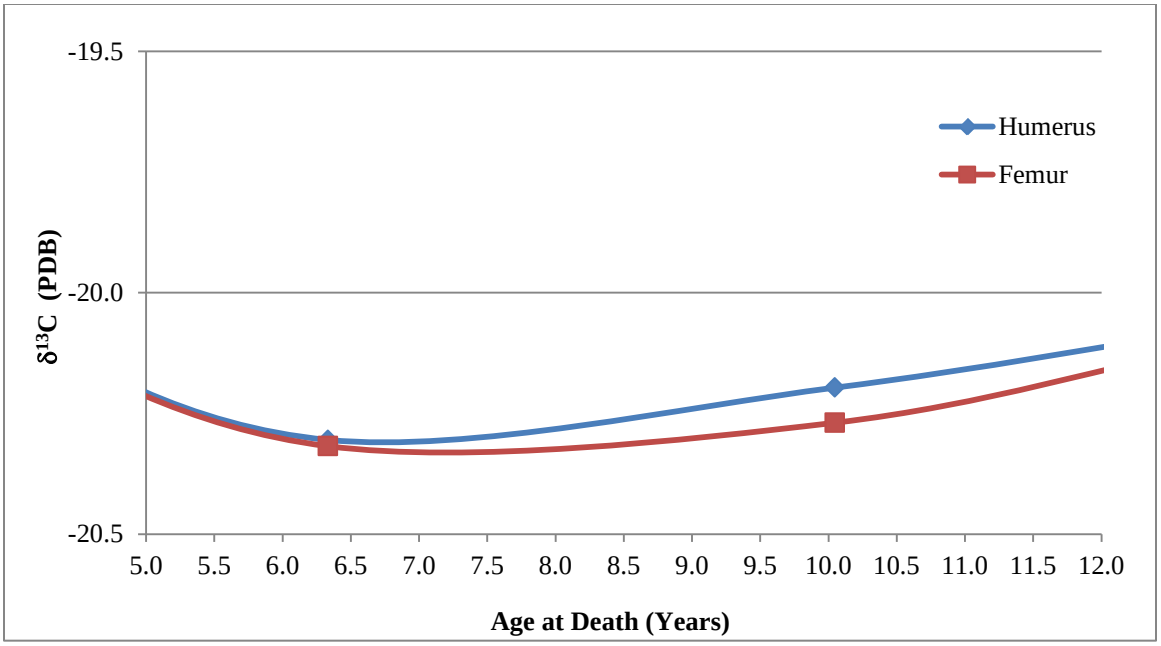


Figure 29. Detail of smooth scatterplot of average cohort $\delta^{13}C$ against age at death every 0.5 year for Child through Juvenile 2 cohorts, post weaning.

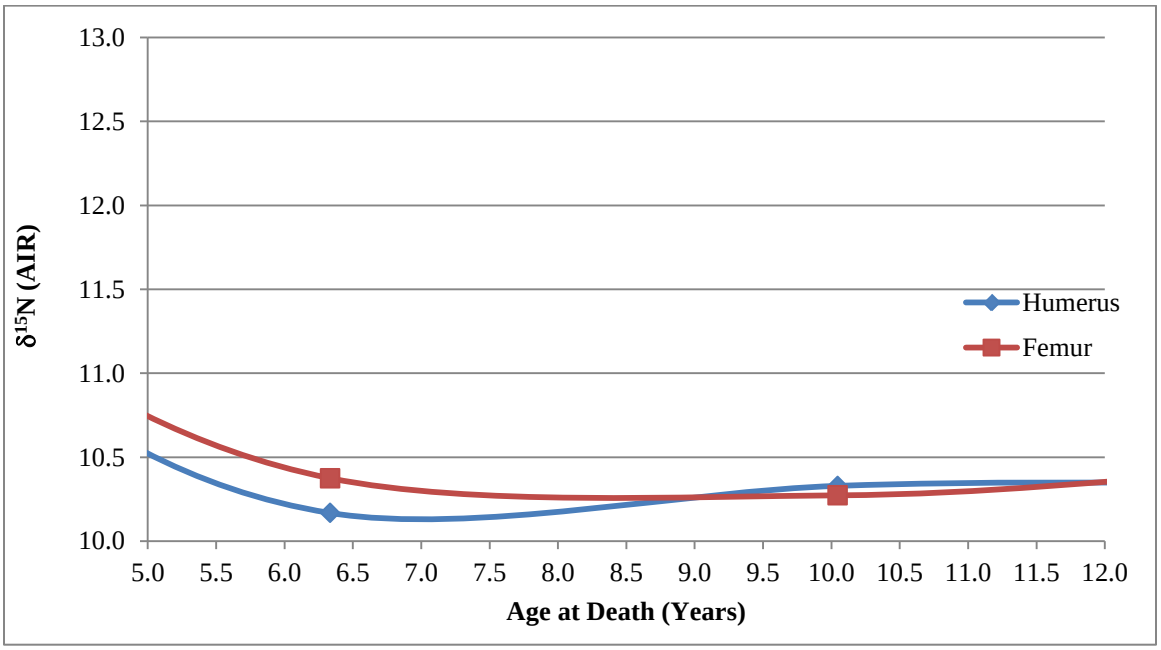


Figure 30. Detail of smooth scatterplot of average cohort $\delta^{15}N$ against age at death every 0.5 year for Child through Juvenile 2 cohorts, post weaning.

By plotting average cohort changes in femoral to humeral carbon and nitrogen an interesting trend in enrichment of one bone over the other is detected (Fig. 31). First, all $\Delta_{F\text{-}H}{}^{13}C‰$ cohort averages demonstrate enrichment in humeral over femoral values for this sample denoted by Δ values less than zero. This may have something to do with carbon metabolism in humans and thus merits future research. Differences in average isotopic values in each cohort follow the same trend of enrichment toward one element over the other when analyzed with age at death. These processes appear to follow those expected during growth when compared to height velocity charts by Šereikienė and Jankauskas (2004).

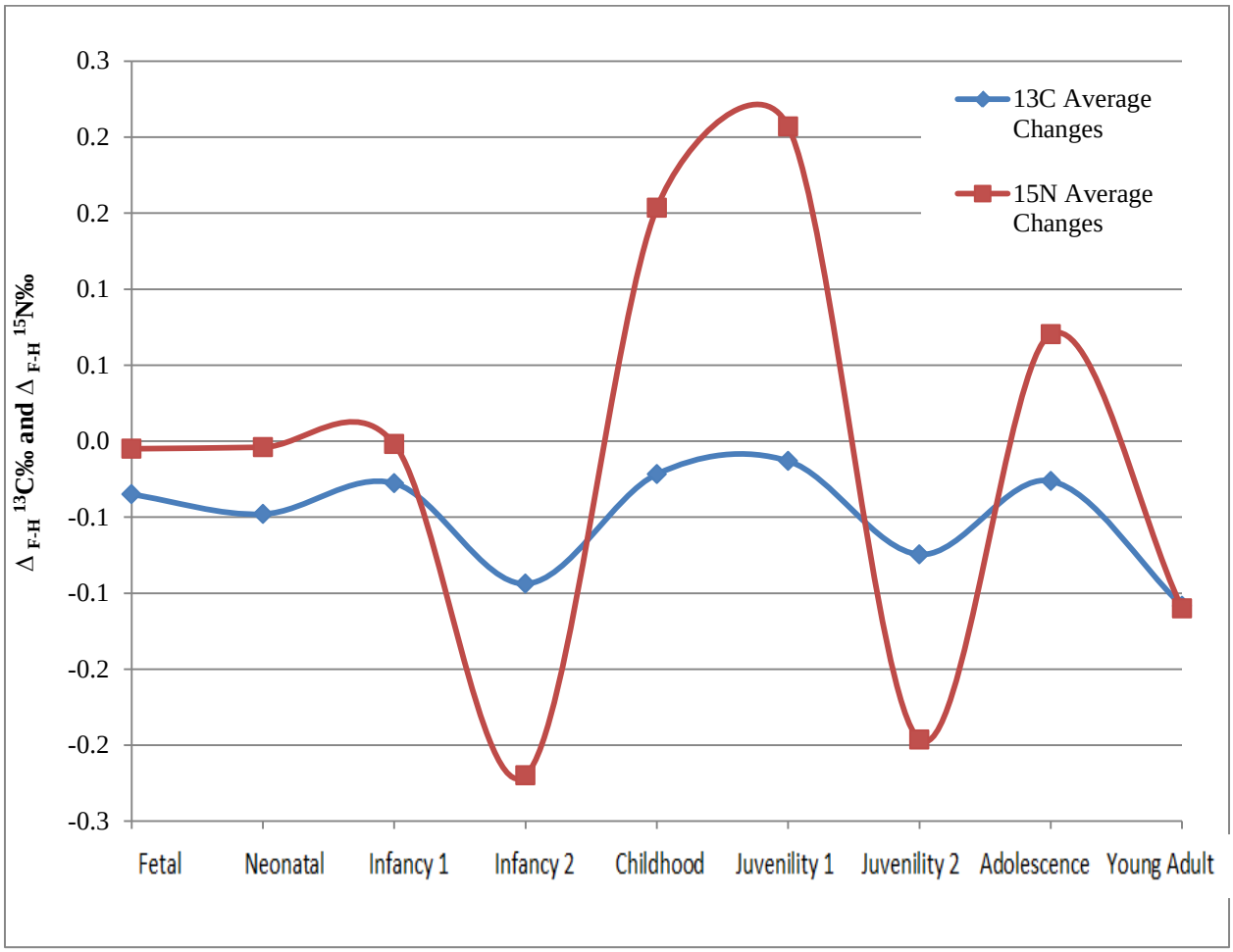


Figure 31. Average change in $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$ by cohorts.

Individuals in cohorts who died between fetal and 12 months of age had intra-individual differences between humeral and femoral isotopic values that were relatively stable with a small enrichment in femoral carbon and nitrogen before the end of the first year of life. However, between the Infant 1 and 2 cohorts there is a sharp increase in $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$ toward more negative values denoting humeral enrichment during a period of predominant humeral mobility. Individuals who died between Infant 2 and Childhood cohorts exhibit a large shift toward greater enrichment in the femur coinciding with ages (1-3 years) of greatest height attainment for individuals at Alytus according to Šereikienė and Jankauskas (2004). Šereikienė and Jankauskas (2004) next identify a period of decreased growth rate, which in turn lines up with the more gradual but steady increase in $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$ towards positive Δ values and therefore femoral enrichment. The next growth spurt occurs between 5-7 years, during the first juvenile cohort but is signified by a slight enrichment but then a trend toward more negative $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$ denoting higher isotopic signatures for the humerus. Finally the graph demonstrates one last positive trend toward enrichment of femoral $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$. It can be argued that antoher juvenile growth spurt likely existed between 8.5 to 12 years at death, peaking in mid-adolescence around 13 years representing a pubertal growth spurt.

Intra-Individual Variation in $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$

For the subadults at Alytus, there is no significant difference between change in bulk isotopic values when humeral values are subtracted from femoral values for either carbon or nitrogen. Therefore we can conclude that in general humeral and femoral isotopic values can be

compared to studies utilizing different sources of bone collagen. However, extreme care must be taken because when doing so as isotopic differences between the bones are magnified during periods of growth and development in the formation of the bone as described above. Only three individuals stood out as having significantly different $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$, though not one demonstrated skeletal stress lesions (Table 18).

Table 18. Age, cohort, and changes in $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$ for outliers.

Burial #	Inventory ID	Age (years)	$\Delta_{F\text{-}H}{}^{13}C‰$	$\delta^{13}C$ Enriched Element	$\Delta_{F\text{-}H}{}^{15}N‰$	$\delta^{15}N$ Enriched Element	Skeletal Stress
1001	1003D	1.7	-1.16	Humerus	-2.31	Humerus	-
1090D	1006K	11.5	-1.00	Humerus	-2.20	Humerus	-
604	637B-1	5.5	0.65	Femur	~~-0.37~~	~~Humerus~~	-
604	637B-2	5.5	-0.78	Humerus	~~0.63~~	~~Femur~~	-

As we saw with $\delta^{13}C$ and $\delta^{15}N$ outliers, individuals who exhibit significant changes between humeral and femoral isotopic values are from the infant and juvenile age categories. Burial 604 is unique and must be addressed separately as this individual has two samples each for their humerus and femur. Upon receiving the bones, sample bags were not labeled as to which pair was meant for comparison and were therefore numbered as they were pulled out of storage with their inventory numbers, bone type, and an additional number as follows: 637B-Hum-1, 637B-Hum-2, 637B-Fem-1, and 637B-Fem-2 (all refer to burial 604). A secondary check of the writing on the original sample bags revealed that the originally labeled 637B-H-2 was taken at the same time as 637B-F-1, as these bags have the same handwriting. The same is true of 637B-H-1 and 637B-F-2. When the data for these are "fixed" to match the handwritings, burial 604 is no longer an outlier in carbon (Table 19).

It is interesting that there is such a large discrepancy between the originally labeled Humeral and Femoral "1" samples and the Humeral and Femoral "2" samples. There is a 0.66‰ ($\Delta_{H\text{-}H}{}^{13}C$‰) and 0.63‰ (for $\Delta_{H\text{-}H}{}^{15}N$‰) enrichment of the first humeral sample over the second humeral sample. The femoral data also show similar difference for $\Delta_{F\text{-}F}{}^{13}C$‰ (0.36‰) and $\Delta_{F\text{-}F}{}^{15}N$‰ (0.36‰). Before anything can be concluded about the samples from this burial, it would be important to know exactly where on the bone the sample derrived (samples are all from right side) and if one sample was from a pathological site specifically that would account for the enrichment of one sample over the other.

Table 19. Age, cohort, and changes in $\Delta_{F\text{-}H}{}^{13}C$‰ and $\Delta_{F\text{-}H}{}^{15}N$‰ for outliers with «fixed» values for individual 604.

Burial #	Inventory ID	Age (years)	$\Delta_{F\text{-}H}{}^{13}C$‰	$\delta^{13}C$ Enriched Element	$\Delta_{F\text{-}H}{}^{15}N$‰	$\delta^{15}N$ Enriched Element	Skeletal Stress
1001	1003D	1.7	-1.16	Humerus	-2.31	Humerus	-
1090D	1006K	11.5	-1.00	Humerus	-2.20	Humerus	-
~~604~~	~~637B-1~~	~~5.5~~	~~-0.13~~	~~Humerus~~	~~0.27~~	~~Femur~~	~~-~~
~~604~~	~~637B-2~~	~~5.5~~	~~-0.01~~	~~Humerus~~	~~0.00~~	~~Humerus~~	~~-~~

For the remaining outliers, 1001 and 1090D, the humeri are enriched over the femora by approximately 1‰ in $\delta^{13}C$ and almost 2.5‰ in $\delta^{15}N$ (Table 19, Fig. 32). The difference between the bones is substantially greater then what could be added due to potential machine error (±0.14) and exhibits a very near trophic level enrichment.

For Infant 1001, a 1.7 year-old at death, these values could be indicative of the breastfeeding trophic level enrichment earlier in infancy and may provide evidence for a sudden shift to a weaning diet based on the notion that the femora are preferentially developed after

walking has commenced. It is likely that 1001 experienced the weanling's dilemma. The introduction of supplementary foods would have been necessary for nourishment and maintenance of life at this age but the process also likely led to the infant's introduction to bacteria in food or on serving containers causing diarrhea and vomiting. Febrile illnesses presenting with fevers, diarrhea, and vomiting would initially deplete the pools of nitrogen used to form the femur of the growing infant as more nutrients were being lost through sickness then are being used to form the bone.

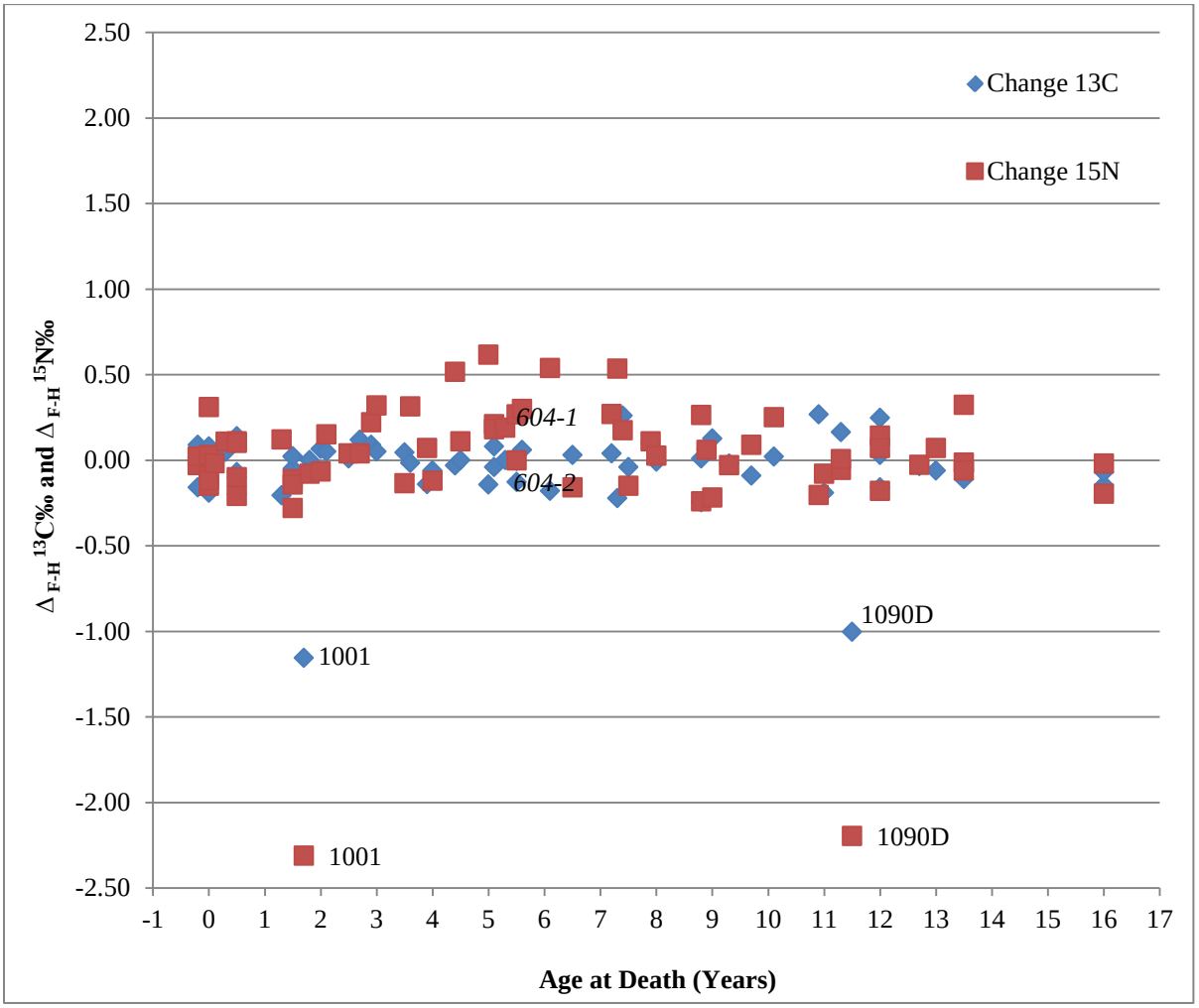


Figure 32. Average change in $\Delta_{F\text{-}H}$ ^{13}C‰ and $\Delta_{F\text{-}H}$ ^{15}N‰ for each individual.

Western Medieval weaning practices call for the use of animal milk and C_3 grains as easy to eat paps for infants. Intolerance by the infant to gluten in available barley and wheat would reduce the body's ability to absorb proteins. Renal failure caused by consumption of cow's milk would concentrate urea in the blood stream, enriching growing bones in nitrogen, while simultaneously hindering calcium absorption in the renal tubules, depleting the body of calcium. Without a positive calcium balance, parathyroid hormone would be released to signal the resorption of bone to release calcium stores with the goal of preserving the body's cellular functions (Marieb and Hoehn, 2010). A deficiency in vitamin D too might also lead to kidney failure, as it is essential to the proper absorption of calcium in the ileum of the small intestine and the renal tubule of the kidneys (Glimcher, 1998; Kini and Nandeesh, 2012; Strewler, 2003). These metabolic assaults result in intestinal malabsorption of what nutrients could even have been consumed by the ailing infant. The absence of skeletal stress markers and the fact that Infant 1001's femoral nitrogen and carbon are drastically depleted relative to its own humeral values suggests that the infant's skeleton was not drastically altered by nutrient deficiencies, that he or she still experienced these stressors, and that they had possibly rebounded from the metabolic offense.

At 11.5 years at death, Juvenile 1090D was past his or her 5-7 year-old childhood growth spurt and coming up on their pubertal growth spurt. The average cohort $\Delta_{F\text{-}H}{}^{13}C‰$ and $\Delta_{F\text{-}H}{}^{15}N‰$ isotopic values indicate that for individuals in this sample, those who died during the second juvenile cohort were most likely growing as femoral isotopic values begin to increase at the end of juvenility and into adolescence (Fig. 31). Goldman and colleagues (2009) note that in modern populations there is a rapid femoral growth spurt for females peri-puberty, around 11.8 years of age (13.8 years males) during which time resorption and deposition of bone are

equalized in the femur. However, Šereikienė and Jankauskas (2004) concluded that the pubertal growth spurt at Alytus was delayed until 15 years. It is possible that not all individuals at Alytus followed the pattern of the standard growth curve and those who died before their own growth was completed might not be bioarchaeologically visible to a model using long bone lengths.

If Juvenile 1090D were growing around their time of death, this would indicate that the femoral nitrogen isotopic values (11.06‰) should be indicative of most recent protein synthesized for bone growth. For a normal growing juvenile in puberty, we would expect this $\delta^{15}N$ value to be representative of positive balance to account for growth (Fuller et al., 2004; Reitsema, 2013). In contrast, humeral $\delta^{15}N$ values in Juvenile 1090 (13.26‰) are substantially enriched signifying a period of bone formation during metabolic negative nitrogen balance, which is most often associated with protein calorie malnutrition or starvation. If the femoral $\delta^{15}N$ values are more recently deposited, then the more enriched humeral $\delta^{15}N$ values might be indicative of a period of earlier stress as was seen in Infant 1001.

CHAPTER 8: CONCLUSIONS AND FUTURE CONSIDERATIONS

Dietary reconstruction of the Alytus individuals reveals that on average, exclusive breastfeeding continued until about two years of age when enriched $\delta^{13}C$ and $\delta^{15}N$ values begin to deplete. Utilizing isotopic data, it is concluded that C_3 grain gruels were supplemented into a breast milk protein diet for infants near the two-year mark. Infants and children who lived beyond this stage likely suffered weaning-associated infirmity that can be detected as faintly enriched humeral over femoral isotopic values. Slightly elevated $\delta^{13}C$ and $\delta^{15}N$ values remained evident in children until the beginning of juvenility indicating a prolonged period of breastfed weaning resulting in protein deficiencies for individuals living and dying within a period of substantial brain and skeletal growth. Infant and childhood feeding practices appear to be fairly consistent and gradual for the sample indicating that dietary characteristics remained relatively unchanged throughout the use of the cemetery. Non-breastfeeding subadult diets were also stable among all individuals where predominant consumption of terrestrial animal protein, possibly with riverine protein, was consumed. This pattern would be expected for rural peasants living on the River Nemunas with similar socioeconomic situations and access to food resources.

The differences between femoral to humeral $\Delta_{F\text{-}H}\,^{13}C$ and $\Delta_{F\text{-}H}\,^{15}N$ were not statistically significant lending credence comparative analyses among bioarchaeological studies utilizing isotopic values derived from various bones. However, comparisons must be performed cautiously under the assumption that bone turnover in growing subadults can acutely effect intra-individual isotopic values reflecting differing metabolic states for one individual. For subadults at Alytus, humeral $\delta^{13}C$ and $\delta^{15}N$ values were, on average, more enriched for the sample and

$\Delta_{F\text{-}H}{}^{13}C$ and $\Delta_{F\text{-}H}{}^{15}N$ differences between the bones changed with patterns of growth. Enrichments in average humeral nitrogen and carbon for cohorts coincided with estimated weaning age found in this study and Šereikienė and Jankauskas' (2004) observations of late infant and early juvenile growth spurts coincided with more enriched femoral $\delta^{13}C$ and $\delta^{15}N$ values.

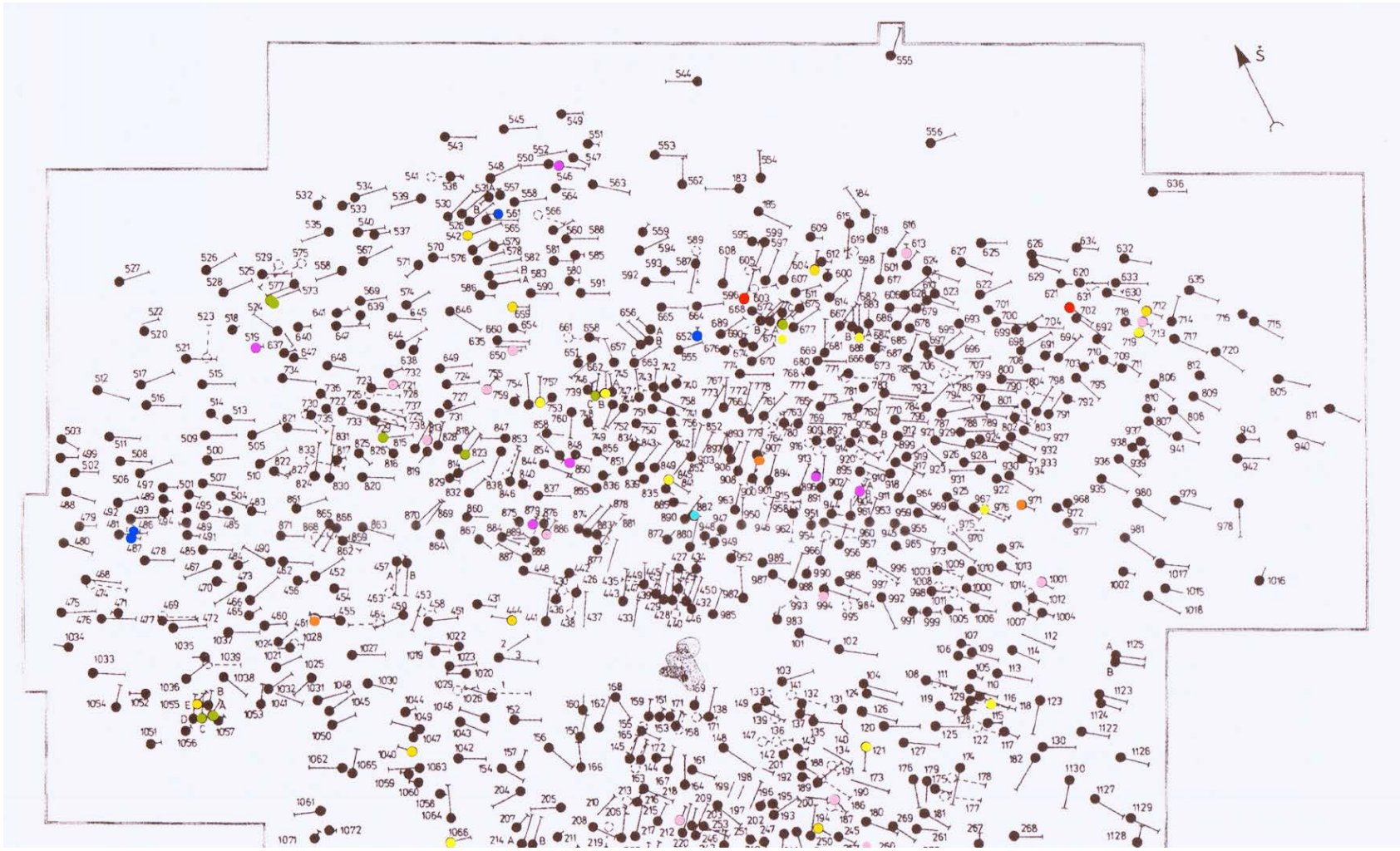
Future research considerations for the subadult sample at Alytus should attempt to refine ages of dietary transitions through considering intra-individual microscopic sampling. Selective macroscopic sampling comparing bulk metaphyseal trabeculae (more recently formed) to bulk midshaft cortical bone has not revealed substantial differences in diet or metabolic status (Waters-Rist and Katzenberg, 2010; Schurr, 1997). A more accurate estimation of the age of bone formation through increased understanding osteon formation on the periosteal and endosteal surface at different phases of growth would certainly provide for a detailed microscopic sampling of isotopic elements at exact ages (Maggiano, 2012; Dentine e.g., Burt and Garvie-Lok, 2013). Amino-acid specific mass spectrometry could be utilized to identify the specific composition of collagen strands with the goal of examining any contribution of aquatic and terrestrial carbon to collagen (Choy et al., 2010) as well as cycling changes of nitrogen in formation of the collagen fibrils (Styring, 2010).

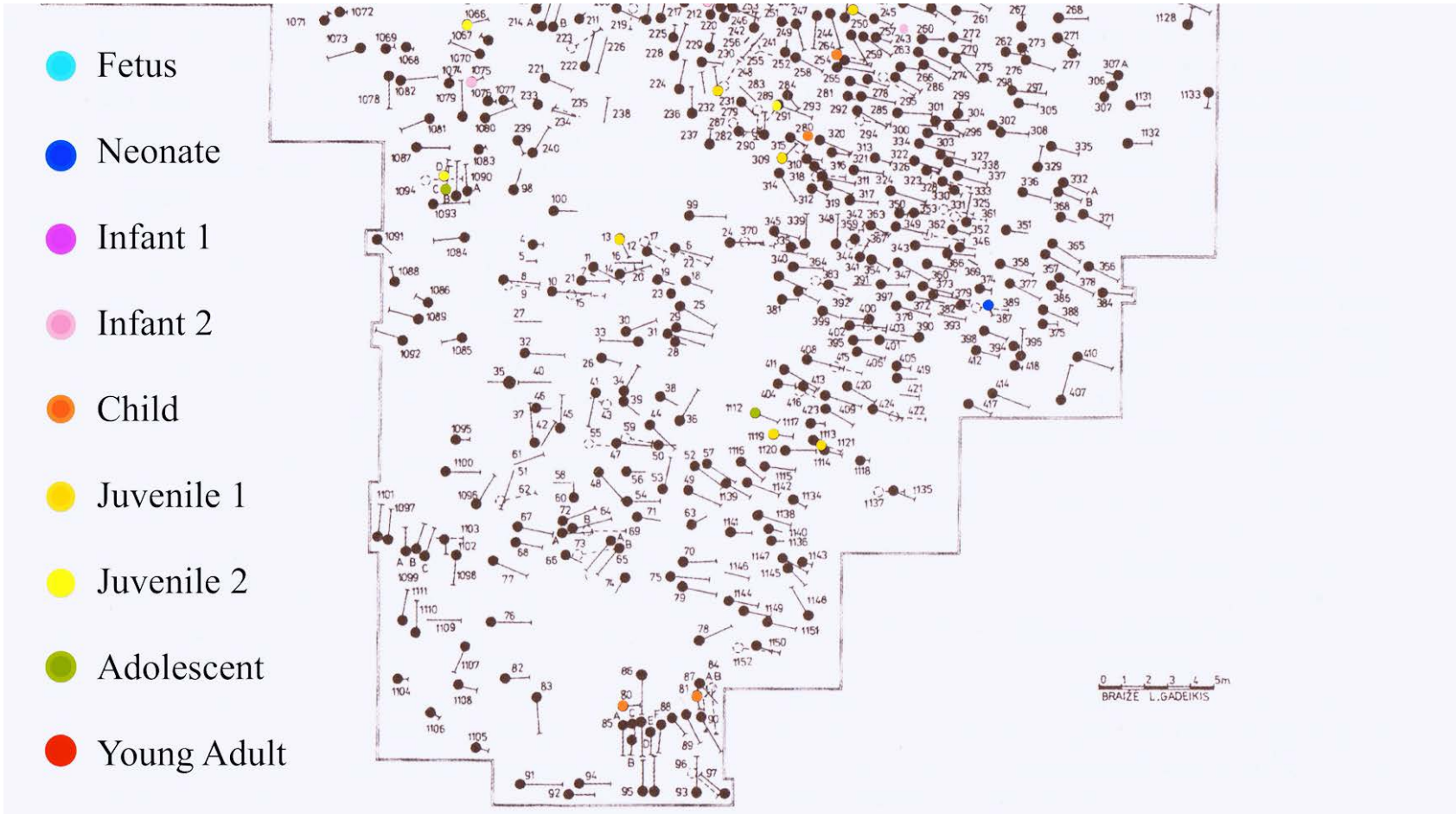
Nutrition, environment, mechanical stress, and genetic predisposition are intrinsic and extrinsic factors responsible for the formation and maintenance of bone during ontogeny (Eleazer, 2013; Hedges and Reynard, 2007; Katzenberg and Lovell, 1999; Mekota et al., 2006; Reitsema, 2013; Sillen et al., 1989). Therefore, future research on the Alytus subadults should compare detailed pathological analyses with nitrogen and carbon isotopic values. It would also be beneficial to compare stature derived from long bone lengths with the specific individuals in

the present study to understand if individuals experienced stunted growth, especially outlying individuals.

The formation of a food-web specific to Medieval Lithuania, including plant remains, would also provide a major contribution to our understanding of general diet among peasants and their children at Alytus. The current sample would also benefit from performing a complete mortuary analysis to consider if there are correlations between burial location, positioning within burial, grave goods, and isotopic values.

APPENDIX A: ALYTUS CEMETERY PLAN WITH SAMPLE INDIVIDUALS




Fetus
Neonate
Infant 1
Infant 2
Child
Juvenile 1
Juvenile 2
Adolescent
Young Adult
0 1 2 3 4 5m
BRAIŽE L.GADEIKIS

APPENDIX B: ISOTOPIC DATA

Burial ID	Inventory Number	Co.	Mean Age	Post cranial Stress	Cranial Stress	Humeral δ13C‰	Humeral δ15N‰	%C	%N	Atomic C:N	% Weight Collagen	Femoral δ13C‰	Femoral δ15N‰	%C	%N	Atomic C:N	% Weight Collagen	Δ13C	Δ 15N
13	314A	J1	7.40	0	0	-20.47	8.57	36.24	12.97	3.26	2.95	-20.21	8.74	43.23	15.75	3.20	20.63	0.26	0.17
80	348A	C	3.50	0	0	-19.89	11.10	44.89	16.27	3.22	25.81	-19.85	10.96	35.41	12.68	3.26	5.40	0.04	-0.14
81 A	348B	C	4.00	0	1	-20.20	11.02	31.97	11.53	3.23	24.59	-20.26	10.90	42.97	15.62	3.21	23.33	-0.06	-0.12
118	350C	J2	8.80	0	0	-20.50	10.24	44.76	16.03	3.26	10.52	-20.49	10.00	43.89	15.71	3.26	16.83	0.01	-0.24
121	350D	J2	9.30	0		-20.06	10.33	43.70	15.68	3.26	20.33	-20.08	10.30	44.25	16.18	3.19	19.32	-0.02	-0.03
186	634R	I2	1.30	0	0	-19.72	12.43	44.55	16.10	3.23	25.06	-19.92	12.55	44.03	16.00	3.21	28.31	-0.20	0.12
194	553C	J1	7.90	1	0	-20.20	10.23	41.20	14.74	3.27	8.10	-20.10	10.34	44.14	15.86	3.24	9.28	0.10	0.11
212	634C	I2	2.10	1	0	-19.56	13.25	44.70	16.17	3.22	23.44	-19.51	13.40	43.47	15.79	3.21	24.84	0.05	0.15
231	553B	J1	7.50	0	0	-20.11	9.80	38.56	13.78	3.27	10.75	-20.15	9.65	38.79	13.72	3.30	7.86	-0.04	-0.15
243	483H	I2	2.70	0	1	-19.47	12.32	44.99	16.27	3.23	21.86	-19.35	12.36	41.26	14.85	3.24	8.84	0.12	0.04
254	492C	C	4.50	0	0	-19.83	11.66	42.29	15.41	3.20	16.52	-19.83	11.77	43.02	15.55	3.23	21.71	0.00	0.11
280	490B	C	3.90	0	0	-19.88	11.70	43.03	15.59	3.22	23.22	-20.02	11.77	43.11	15.44	3.26	6.35	-0.14	0.07
291	465A	J2	10.10	1	0	-20.05	9.68	41.15	14.66	3.28	7.32	-20.03	9.93	41.10	14.85	3.23	6.09	0.02	0.25
309 A	478D	J1	5.10	0	0	-20.89	10.00	41.79	15.06	3.23	7.37	-20.81	10.21	41.10	15.02	3.20	3.42	0.08	0.21
387	463E	N	0.00	0	0	-20.14	12.56	44.62	16.01	3.26	19.02	-20.09	12.41	45.63	16.34	3.26	17.65	0.05	-0.15
444	470B	J1	7.20	1	1	-19.92	10.26	38.70	13.80	3.27	7.41	-19.88	10.53	43.95	15.90	3.22	4.78	0.04	0.27
461	464G	C	3.00	1	1	-20.10	10.73	43.65	15.73	3.24	23.18	-20.05	11.05	43.34	15.88	3.19	21.02	0.05	0.32
482	469B	N	0.00	0	1	-20.19	11.99	42.52	15.36	3.23	22.40	-20.38	11.90	43.69	15.74	3.24	22.22	-0.19	-0.09
487 A	466B	N	0.00	1		-19.62	10.84	44.46	16.14	3.21	21.64	-19.71	10.72	45.93	16.49	3.26	20.58	-0.09	-0.12
519	478E	I1	0.50	0	1	-20.38	11.58	44.36	15.94	3.24	19.16	-20.24	11.69	43.57	15.71	3.23	22.84	0.14	0.11
542 B	478F	J1	5.30	0	0	-20.17	10.61	43.23	15.58	3.23	22.63	-20.17	10.80	43.52	15.83	3.21	24.57	0.00	0.19
546	465C	I1	0.50	0	1	-19.31	13.18	39.89	14.28	3.26	20.35	-19.38	13.28	45.77	16.67	3.20	22.73	-0.07	0.10
561	483B	N	0.00	0	1	-19.30	12.29	45.15	16.33	3.23	21.72	-19.39	12.32	44.40	16.03	3.23	22.12	-0.09	0.03
572	674B	J2	10.90	0	1	-20.66	10.56	38.21	13.55	3.29	20.60	-20.39	10.36	38.49	14.02	3.21	5.55	0.27	-0.20
573	676A	A	15.00	1	0			17.27	5.46	3.69	20.67	-20.12	10.57	42.07	15.30	3.21	3.54		
604	637B-1	J1	5.50	0	0	-20.55	10.80	39.06	14.34	3.18	25.35	-19.90	10.44	44.80	16.32	3.20	20.72	0.65	-0.37
604	637B-2	J1	5.50	0	0	-19.89	10.17	43.55	15.93	3.19	23.14	-20.68	10.80	41.28	14.81	3.25	25.69	-0.78	0.63
613	478C	I2	1.80	0	1	-19.28	12.75	44.53	16.17	3.21	23.72	-19.28	12.67	45.45	16.48	3.22	23.81	0.00	-0.08
650	465B	I2	1.50	1	1	-18.80	14.28	43.95	15.68	3.27	24.47	-18.85	14.17	41.90	14.96	3.27	23.85	-0.05	-0.11
652	465E	N	0.00	1	1	-20.15	11.74	44.40	15.79	3.28	19.70	-20.07	12.05	43.16	15.63	3.22	19.87	0.08	0.31
659	474B	J1	5.60	1	0	-20.74	10.12	37.98	13.63	3.26	5.91	-20.68	10.42	36.88	13.22	3.26	6.70	0.06	0.30
671	736B	J2	9.00	1	1	-19.89	11.11	43.60	15.50	3.28	22.40	-19.76	10.89	44.51	16.30	3.19	13.50	0.13	-0.22
672 A	824A	A	12.00	0	0	-20.44	10.50	46.20	16.53	3.26	20.41	-20.19	10.64	35.35	12.96	3.19	4.46	0.25	0.14

Burial ID	Inventory Number	Co.	Mean Age	Post cranial Stress	Cranial Stress	Humeral δ13C‰	Humeral δ15N‰	%C	%N	Atomic C:N	% Weight Collagen	Femoral δ13C‰	Femoral δ15N‰	%C	%N	Atomic C:N	% Weight Collagen	Δ13C	Δ 15N
682B	980B	J2	9.70	0	0	-20.19	9.88	43.35	15.98	3.16	15.28	-20.28	9.97	42.60	15.51	3.21	9.51	-0.09	0.09
688	885	YA	16.00	0	0	-20.19	10.52	37.56	13.42	3.27	1.41	-20.34	10.32	45.72	16.36	3.26	19.21	-0.15	-0.20
702	944A	YA	16.00	1		-20.44	9.93	37.66	13.71	3.21	5.92	-20.51	9.91	44.36	16.20	3.20	6.36	-0.07	-0.02
712	991C	J1	6.10	0	0	-20.43	9.93	43.45	15.95	3.17	23.46	-20.61	10.47	46.03	16.43	3.27	22.47	-0.18	0.54
713	1002C	I2	1.50	0		-20.02	12.88	41.52	14.72	3.29	25.22	-19.99	12.74	45.11	16.14	3.26	25.08	0.02	-0.14
719	1005A	J2	8.90	1	0	-20.44	10.21	44.60	16.02	3.25	22.74	-20.37	10.27	44.26	15.89	3.25	19.90	0.06	0.06
721	999B	I2	1.50	1	0	-19.46	12.11	42.12	15.43	3.19	24.94	-19.44	11.83	43.07	15.77	3.19	25.45	0.02	-0.28
729	969A	A	13.50	1	0	-20.03	11.11	46.06	16.40	3.28	20.90	-20.10	11.10	45.86	16.36	3.27	21.29	-0.06	-0.01
745 B	979A	J2	11.00	0	0	-19.96	10.53	42.59	15.52	3.20	8.70	-20.15	10.45	43.61	16.01	3.17	11.78	-0.19	-0.08
745 C	983B	A	12.00	0	0	-20.02	10.66	42.07	15.27	3.21	9.47	-19.99	10.73	39.61	14.64	3.15	9.81	0.03	0.07
755	1004D	I2	2.00	0	1	-19.50	13.30	41.43	14.75	3.28	25.64	-19.43	13.24	43.38	15.70	3.22	26.97	0.07	-0.07
757	1005D	J2	10.00	1	0							-20.25	11.95	45.91	16.49	3.25	21.28		
813	994H	I2	2.50	1	1	-20.17	12.52	32.38	11.83	3.20	35.29	-20.16	12.56	43.86	16.15	3.16	26.48	0.01	0.04
823	730B	A	13.00	0	1	-19.83	9.81	42.43	15.32	3.23	7.08	-19.89	9.88	42.30	15.31	3.22	5.26	-0.06	0.07
841	1000H	J2	8.80	0	1	-20.02	9.21	42.59	15.38	3.23	18.23	-20.26	9.47	45.72	16.46	3.24	16.29	-0.25	0.26
850	1000E	I1	0.50	0	0	-20.05	11.40	43.13	15.74	3.20	24.14	-20.25	11.30	43.96	16.07	3.20	24.96	-0.20	-0.10
879	1000B	I1	0.50	1	0	-19.07	12.09	44.05	16.15	3.19	23.89	-19.17	11.88	44.29	16.22	3.19	25.31	-0.10	-0.21
882	997F-Prox	F	-0.20	0	0	-20.18	11.81	43.44	15.73	3.22	23.08	-20.09	11.78	43.30	15.67	3.22	21.17	0.09	-0.03
882	997F-Distal	F	-0.20	0	0	-19.99	11.86	42.35	15.37	3.22	22.26	-20.15	11.88	43.40	15.70	3.22	21.14	-0.16	0.02
886	1000F	I2	2.50	0	0							-19.89	11.36	43.54	15.84	3.21	9.98		
896	1006H	I1	0.10	0	1	-19.70	12.03	41.71	14.84	3.28	11.19	-19.68	12.01	43.75	15.82	3.23	24.90	0.02	-0.02
904 B	1008G	I1	0.30	0	0	-19.68	11.46	43.26	15.46	3.26	21.02	-19.63	11.57	43.84	15.68	3.26	22.69	0.05	0.11
907	1008E	C	3.60	0	0	-20.03	10.97	46.03	16.71	3.21	22.38	-20.05	11.28	45.01	16.24	3.23	21.61	-0.02	0.31
971	1005C	C	4.40	1	0	-20.25	10.76	45.00	16.26	3.23	18.47	-20.28	11.27	43.98	15.94	3.22	20.53	-0.03	0.52
976	753B	J2	11.30	0	0	-20.12	9.38	44.39	16.05	3.23	19.30	-20.11	9.32	45.79	16.47	3.24	19.75	0.00	-0.06
994	1007D	I2	2.80	0	1							-19.65	13.40	41.68	14.80	3.28	6.60		
1001	1003D	I2	1.70	0	0	-19.35	13.37	43.38	15.58	3.25	26.71	-20.50	11.06	42.93	15.37	3.26	8.85	-1.16	-2.31
1040	1011A	J1	5.00	1	0	-20.00	11.36	36.89	13.28	3.24	8.17	-20.14	11.98	44.30	15.98	3.23	22.07	-0.14	0.62
1055 A	745A	A	12.00	1	0	-20.04	10.10	42.22	15.24	3.23	6.70	-20.20	9.92	44.22	16.23	3.17	13.97	-0.16	-0.18
1055 C	790A	A	12.70	1	1	-20.13	10.43	45.50	16.39	3.24	16.74	-20.16	10.40	45.05	16.27	3.23	16.47	-0.03	-0.03
1055 E	746B	J1	7.30	0	1	-19.77	10.95	43.13	15.66	3.21	11.66	-19.99	11.48	44.73	16.06	3.25	22.49	-0.22	0.53
1066	742B	J2	11.30	1	1	-21.03	9.57	44.45	15.97	3.25	23.43	-20.87	9.57	45.20	16.25	3.24	18.95	0.16	0.01
1075	1007B	I2	2.90	1	0	-19.77	11.70	41.06	14.47	3.31	7.19	-19.68	11.93	45.61	16.36	3.25	22.24	0.09	0.22
1090 C	976A	A	13.50	1	0	-19.69	10.91	44.01	16.03	3.21	7.14	-19.80	10.85	45.96	16.78	3.20	7.72	-0.11	-0.06
1090 D	1006K	J2	11.50	0	0	-19.46	13.26	42.70	15.23	3.27	6.30	-20.46	11.06	42.79	15.32	3.26	7.71	-1.00	-2.20

Burial ID	Inventory Number	Co.	Mean Age	Post cranial Stress	Cranial Stress	Humeral δ13C‰	Humeral δ15N‰	%C	%N	Atomic C:N	% Weight Collagen	Femoral δ13C‰	Femoral δ15N‰	%C	%N	Atomic C:N	% Weight Collagen	Δ13C	Δ 15N
1112	956A	A	13.50	0	0	-20.35	9.30	45.90	16.40	3.26	19.80	-20.41	9.62	44.91	16.03	3.27	6.28	-0.06	0.32
1119	1000G	J1	5.10	0	0	-20.53	9.81	42.92	15.80	3.17	23.51	-20.57	9.99	45.10	16.44	3.20	5.94	-0.04	0.18
1121	1008H	J1	8.00	1	1	-20.86	9.08	43.87	15.82	3.24	23.14	-20.87	9.11	44.90	16.21	3.23	23.14	-0.01	0.03
pl59k4	486B	J1	6.50	0	1	-20.04	10.82	39.84	14.31	3.24	12.46	-20.01	10.66	44.45	16.01	3.24	7.56	0.03	-0.16

REFERENCES

Adamson MW. 2004. Food in Medieval Times. Westport, Connecticut: Greenwood Press.

Ambrose SH. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. J Archaeol Sci 17:431-451.

Ambrose SH, DeNiro MJ. 1986. The isotopic ecology of East African mammals. Oecologia 69:395-406.

Ambrose SH, Krigbaum J. 2003. Bone chemistry and bioarchaeology. J Anthropol Archaeol 22:193-199.

Antanaitis-Jacobs I, Girinikas A. 2002. Periodization and chronology of Neolithic Lithuania. Archaeologia Baltica 5:9-39.

Antanaitis-Jacobs I, Richards MP, Daugnora L, Jankauskas R, Ogrinc N. 2012. Diet in early Lithuanian prehistory and the new stable isotopic evidence. Archaeolgica Baltica 12-30.

Aufderheide AC, Rodriguez-Martin C. 1998. The Cambridge Encyclopedia of Human Paleopathology. Cambridge: Cambridge University Press.

Baker BJ, Dupras TL, Tocheri MW, Wheeler SM. 2005. The Osteology of Infants and Children. Texas: Texas A & M University anthropology series.

Baker SL, Butterwoth EC, Langley FA. 1946. The calcium and nitrogen content of human bone tissue cleaned by micro-dissection. Biochem 40:391-396.

Brickley and Ives. 2008. The Bioarchaeology of Metabolic Bone Disease. Amsterdam: Elsevier.

Bogin B. 1997. Evolutionary hypotheses for human childhood. Yearb Phys Anthropol 40:63-89.

Bourbou C, Fuller BT, Garvie-Lok SJ, Richards MP. 2013. Nursing mothers and feeding bottles: Reconstructing breastfeeding and weaning patterns in Greek Byzantine populations (6^{th}-15^{th} centuries AD) using carbon and nitrogen stable isotope ratios. J Archaeol Sci 40:3903-3913.

Burt NM. 2013. Stable isotope ratio analysis of breastfeeding and weaning practices of children from Medieval Fishergate House York, UK. Am J Phys Anthropol 152:407-416.

Burt NM Garvie-Lok S. 2013. A new method of dentine micro-sampling of deciduous teeth for stable isotope ratio analysis. J Archaeol Sci 40:3854-3864.

Brown, T.A., Nelson, D.E., Southon, J.R., 1988. Improved collagen extraction by modified Longin method. Radiocarbon 30, 171-177

Choy K, Smith CI, Fuller BT, Richards MP. 2010. Investigation of amino acid δ13C signatures in bone collagen to reconstruct human palaeodiets using liquid chromatography–isotope ratio mass spectrometry. Geochim Cosmochim Acta 74:6093-6111.

DeNiro MJ. 1985. Postmortem preservation and the alteration of in vivo bone collagen isotope ratios in relation to paleodietary reconstruction. Nature 317:806-809.

DeNiro MJ, Epstein D. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495-506.

DeNiro MJ, Epstein D. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341-351.

Dettwyler KA Fishman C. 1992. Infant feeding practices and growth. Annu Rev Anthropol 21:171-204.

Dupras TL, Schwarcz HP, Fairgrieve SI. 2001. Infant feeding and weaning patterns in Roman Egypt. Am J Phys Anthropol 115:204-212.

Eleazer CD. 2013. The interaction of mechanical loading and metabolic stress on human cortical bone: Testing anthropological assumptions using cross-sectional geometry and histomorphology. PhD Dissertation, Department of Anthropology, University of Tennessee, Knoxville.

Fogel ML, Tuross N, Owsley D. 1989. Nitrogen isotope tracers of human lactation in modern and archaeological populations. Carnegie Inst Yr Bk 88:111–117.

Fuller BT, Fuller JL, Harris DA, Hedges REM. 2006a. Detection of breastfeeding and weaning in modern human infants with carbon and nitrogen stable isotopes. Am J Phys Anthropol 129:279-293.

Fuller BT, Molleson TI, Harris DA, Gilmour LT, Hedges REM. 2006b. Isotopic evidence for breastfeeding and possible adult dietary difference from Late/Sub-Roman Britain. Am J Phys Anthropol 129:45-54.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2004. Nitrogen Balance and $\delta^{15}N$: Why you're not what you eat during pregnancy. Rapid Commun Mass Spectrum 18:2889-2896.

Fuller BT, Richards MP, Mays SA. 2003. Stable carbon and nitrogen isotope variation in tooth dentine serial sections from Wharram Percy. J Archaeol Sci 30:1673-1684.

Fujita M, Roth E, Yun-Jia L, Hurst C, Vollner J, Kendell A. In poor families, mother's milk is richer for daughters than sons: A test of Trivers-Willard Hypothesis in agropastoral settlements in Northern Kenya. Am J Phys Anthropol 149:52-59.

Glimcher MJ. 1998. The nature of the mineral phase in bone: Biological and clinical implications. In: Avioli LV, Krane SM, eds. Metabolic Bone Diseases and Clinically Related Disorders. 3rd ed. Amsterdam: Elsevier. p 1-22

Goldman HM, McFarlin SC, Cooper DML, Thomas CDL, Clement JG. 2009. Ontogenetic patterning of cortical bone microstructure and geometry at the human mid-shaft femur. Anat Rec 292:48-64.

Halcrow SE, Tayles N. 2008. The bioarchaeological investigation of childhood and social age: Problems and perspectives. J Archaeol Method Th 15:190-215.

Heaton THE, Vogel JC, von la Chevallerie G, Collett G. 1986. Climatic influence on the isotopic composition of bone nitrogen. Nature 322:822-823.

Hedges REM, Clement JG, Thomas DL, O'Connell TC. 2007. Collagen turnover in the adult femoral mid-shaft: Modeled from anthropogenic radiocarbon tracer measurements. Am J Phys Anthropol 133:808-816.

Hedges REM, Reynard LM. 2007. Nitrogen isotopes and the trophic level of humans in archaeology. J Archaeol Sic 34:1240-1251.

Herring DA, Saunders SR, Katzenberg MA. 1998. Investigating the weaning process in past populations. Am J Phys Anthropol 105:425–439.

Hoefs J. 1997. Stable Isotope Geochemistry. Berlin: Springer-Verlag.

Holloway KL, Henneber RJ, de Barros Lopes M, Henneberg M. 2011. Evolution of human tuberculosis: A systematic review and meta-analysis of paleopathological evidence. Homo 62:402-458.

Jankauskas R. 1998. History of human tuberculosis in Lithuania: Possibilities and limitations of paleoosteological evidence. B Mem Soc Anthro Par 10: 357-374.

Jankauskas R, Schultz M. 1995. Meningeal reactions in a Late Medieval- Early Modern child population from Alytus, Lithuania. J Paleopath 7:106.

Jankauskas R, Schultz M. 2009. Infant diseases in Eastern Europe during the Late Middle Ages and Early Modern times. Am J Phys Anthropol 138: 160-161.

Jankauskas R, Urbanavicus A. 2008. Possible indication of metabolic syndrome in Lithuanian paleoosteological materials. Papers on Anthropol 17:103-112.

Jankauskas R, Gerhards G. 2012. History of paleopathology in Lithuania, Lativa, and Estonia. In: The Global History of Paleopathology: Pioneers and Prospects. Buikstra JE, Roberts C, eds. Oxford: Oxford University Press. p 469-475.

Jay M, Fuller B, Richards M, Knüsel C, King S. 2008. Iron Age breastfeeding practices in Britain: isotopic evidence from Wetwang Slack, East Yorkshire. Am J Phys Anthropol 136:327–337.

Katzenberg MA. 2008. Stable isotope analysis: A tool for studying past diet, demography, and life history. In: Katzenberg MA, Saunders SR, editors. Biological Anthropology of the Human Skeleton. 2nd ed. New York: Wiley-Liss. p 413-441.

Katzenberg MA, Herring DA, Saunders SR. 1996. Weaning and infant mortality: Evaluating the skeletal evidence. Yearb Phys Anthropol 39:177-179.

Katzenberg MA, Lovell NC. 1999. Stable isotope variation in pathological bone. Int J Osteoarchaeol 9:316-324.

Katzenberg MA, Schwarcz HP, Knyf M, Melbye FJ. 1995. Stable isotope evidence for maize horticulture and paleodiet in southern Ontario, Canada. Am Antiquity 60:335-350.

Kini U, Nandeesh BN. 2012. Physiology of bone formation, remodeling, and metabolism. In: Fogleman et al., eds. Radionuclide and Hybrid Bone Imaging. Heidleberg, Springer-Verlag. p 29-57.

Kisieliene D. 2012. Klaipeda town, Western Lithuania, in XVI-XVII centuries AD: Paleobotanical, osteological, and archaeological data. Quatern Int 279-280: 233-345.

Knight K. 2002. A precious medicine: Tradition and magic in some seventeenth-century household remedies. Folklore 113:237-259.

Koerner W, Dambrine E, Dupouey JL, Benoit M. 1999. $\delta^{15}N$ of forest soil and understory vegetation reflect the former agricultural land use. Osteologia 121:421-425.

Kozakite J. 2011 The analysis of long bone fractures and dislocations in 14th- 17th century Alytus, Lithuania. MSc Thesis, Department of Archaeology, Durham University, England.

Kreuger HW, Sullivan CH. 1984. Model for carbon isotope fractionation between diet and bone. ACS symposium series: Stable isotopes in nutrition 258:205-220.

Lee-Thorp JA. 2008. On isotopes and old bones. Archaeometry 50:925-950.

Lewis ME. 2007a. Endocranial lesions in non-adult skeletons: Understanding their aetiology. Int J Osteoarchaeol 14:82-97.

Lewis ME. 2007b. The Bioarchaeology of Children. Cambridge: Cambridge University Press.

Longin R. 1971. New method for collagen extraction for radiocarbon dating. Nature 230:241-242.

Maggiano CM. 2012. Making the mold: A microstructural perspective of bone modeling during growth and mechanical adaptation. In: Crowder C, Stout S, eds. Bone Histology: An Anthropological Perspective. Boca Raton: CRC Press. p 45-90.

Marieb EN, Hoehn A. 2010. Human Anatomy and Physiology. 8th ed. San Francisco, Benjamin Cummings.

Mays SA, Richards MP, Fuller BT. 2002. Bone stable isotope evidence for infant feeding in Mediaevel England. Antiquity 76:654-656.

Mekota AM, Grupe G, Ufer S, Cuntz U. 2006. Serial analysis of stable nitrogen and carbon isotopes in hair: Monitoring starvation and recovery phases of patients suffering from anorexia nervosa. Rapid Commun Mass Sp 20:1604-1610.

Monter W. 1983. Ritual, Myth, and Magic in Early Modern Europe. Athens, OH: Ohio University Press.

Myllyharju J. 2004. Molecular biology and biosynthesis of collagens. In: Massaro EJ and Rogers JM, eds. The Skeleton: Biomechanical, Genetic, and Molecular Interactions in Development and Homeostasis. Totowa, NJ: Humana Press Inc.

Nelson GJ. 1969. Isolation and purification of lipid from animal tissues. In: Perkins EG, editor. Analysis of Lipids and Lipoprotein. Illinois: American Oil Chemical Society. p 1-22

Orme N. 2003. Medieval Children. New Haven, CT: Yale University Press.

Ortner DJ. 2003. Identification of Pathological Conditions in Human Skeletal Remains. 2nd ed. Amsterdam: Elsevier.

Orwoll ES. 1992. The effects of dietary protein insufficiency and excess on skeletal health. Bone 13:343-350.

Pearson JA, Hedges REM, Molleson TI, Özbek M. 2010. Exploring the relationship between weaning and infant mortality: An isotope case study from Aşikli Höyük and Çayönü Tepesi. Am J Phys Anthropol 143:448-457.

Perry MA. 2006. Redefining childhood through bioarchaeology: Toward an archaeological and biological understanding of children in antiquity. Archaeological Papers of the American Anthropological Association 15:89-111.

Pollard AM, Batt CM, Stern B, Young SMM. 2007. Analytical Chemistry in Archaeology. Cambridge: Cambridge University Press.

Reitsema LJ. 2013. Beyond diet reconstruction: Stable isotope applications to human physiology, health, and nutrition. Am J Hum Biol 25:445-456.

Reitsema LJ, Kozlowski T, Makowiecki D. 2013. Human-environment interaction in Medieval Poland: A perspective from the analysis of faunal stable isotope ratios. J Archaeol Sci 40:3636-3646.

Richards MP, Mays S, Fuller B. 2002. Stable carbon and nitrogen isotope values of bone and teeth reflect weaning age at medieval Wharram Percy site, Yorkshire, UK. Am J Phys Anthropol 119:205-210.

Roberts C, Manchester K. 2007. The Archaeology of Disease. 3rd ed. New York: Cornell University Press.

Rowell SC. 1994 Lithuania Ascending: A Pagan Empire within East-Central Europe, 1295-1345. Cambridge: Cambridge University Press.

Scheuer L, Black S. 2000. Developmental Juvenile Osteology. Amsterdam: Elsevier Academic Press.

Scheuer L, Black S. 2004. The Juvenile Skeleton. Amsterdam: Elsevier Academic Press.

Schoeninger MJ, DeNiro MJ. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim Cosmochim Ac 48:625-369.

Schoeninger MJ, DeNiro MJ, Tauber H. 1983. Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. Science 220:1381-1383.

Schurr MR. 1997. Stable nitrogen isotopes as evidence for the age of weaning at the Angel site: a comparison of isotopic and demographic measures of weaning age. J Archaeol Sci 24:919-927.

Schwarcz HP, Schoeninger MJ. 1991. Stable isotope analyses in human nutritional ecology. Yrbk Phys Anthropol 34:283-311.

Šeškauskaite D, Gliwa B. 2006. Some Lithuanian ethnobotanical taxa: A linguistic view on the Thorn Apple and related plants. J Ethnobio Ethnomed 2:13: 1-4.

Šereikienė I, Jankauskas R. 2004. Lithuanian children's growth patterns in the past- An updated Medieval sample. Pap Anthropol 13:226-238.

Sillen A, Sealy JC, van der Merwe NJ. 1989. Chemistry and paleodietary research: No more easy answers. Am Antiquity 54:504-512.

Smith BN, Epstein S. 1971. Two categories of $^{13}C/^{14}C$ ratios for higher plants. Plant Physiol 47:380-384.

Stancikaite M, Kisieliene D, Mazeika J, Blazevicus P. 2008. Environmental conditions and human interference during the 6^{th} and 13^{th}-15^{th} centuries A.D. at Vilnius, Lower Castle, East Lithuania. Veget Hist Archaeobot 17:S239-S250.

Strewler GJ. 2003. Parathyroid hormone and calcium homeostasis. In: Glorieux FH, Pettifor JM, Jüppner H, eds. Pediatric Bone: Biology and Diseases. Amsterdam: Elsevier. p 135-172.

Styring AK, Sealy JC, Evershed RP. 2010. Resolving the bulk ^{15}N values of ancient human and animal bone collagen via compound-specific nitrogen isotope analysis of constituent amino acids. Geochim Cosmochim Acta 74:241-251.

Tauber H. 1981. ^{13}C evidence for dietary habits of prehistoric man in Denmark. Nature 292:332-333.

Turner BL, Edwards JL, Quinn EA, Kingston JD, van Gerven DP. 2007. Age-related variation in isotopic indicators of diet at medieval Kulubnarti, Sudanese Nubia. Int J Osteoarchaeol 17:1-25.

Ulijaszek SJ. 1990. Nutritional status and susceptibility to infectious disease. In: Harrison GA and Waterlow JC, editors. Diet and Disease in Traditional and Developing Societies. Cambridge: Cambridge University Press p137-154.

Ulizzi L, Zonta L. 2002. Sex differential patterns in perinatal deaths in Italy. Hum Biol 74:879-888.

Waters-Rist AL, Bazaliiskii VI, Weber AW, Katzenberg MA. 2011. Infant and child diet in Neolithic Hunter-Fisher-Gatherers from Cis-Baikal, Siberia: Intra-long bone stable nitrogen and carbon isotope ratios. Am J Phys Anthropol 146:225-241.

Waters-Rist AL, Katzenberg MA. 2010. The effect of growth on stable nitrogen isotope ratios in subadult bone collagen. Int J Osteoarchaeol 20:172-191.

Wood JW, Milner GR, Harpending HC, Weiss KM. 1992. The osteological paradox. Problems of inferring prehistoric health from skeletal samples. Curr Anthropol 33: 343-370.

World Health Organization. 1991. Indicators for assessing breastfeeding practices. Rep Informal Meet June 11-12, Geneva.

Wheeler SM. 2012. Nutritional and disease stress of juveniles from the Dakhleh Oasis, Egypt. Int J Osteoarchaeol 22:219-234.

Wright LE, Schwarcz HP. 1998. Stable carbon and oxygen isotopes in human tooth enamel: Identifying breastfeeding and weaning in prehistory. Am J Phys Anthropol 106:1-18.

Wright LE, Yoder CJ. 2003. Recent progress in bioarchaeology: Approaches to the osteological paradox. J Archaeol Res 11: 43-70.

van der Merwe, NJ. 1982. Carbon isotopes, photosynthesis, and archaeology. Am Sci 70:596-606.

van Klinken GJ. 1999. Bone collagen quality indicators for paleodietary and radiocarbon measurements. J Archaeol Sci 26:687-695.

van Klinken GJ, Richards MP, Hedges REM. 2000. An overview of causes for stable isotope variation in past European human populations: Environmental, ecophysical, and cultural effects. In: Ambrose SH, Katzenberg MA, eds. Biochemical Approaches to Paleodietary Analysis. New York: Kluwer Academic/ Plenum Publishers p 39-63.

Vogel JC, van der Merwe, NJ. 1977. Isotopic evidence for early maize cultivation in New York State. Am Antiq 42:238-242.