THE CRYPT PEOPLE FROM THE CATHEDRAL BASILICA OF SAINTS STANISLAUS AND VLADISLAUS, VILNIUS, LITHUANIA: RECONSTRUCTION OF LIFE HISTORIES USING STABLE ISOTOPE ANALYSIS

by

KAYLEE LAVONNE SCHOTTEN
B.A. University of Central Florida, 2011

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Arts
in the Department of Anthropology
in the College of Sciences
at the University of Central Florida
Orlando, Florida

Summer Term
2015

ABSTRACT

Stable isotope analyses of multiple tissue types have been used to reconstruct life histories of individuals from past populations. This thesis presents the life history reconstructions of a unique population recovered from a 16th to 18th century cathedral crypt located in Vilnius, Lithuania. The sample consists of 23 individuals (21 males, one possible female, and one juvenile). Stable carbon, nitrogen, and oxygen isotope analyses were performed on bone and dentin collagen, and on bone and enamel hydroxyapatite, resulting in eight isotope values per individual, providing both childhood and adult dietary and migration information.

For bone collagen the average $\delta^{13}C$ isotope ratio is -19.9‰ +/- 0.4 and for $\delta^{15}N$ is 11.8‰ +/- 0.9. The average $\delta^{13}C$ isotope ratio for collagen extracted from dentin is -19.5‰ +/- 0.4 and for $\delta^{15}N$ is 11.4‰ +/- 0.9. The bone apatite average $\delta^{13}C$ ratio is -14.2‰ +/- 0.9, and the average $\delta^{13}C$ enamel ratio enamel is -13.5‰ +/- 1.5. These values indicate a diet in both early childhood and adulthood that was heavily reliant on C_3 plants; a result supported by previous isotopic studies from Lithuania and surrounding countries. The average $\delta^{18}O_{VSMOW}$ bone apatite ratio is 26.1‰ +/- 1.0 and the average enamel $\delta^{18}O_{VSMOW}$ ratio is 24.9‰ +/- 1.5. The $\delta^{18}O$ isotope values suggest that the majority of these individuals were born elsewhere and migrated to the city of Vilnius sometime after their childhood years. Stable isotope analyses, in combination with macroscopic examination, aids in the understanding of those who were buried in this unique location.

To my sweetheart, Steven Biegler. I cannot wait to call you “husband”.

ACKNOWLEDGMENTS

Where to start? I want to thank all the professors who opened my mind to anthropology during my undergraduate career and then guided me throughout my graduate career. You all made my time at UCF one of fascinating discovery and intellectual growth. Thank you, Dr. Williams, for the practical know-how in the isotope lab and for being an advocate in my desire to continue my education. Descriptions of wet gummy bears, rubber floor mats, and soggy bread will serve me well in future research. Thank you, Dr. Schultz, for always being available to your students (I regret not taking greater advantage of that) and for genuine passion for what you do. Any ability I have at writing and thinking scientifically I owe to you. Lastly, I would like to extend my heartfelt thanks to Dr. Dupras. You are a saintly example of patience and understanding. You never gave up on me, at least not that I am aware of, and were always willing to give me much needed pep talks.

On a more personal note, I want to thank my cohort in the anthropology program for showing me different points of view and teaching (forcing) me to say what I was thinking. Of course, I have to thank my parents. You have an unshakeable faith in me and your pride that I am your daughter means so much to me. Lastly, I have to thank my wonderful fiancé. You sat patiently through much rambling (or was it complaining?) on stable isotopes and anthropology and managed to keep a glazed look from coming over your face. Your constant love and support makes my world go round.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE: INTRODUCTION

Despite stable isotopes first being detected in the beginning of the 20th century, it is only relatively recently that the field of anthropology began to incorporate this type of analysis into their work (Fry, 2006; Katzenberg, 2008). Since the mid-1970s, stable isotope analysis has become an expected component of archaeological and bioarchaeological research involving dietary habits, health, and origin (DeNiro, 1987; Ambrose and Krigbaum, 2003; Katzenberg, 2008). Stable isotope studies have been conducted with particular interest in carbon, nitrogen, and oxygen (Schoeninger and Moore, 1992; Katzenberg, 2008). The stable isotopes of carbon and nitrogen are analyzed in conjunction with each other to offer insight on diet and physiological state (i.e. Mays, 1997; Harrison and Katzenberg, 2003; Le Huray and Schutkowski, 2005; Chenery et al., 2010; Reitsema, 2010; Reitsema and Kozłowski, 2013), while oxygen is used in studies of migration and origin through the provision of climatic and ecological baselines (White et al, 1998, 2004; Dupras and Schwarcz, 2001; Darling, 2004; Arppe and Karhu, 2010). It should be noted that while stable isotope analysis can certainly provide new information and ways of understanding past populations, this analysis can be described as peering "through a glass darkly" (Schoeninger and Moore, 1992 pp. 248) in that while not offering a clear and concise picture, these analyses provide valuable clues into past lifeways, which aid bioarchaeologists and archaeologists in search of answers for their target populations.

Stable isotopes can be analyzed from a variety of materials and compounds, including human and non-human animal tissues and other organic matter such as plants, and these are commonly analyzed to reconstruct the environment that people would have lived in. The present

research is based on samples taken from human bone and teeth to create life histories for a unique population recovered from a cathedral crypt in Vilnius, Lithuania. The analyses will provide dietary habits from childhood to adulthood in addition to highlighting potential migration patterns. The information gained from the stable isotope analyses will then be compiled with information gathered from a morphological analysis of the individual skeletons to provide as detailed of a life history as possible under the present circumstances.

This study, in conjunction with present isotopic projects in Lithuania, will provide valuable information that will aid in filling the void of Lithuanian research. Due to the tumultuous history of Lithuania much of their history has been lost or forgotten; since they gained their independence in 1991 (Lane, 2002) many researchers are working to provide knowledge and a national identity to Lithuanians, as well as to the rest of the world. This study will continue this effort by giving an analysis that will be available to a global audience.

Research Questions

The research questions to be considered in this project are based upon the idea of comparative analyses, meaning that the stable isotope values resulting from this research will have little meaning unless they can be compared and contrasted against other related values. These comparisons will occur between the sample population and previous research as well as within the sample population itself. With this comparative framework in mind the following questions will be addressed:

1. Are there any differences between childhood and adult diet within individuals?
2. Did any individuals migrate into Lithuania after their childhood?

3. Does this unique urban population have isotopic values that indicate an "elite" diet in comparison to recently published information centered on rural populations?

Thesis Chapter Summaries

The first chapter provides the foundational information on which this research will stand. The physiological and histological characteristics of bone and teeth that are pertinent to the analysis of stable isotope studies are addressed. Following this is an overview of the basic properties of stable isotopes and an explanation of their analysis. A more detailed discussion of the stable isotopes to be studied – nitrogen, carbon, and oxygen – establishes the respective strengths, weaknesses, and research value of each isotope.

The second chapter details the materials and methods utilized in this research. A physical analysis of the individual remains was conducted at the University of Vilnius, while the stable isotope analyses were carried out at the University of Central Florida, Northern Arizona University, and the University of Florida. The third chapter presents the results of the stable isotope and morphological analyses conducted upon the study sample. The level of sample preservation will be discussed first. Following this will be the quantitative results of the nitrogen, carbon, and oxygen stable isotopes for the available samples. As mentioned previously, no statistical methods were conducted on the samples however the noted differences between results representing childhood years and adulthood years are discussed.

The fourth chapter presents an integration of the results obtained from both the stable isotope and the osteological analysis into a comprehensive discussion of the sample population. The main feature of this chapter is the comparative analyses taken within the sample as well as

between this sample and others of similar geographical and/or temporal characteristics. Chapter Two presented the multitude of exceptions to the general rules of meaning in stable isotope analysis and this chapter will continue that discussion in an effort to uncover the significance of the values and how they relate to the unique sub-population that is the focus of this research. The final chapter highlights the main findings of this research and suggests ways to improve the methodology discussed. Additionally, some hopeful future directions will be mentioned.

CHAPTER TWO: LITERATURE REVIEW

Bone

Bone is composed of different components that contain carbon, nitrogen, and/or oxygen that are used in diet reconstruction studies (Schoeninger and Moore, 1992; Hedges et al., 2005) (Fig. 1). Collagen, the main protein component of bone, comprises roughly a quarter of the bone weight and its molecular structure, and it often survives even in highly degraded bone thus making it the primary source of carbon and nitrogen for isotopic studies.

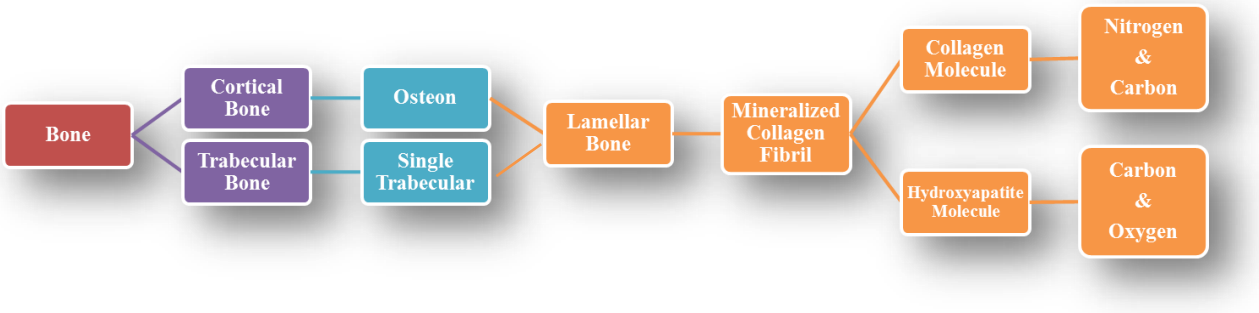

Figure 1: Simple flow chart showing the progression of skeletal tissue from a large to small scale, as well as the use of the tissues in stable isotope analysis.

Like most tissues in the human body, bone constantly remodels and is fully replaced at different periods throughout an individual's lifetime (Hollinger, 2005; Hedges et al., 2007). Research is still being conducted on turnover rates in different tissues in an effort to acquire more accurate and finite time periods but it is widely accepted that bone collagen undergoes

complete replacement every 10-20 years (Hedges et al., 2007; Katzenberg, 2008), with 0.05 mm^3 of bone being turned over every four months (Hollinger, 2005). As with many systems in the body, turnover rate will slow throughout an individual's lifetime. Males in particular have a slower turnover rate than females at about 3% of the bone collagen being replaced every year at about 20 years of age, with a decline to 1.5% at about 80 years of age (Hedges et al., 2007). These values reflect collagen turnover rates of the femoral mid-shaft and are applicable to the bone samples analyzed in this study.

The mineral fraction of bone apatite represents the isotopic carbon value derived from the whole diet (isotope values from carbohydrates, lipids, and proteins) and is not solely a reflection of protein intake as seen in bone collagen (Lee-Thorp et al., 1989; Schoeninger and Moore, 1992; Ambrose and Krigbaum, 2003; Mays and Beavan, 2012). The bone apatite is composed of a crystalline matrix of carbonate ions (Lee-Thorp et al., 1989) which bind themselves to the preexisting collagen fibers (Hedges et al., 2006). This mineral portion is analyzed not only for carbon but also for oxygen, which is used to determine geographic location of an individual at the approximate time that the respective tissue represents.

Bone apatite also displays preferential preservation in that, even in poorly preserved bones where the collagen has been degraded to a state that does not allow for isotopic analyses, the mineral portion may still be viable (Lee-Thorp et al., 1989; Schoeninger and Moore, 1992; Katzenberg, 2008). This preferential preservation is due to the crystalline structure of bone apatite being supported and embedded within the bone collagen matrix (Hedges, et al, 2006). In some cases the use of Fourier Transform Infrared Spectroscopy (FTIR) is utilized to analyze the

crystalline structure of the apatite to detect any diagenetic changes within the bone apatite (Yoder and Bartelink, 2010). Along with other statistical analyses, FTIR can provide a good estimation on the quality of the bone apatite; unfortunately this method is not available for use in this study. Instead the preservation of the apatite will be estimated via the percent yield of apatite after pretreatment; enamel, with a much lower organic content, consists of 95-97% apatite while bone consists of 75-80% apatite (Katzenberg, 2008; Crowley and Wheatley, 2014).

Teeth

Teeth have two primary components, enamel and dentin (Hillson, 2014). Dentin is a dull, yellow porous substance that makes up internal portion of the tooth crown and roots. Enamel, the strongest material in the human body, is a shiny, white nonporous substance that forms the crown of the tooth, the portion of the tooth that is above the gum line, and forms the external layer around the pulp chamber (Fig. 2). Just as in bone, teeth also have apatite and collagen portions; apatite is found in the enamel, providing information on the carbon taken from the whole diet and on oxygen, and collagen in the dentin, providing information on the carbon and nitrogen from the protein portion of the diet (Hillson, 2005).

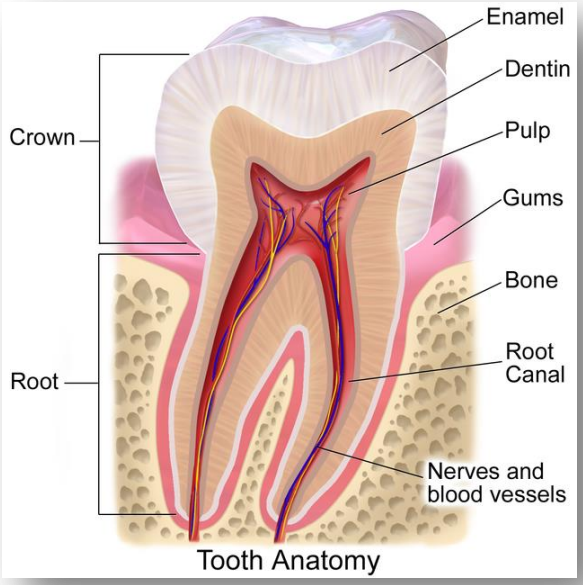

Figure 2: Image depicting the gross anatomy of a typical human molar; https://en.wikipedia.org/wiki/Human_tooth#/media/File:Blausen_0863_ToothAnatomy_02.png (Image public domain)

Initial tooth formation consists of a dental papilla encased within an enamel organ (Hillson, 2005:208). The first dental tissue to be laid down consists of a dentin matrix from the dental papilla and the cells on the edge of the papilla differentiate into odontoblasts which then lay down the first layer of enamel matrix (Hillson, 2005:208-209).The interest of analyzing both bone and teeth together is that it is possible to reconstruct different periods in an individual's lifetime. Teeth are fully formed during different stages of childhood and do not remodel (Hedges et al., 2005) thus reflecting this time period of a person's life; bone continually remodels and its isotopic composition is a heterogeneous reflection of the last several years of life (Chenery et al.,

2010). The development of teeth is well understood and allows the researcher to pinpoint at what stage in an individual's life that the isotope values correspond to depending on what tooth was selected for analysis (Hedges et al., 2005). For example, the present study includes the analysis of the first molars. Initial crown formation begins shortly after birth with crown formation completed by approximately 2.1 years of age (Moorrees et al., 1963; Smith, 1991). Root formation begins once the crown has fully formed and the apical ends of the roots close at approximately nine years of age (Moorrees et al., 1963; Smith, 1991). This means that the isotope ratios of oxygen will reflect an individual's average geographic location from birth to 2.1 years of age and the isotope ratios of nitrogen will present an average representation of their protein consumption from 2.1 years until nine years of age.

Stable Isotopes – The Fundamentals

The majority of elements occur as two or more stable, or nonradioactive, isotopes (Fry, 2006). An isotope is an element with the same amount of protons and electrons but a different number of neutrons (DeNiro, 1987; Schoeninger and Moore, 1992; Larson, 2002). There are several hundred isotopes that have been identified but the use of isotopes to study past populations is mostly limited to carbon, hydrogen, nitrogen, oxygen, sulfur, and strontium (Schoeninger and Moore, 1992). Isotope values are represented by the symbol δ, which indicates the difference between a sample's isotope value and that of the standard (DeNiro, 1987; Fry, 2006). Understanding isotope ratios can be confusing and negative values will often be interpreted as meaning there is "less" of the target isotope in that sample; however, this is not the case. The negative values indicate that there is less of the heavy isotope in the sample or that the

sample is enriched in the lighter isotope in comparison to the reference (Schoeninger and Moore, 1992; Fry, 2006). So, for example, a $\delta^{13}C$ value of -10.00‰ simply means that there is an enrichment of the ^{12}C isotope and a depletion of the lighter isotope, ^{13}C. Thus, δ expresses a particular concentration, or percent, of a heavy isotope.

Another notation used when reporting isotope values is the symbol ‰ (per mil), which denotes that a value is in "parts per thousand" (DeNiro, 1987; Fry, 2006). The difference in the sample isotope value from the reference standard is often very small and the ‰ notation serves to magnify that difference and make it easier on the researcher to extrapolate patterns and relationships. Commonly used standards are as follows: carbon values are reported against PeeDee Belemnite (PDB); nitrogen values utilized Air (AIR) reference standards; and oxygen values are most commonly based upon the Vienna Standard Mean Ocean Water (VSMOW) but may also be reported in relation to Vienna-PDB (VPDB) (DeNiro, 1987; Schoeninger and Moore, 1992; Fry, 2006). The relationship between the standard value and the sample isotopic value is calculated by the following equation (Fry, 2006:31):

$$\delta = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 1000 \qquad \textbf{(1)}$$

In this equation R represents the ratio of fractionation for the heavy and light isotope of the element being analyzed.

In addition to specific notations, there are also specific processes that one must keep in mind when dealing with isotopes: mixing and fractionation. Mixing is a much simpler concept than fractionation and can often be thought of as combining the isotopes into a homogenous compound, like what is done in baking. Fractionation is a simple process as well, if mixing combines isotopes, then fractionation breaks them apart (Fry, 2006). While this is essentially true it should be noted that the heavy and light isotopes behave differently during fractionation. There are two rules for fractionation: during a forward moving reaction "the light isotopes usually react faster" (Fry, 2006:12), and in a reaction reaching equilibrium (one that moves forward and backward), the "heavy isotopes concentrate where bonds are strongest" (Fry, 2006:12). This is important because fractionation is the process that establishes the distribution of isotopes around the planet and throughout organic matter, making it a crucial step in stable isotope cycling.

Nitrogen

The use of ^{15}N in diet reconstruction is much in debate (Hedges and Reynard, 2007). It has been proposed that an enriched $\delta^{15}N$ value equates to a diet high in protein but the reverse has also been argued (Hedges and Reynard, 2007). It is known, however, that $\delta^{15}N$ values will increase for an animal at successive trophic levels, as an animal moves up the food chain, e.g., a deer eating grass, and then a human that eats the deer will have a $\delta^{15}N$ value that is two trophic levels above the grass. Current research supports an approximate increase of 3‰ (Hedges and Reynard, 2007; Adams and Sterner, 2000; Mays and Beavan, 2012). Due to these trophic level increases, $\delta^{15}N$ values are often higher in animals that eat a diet rich in marine-based proteins compared to those that eat a terrestrial-based diet because of the longer food chain found in the

former (Mays and Beavan, 2012). Additional factors that can influence $\delta^{15}N$ values are nutritional stress, pregnancy, pathology, and environmental factors such as precipitation (Cormie and Schwarcz, 1996; Katzenberg and Lovell, 1999; Fuller et al., 2005; Olsen et al., 2014).

As might be inferred, ^{15}N is one of the most heavily and broadly impacted of the isotopes often analyzed (Fuller et al. 2005; Hedges and Reynard 2007; Olsen et al. 2014). Despite the mercurial nature of nitrogen there are some known patterns, including 1) nitrogen is an indicator of trophic level, i.e. the consumer's tissue will show a 3‰ increase over the consumed organism's tissue (Hedges et al. 2005; Hedges and Reynard 2007), and 2) the concept of a nitrogen pool or nitrogen balance is closely associated with nutrition, and when an individual is under stress this balance will reflect that stress (Katzenberg and Lovell 1999; Fuller et al. 2005; Mekota et al. 2006; Olsen et al. 2014). These patterns become important when one considers that nitrogen is primarily derived from the protein portion of the diet and pathological or other metabolic processes often disrupt protein metabolism (Olsen et al. 2014) (Fig. 3).

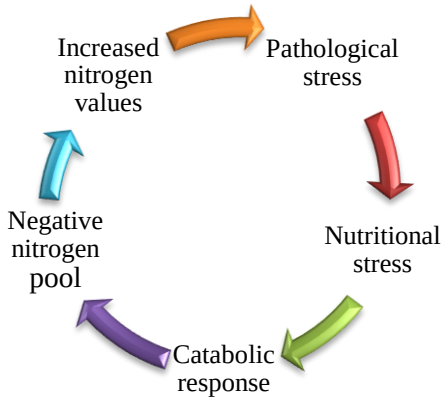

Figure 3: Chart showing the impact of pathological conditions on $\delta^{15}N$ values.

Many researchers choose to avoid taking samples from morphologically pathological bone (Katzenberg and Lovell 1999) because they realize that it will skew the nitrogen values and result in inaccurate diet reconstructions, i.e. an over estimation of marine protein source contribution. Unfortunately, it is the pathological bones that truly need to be sampled in order to detail the impact of disease on stable isotopes. In an effort to untangle this relationship Katzenberg and Lovell (1999) conducted a small scale study analyzing the stable ^{15}N isotopes from samples of pathological bone. More recently Olsen et al. (2014) built upon Katzenberg and Lovell's (1999) research with a larger sample size and more systematic approach.

Katzenberg and Lovell (1999) set out to determine whether there was a difference in $\delta^{15}N$ values in pathological bone. Four pathological samples were analyzed – a periostitic lesion, a healed fracture callus, a segment of atrophied bone, and a sample from an osteomyelitic lesion. They found that the individual with osteomyelitis had a significant increase in their $\delta^{15}N$ values. Additionally, the atrophied bone showed significantly decreased nitrogen values. In an effort to expand upon this research and add reference data, Olsen et al. (2014) conducted another study to determine intra-skeletal differences of $\delta^{15}N$ values in pathological bone. A larger sample size of 59 individuals was grouped into broad disease categories: metabolic, degenerative joint disease, trauma (fracture), nonspecific infection, and nonspecific inflammation. Samples were taken from the lesion itself, from normal looking bone near the lesion, and from a bone far distant from the lesion site. Their results supported those found by Katzenberg and Lovell (1999) in that the

difference in isotope value for the osteomyelitic lesion and fracture callus and their corresponding distant-bone sample showed the greatest significance.

Other studies (e.g. Fuller et al. 2005 and Mekota et al. 2006), have analyzed the correlation between nutritional stress and nitrogen isotope values by using hair. Mekota et al. (2006) conducted an isotopic analysis for $\delta^{15}N$ values for six patients recovering from anorexia, a severe and often prolonged state of malnutrition. It was found that these patients had significantly decreased nitrogen values. Fuller et al. (2005) looked at less severe cases of nutritional stress. Analysis was conducted on hair taken from eight pregnant women five days after they had given birth. It was found that those women who had suffered severe enough morning sickness to cause restricted weight gain or weight loss had a corresponding increase in their nitrogen isotope values.

These studies are vital to the discussion of the pathological influence on $\delta^{15}N$ values because often an individual plagued by an illness will suffer malnutrition or nutritional stress (Norman et al. 2008). Nutritional stress may be even more invisible than a pathological condition in regards to skeletal markers but also has as great, if not greater, of an impact on nitrogen values.

Table 1: Sources of Nitrogen Enrichment/Depletion in Human Tissue

Consumer Condition	**δ^{15}N Value**	**Sample Tissue**	**Reference**
Diet → Consumer *Terrestrial* *Marine*	+ 3‰ 6‰ to 12‰ 17‰ to 20‰	Human Bone Collagen	Schoeninger et al., 1983
Morning Sickness during Pregnancy	+0.4‰ to 0.7‰	Human Hair Keratin	Fuller et al., 2005
Anorexia	+0.6‰ ± 0.2‰	Human Hair Keratin	Mekota et al., 2006
Anorexia and Bulimia	+0.2‰ ± 0.4‰	Human Hair Keratin	Mekota et al., 2006
Pathological Bone *Atrophy* *Osteomyelitis* *Degenerative Joint Disease* *Fracture* *Osteomyelitis*	 -3‰ (6.9‰) +3‰ (12.9‰) -0.1‰ (10.8‰) +0.8‰ (11.7‰) +0.9‰ (11.8‰)	Human Bone Collagen	 Katzenberg and Lovell, 1999 Olsen et al., 2014

Carbon

Carbon isotopes are used to help determine dietary patterns by differentiating between major food groups, namely C_3 and C_4 plants (DeNiro, 1987; Ambrose and Krigbaum, 2003; Chenery et al., 2010). C_3 and C_4 plants are categorized based on the photosynthetic pathway utilized by the plant. C_4 plants tend to be found in more tropical climates and consist of plants such as maize, sorghum, sugar cane, and some millets (DeNiro, 1987). C_3 plants are more common as food plants and are found in temperate climes; they include plants such as wheat, barley rice, and beans (DeNiro, 1987; Chenery et al., 2010). The δ^{13}C values of C_3 plants range from -32 to -21‰ with an average value of -26‰, and C_4 plants have δ^{13}C values that run between -15 to -8‰ with an average value of -11.5‰ (DeNiro, 1987; Katzenberg, 2000). When looking at the relationship between an organism's diet and the δ^{13}C values extracted from

collagen there is a difference of 3-5 ‰ (Schoeninger and Moore, 1992; Dupras et al., 2001). This is to say that an individual with a diet made up primarily of C_3 plants, which possess a mean isotopic value of -26‰, would have a $\delta^{13}C$ value of approximately -21‰ and an individual with a diet heavy in C_4 plants, which possess a mean isotopic value of -11.5‰, would have a $\delta^{13}C$ value of approximately -6.5‰.

An important distinction to bear in mind in regards to this research is that the above values are reflective of $\delta^{13}C$ values taken from collagen. This study, however, also utilizes carbon analysis from carbonate sources, the hydroxyapatite or mineral portion of skeletal tissues. In spite of an acknowledged difference in the isotopic composition of these two tissues relatively few studies have analyzed this difference (Lee-Thorp et al., 1989; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Hedges, 2003; Crowley et al., 2010). However, current research suggests that the carbon found in proteinaceous tissues, collagen, is derived primarily from dietary protein sources (Lee-Thorp et al., 1989; Ambrose and Norr, 1993). Ambrose and Norr (1993) discuss the possibility that in cases of insufficient protein in the diet other forms of energy, such as from carbohydrates or lipids, may be used in the formation of collagen. Carbon from the mineral portion of bone, also called carbonate carbon, represents dietary habits on a whole, in that the ^{13}C is derived from dietary carbohydrates, lipids, and sometimes protein (Lee-Thorp et al., 1989; Ambrose and Norr, 1993).

As mentioned above, the fractionation values of carbon taken from collagen are typically +5‰ higher than the consumed plants, while carbon taken from carbonate typically has a fractionation value of around +12‰ (Lee-Thorp et al., 1989). Ambrose and Norr (1993)

suggested a value of +9.4‰ based upon a controlled-feeding experiment using small mammals, namely rats. Additionally, the offset between diet and carbonate was found to be consistent at +9.4‰ regardless of diet but that the offset between diet and collagen would vary when the dietary source of protein and energy differed (Ambrose and Norr, 1993; Ambrose et al., 1997; Harrison and Katzenberg, 2003). Information pertaining from the diet can also be gleaned from the offset between carbonate and collagen ($\Delta^{13}C_{CA\text{-}CO}$) (Lee-Thorp et al., 1989; Ambrose et al., 1997; Harrison and Katzenberg, 2003; Kellner and Schoeninger, 2007). When the difference between the carbonate and collagen values is greater than 4.4‰, then it is suggested that dietary carbohydrates were C_4-sourced and that dietary protein was C_3-sourced. However, when the difference is less than 4.4‰, then the carbohydrates are C_3-sourced and the protein is marine-sourced. Keeping in mind that the +9.4‰ fractionation value determined from Ambrose and Norr (1993) was from a study of small mammals, Garvie-Lok (2001) reexamined published studies and suggested that the +12‰ fractionation value given by Lee-Thorp et al. (1989) was more appropriate for large mammals, such as humans.

Continuing with the fact that collagen represents the protein portion of the diet and carbonate represents the energy portion, Kellner and Schoeninger (2007) developed a model that would allow for a more definitive determination of protein sources, e.g. C_3 or C_4. The model consists of plotting the collagen and carbonate carbon stable isotope values against three regression lines representing a diet based on C_3 protein:

$$y = 1.74x + 21.4$$

(2)

A diet based upon C_4 protein:

$$y = 1.71x + 10.6$$

(3)

A diet based on marine protein:

$$y = 2.18x + 18.6$$

(4)

These regression lines provide information not only about protein consumption in the diet but also about energy consumption. The upper endpoint of the lines indicate a diet of 100% C_4-sourced lipids and carbohydrates while the lower endpoint indicates a diet of 100% C_3-sourced lipids and carbohydrates (Kellner and Schoeninger, 2007). As Reitsema and Kozlowski (2013) point out, these regressions lines were developed from animal studies during modern time periods resulting in lower $\delta^{13}C$ because of the difference in modern and pre-industrial atmospheric CO_2 values. In order to apply these lines to archaeological samples, such as this study, the regression lines are shifted up by 1.5‰ (Reitsema and Kozlowski, 2013).

Oxygen

Stable oxygen isotopes are often used to reconstruct climate systems (Chenery et al., 2010). This isotope can also provide information on the geographical location of an individual at the time when the respective sample tissue was being formed (White et al., 1998, 2004) because environmental and body water composition is nearly identical (Fricke et al., 1995; Iacumin et al.,

1996). This is only possible if an individual's primary source of water intake is from the local environment as the $\delta^{18}O$ value relies mainly on the isotopic composition of drinking water and water in consumed food. The water found in food is often slightly more enriched in the ^{18}O values than meteoric, or environmental, water due to the fractionation occurring within the diet source (Daux et al., 2008). Since this internal fractionation of water is related to body mass, animals, such as humans, are less impacted by the water derived from food sources (White et al., 1998; Daux et al., 2008).

The $\delta^{18}O$ values decrease with distance from the sea as well as elevation and falling temperatures; conversely these values can increase with decreasing humidity (Dansgaard, 1964; White, 1998; Dupras and Schwarcz, 2001). While stable carbon and oxygen isotopic values can be taken from both bone collagen (Koon and Tuross, 2013) and bone/teeth apatite sources, the preferred source of ^{18}O is the apatite found in the enamel to determine childhood location, allowing for a comparison with bone values that represent adult location and migration (Chenery et al., 2010). Oxygen isotopes can enter the body by one or more of three ways: through the intake of drinking water, water contained and metabolically derived from the food eaten, and through atmospheric oxygen; although water consumption through drinking has the biggest impact on $\delta^{18}O$ values (Luz et al., 1984; White, 1998). Even though the ^{18}O composition in human tissues is primarily influenced by drinking water, the consumption of cooked food also has an impact. This is due to processed food having a higher δ18O value than drinking water (Daux et al., 2008). Another influencing factor is the possibility that the type of plant, C_3 or C_4, consumed may have an impact on the $\delta^{18}O$ values (White et al., 2004), such that the consumption

of C_4 plants leads to higher $\delta^{18}O$ values. This is by virtue of the hardiness of C_4 plants that allows them to continue to photosynthesize even in dry conditions which leads to an enrichment in ^{18}O; the reverse has also been speculated (White et al., 2004).

Historical Background

Lithuania is one of the three countries that comprise the Baltic states (Fig. 4) and is located to the northeast of the European continent with the Baltic Sea to its west, Latvia to the north, Belarus along its southeastern border, Poland to the southwest, and the Russian territory of Kaliningrad to the west. The capital city of Vilnius is located in the eastern highlands of the country, roughly thirty kilometers west of its border with Belarus (Fig. 5).

Figure 4: Map of Europe with the country of Lithuania highlighted in light blue; adapted from; http://www.mapcruzin.com/free-maps-europe/europe_ref_2007.jpg (Map public domain)

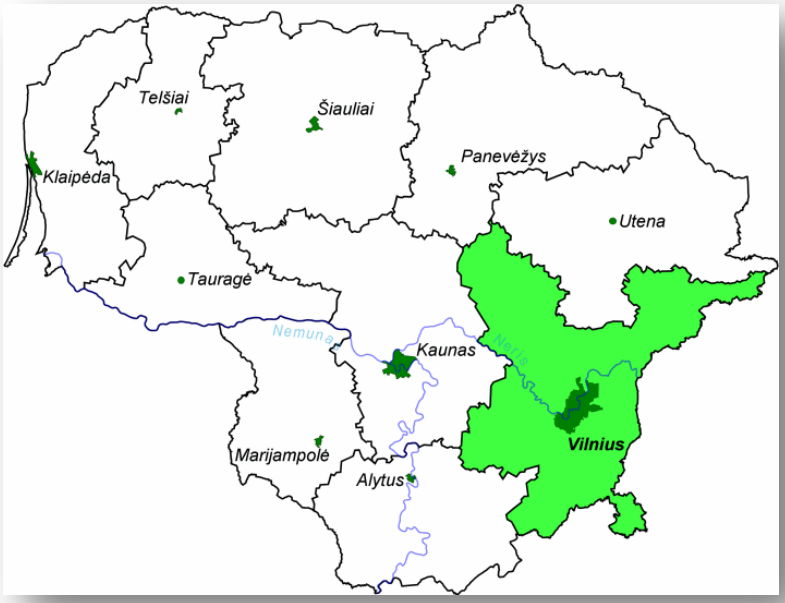

Figure 5: Map of the modern counties of Lithuania with the capital and site city, Vilnius highlighted in the green section; https://commons.wikimedia.org/wiki/Maps_of_Lithuania#/media/File:Vilniaus_apskritis.png (Map public domain)

Of the three Baltic nations Lithuania, or *Lietuva*, is the largest, oldest and most populated at about 2.88 million in 2015 (CIA, 2015). The country is roughly the size of West Virginia at 25,200 square miles. Lithuania is heavily forested and enjoys a temperate climate which results in damp cool summers and moderate but snowy winters (O'Connor, 2006). In addition to being heavily forested, Lithuania is covered with hundreds of lakes, streams, and rivers. There are no mountains and the country is fairly flat, this in conjunction with excellent irrigation allows for almost half of the country to be arable land.

Lithuanian agriculture, while changing in how it is carried out, has seen relatively little change in what is grown (French, 1970; CCET, 1996), supporting the use of modern agricultural data to stand as proxy for past practices. During the sixteenth century many gardens consisted of cabbage, turnips, onions, beets, parsnips, and parsley for vegetables as well as orchards of apples, pears, cherries, and plums (French, 1970). Additionally, the country has cultivated barley, wheat, and rye, in order of decreasing percentage of land given over to the cultivation of each grain (CCET, 1996). Additional archaeobotanical evidence excavated from the Vilnius Lower Castle indicates that rye, millet, flax, and buckwheat were being cultivated during the 13th and 14th centuries (Stancikaite et al., 2008).

Lithuania first established itself as a state in the 13th century but the area has been occupied since roughly 3500 B.C. The Baltic languages are among Europe's oldest living languages with Lithuania and Latvia the only surviving Baltic languages of Indo-European language family (O'Connor, 2006). Although the dominant religion in Lithuania is Roman Catholic, the capital city of Vilnius alone has 40 churches, and about five percent of the population practices Russian Orthodoxy and while Vilnius was at one time known as the "Jerusalem of Europe" but the once vibrant population of Judaism has all but disappeared (O'Connor, 2006).

As was briefly mentioned previously the history of Lithuania has been turbulent. The isolated geographical location of the Baltic states has played a large role in the history of the region in that they have become a natural corridor for the often competing powers of Russia and Germany (Bojtár, 1999). Conflicts began with the advent of Christianity that swept through

Europe beginning around 371 A.D. (Smith et al., 2002). The Catholic Church was ruthless in its push for conversion and established the Teutonic Order at the end of the twelfth century. The order was a band of knights bent on conversion and where conversion failed extermination prevailed. Lithuania resisted the Order and conversion until 1387, just over a millennium after the conversion from paganism to Christianity began Europe. The last third of the 18th century saw the union of Poland and Lithuania torn asunder as Lithuania was given over to the Russian sphere of influence (Plakans, 2011).

The history of the Vilnius Cathedral Basilica of Saints Stanislaus and Vladislaus is almost as tumultuous as that of the country itself. The first Cathedral of Vilnius is assumed to have been built when Lithuanians first accepted Christianity in the mid-thirteenth century (Jankauskas, personal comm.). The Cathedral was built in the area of the Lower Castle on top of a pagan temple (Fig. 6). Archaeological excavations have determined that this first Cathedral was rebuilt four separate times: in the middle of the 13th century, during the late 13th and early 14th century, the late 14th century, and the early 15th century. When King Mindaugas died in 1263 the Cathedral was burned to the ground and a pagan temple was erected and existed until 1386. Upon the second adoption of Christianity (1387) a new Cathedral was built and became the episcopal seat of the diocese of Vilnius in 1388 (Jankauskas, personal comm.).

Figure 6: Google map of Vilnius showing the location of the Cathedral. Note the proximity of the Neris River, just to the north of the Cathedral, which has routinely flooded the crypts.

The Cathedral was continuously rebuilt as fires destroyed it in 1419, 1530, and 1610; each time the Cathedral reflected the architecture of the city, i.e. Gothic, Renaissance, and Baroque. In 1769 a storm caused the destruction of corner chapel and tower resulting in the reconstruction of the Vilnius Cathedral from 1782-1801. Soviet authorities closed the Cathedral in 1950, turning it into a warehouse and then a concert hall. The Cathedral was given back to the Catholic Church in 1988 and several statues that had been knocked down were replaced in 1997.

Several crypts exist under the Cathedral and because of routine flooding by the Neris River many were excavated during 1931 to 1937 when the foundations of the Cathedral were fortified. Crypts located in the side chapels are thought to have been burial locations for the

nobility and their families. Burials of past Grand Dukes were also found underneath these side chapels. There are now 27 known crypts in the Cathedral with 20 of them being used for burials. Many additional graves were found outside of the crypts. The latest excavations, which took place from 1987 to 1989, were of burials in the crypts or under the floor that date to the 17th and 18th centuries and are thought to be the graves of the church and secular elite.

A report for the excavation of the individuals analyzed in this study was not available, and beyond a general burial location very little is known about the burial context of these individuals. The skeletal remains of the individuals analyzed in this study were found buried in the northern nave, or main aisle, of the Cathedral (Jankauskas, personal comm.). The individuals were buried directly into the soil with no evidence of grave goods or burial accessories, e.g. coffins, present. Some of the burials, it is not known which ones, had a brick lining. Despite this lack of grave goods, these individuals have been tentatively labeled as "elite" members of society (Jankauskas, personal comm.).

Defining the word "elite" is rife with difficulties, thus this study will rely upon the term "high status." Determining which, if any, individuals held a socially-deemed high status during their life will be reliant upon comparing the stable isotope values within the study sample. Individuals with higher status are expected to have overall better health and better access to quality dietary sources, particularly to meat (Jankauskas, 2003; Le Huray and Schutkowski, 2005; Zvelebil and Pettitt, 2013). Two other terms used in relation to a higher social status are achieved and attained status. Lin (1999) states that achieved status relates to individual accomplishments, such as education or occupation, that results in greater access to resources;

ascribed status is given to an individual based upon the attained (cumulative) status of their parents.

CHAPTER THREE: MATERIALS AND METHODS

The sample used in this study consists of a unique population recovered from a crypt in the Cathedral Basilica of Saints Stanislaus and Vladislaus in the capital city of Vilnius. The individuals were buried in the crypt during the 16th to the 18th centuries (Jankauskas, personal comm.). The sample set is comprised of 23 individuals – 21 males, one possible female, and one juvenile. While these individuals were not buried with any grave goods nor were they interred in any type of coffin, they are assumed to have been of higher status based upon their burial in the northern aspect of the nave, or main aisle, of the cathedral (Fig. 7).

Figure 7: Cathedral Basilica of Saints Stanislaus and Vladislaus; https://commons.wikimedia.org/wiki/Category:Exterior_of_Vilnius_Cathedral#/media/File:2010_02_07Vilnius16Arkikatedra.JPG (left). Interior layout of the Cathedral; http://en.wikipedia.org/wiki/Vilnius_Cathedral#/media/File:Vilnius_Cathedral_Interior_2,_Vilnius,_Lithuania_-_Diliff.jpg (right) (Images public domain)

A morphological analysis was performed at the Department of Anatomy, Histology and Anthropology, Faculty of Medicine at the University of Vilnius in Vilnius, Lithuania. The stable isotope analyses were performed on both bone and teeth; samples were prepared at the University of Central Florida and analyzed at the Colorado Plateau Stable Isotope Lab at Northern Arizona University and at the Light Stable Isotope Mass Spec Lab in the Department of Geological Sciences at the University of Florida. Fifteen individuals have paired bone (collagen and apatite) and tooth samples (enamel and dentin) available for analysis, while seven individuals are only represented by a bone sample, and one individual has only a tooth sample (Table 2). The bone used was a sample taken from the mid-shaft of the femur and the teeth are upper, right first molars with upper, left first molars used when the right was missing or too worn; additionally some of the teeth are lower left or right first molars. Those individuals that did not have first molars available for analysis are only represented by bone samples.

As with any study, the researcher must work with what sample is available. Therefore, it is acknowledged that the small sample size places several constraints on what the data gained from the study can inform upon. No statistical analysis will be performed upon the samples in this study because any results gathered from this method will lack statistical significance due to sample size. Instead, the data will be given meaning and value through comparative analysis with pre-existing related stable isotope research. In particular, the data from this study will be compared and contrasted with previous work in similar geographical and/or temporal frames.

Table 2: Summary of Thesis Samples

Sample ID	**Sex**	**Age**	**Bone Only**	**Tooth Only**	**Bone & Tooth**	**Tooth Type**
KAT-2B	M	30-35			X	M_1 U.R.
KAT-3	M	55+	X			
KAT-4	M	45-50			X	M_1 L.R.
KAT-5	M	40-45			X	M_1 U.L.
KAT-5A	M	25-30		X		M_1 U.L.
KAT-6	M	55+			X	M_1 L.R.
KAT-8	M	50-55	X			
KAT-9	M	40-50	X			
KAT-10	M	35-40			X	M_1 U.R.
KAT-11	M	45-50			X	M_1 U.L.
KAT-11a	M	45-50	X			
KAT-12	M	25-30			X	M_1 U.L.
KAT-13	M	25-30	X			
KAT-14a	-	9.5-10.5	X			
KAT-15	M	40-45			X	M_1 U.R.
KAT-16	F(?)	20+	X			
KAT-17	M	18-20			X	M_1 U.L.
KAT-18	M	40-45			X	M_1 U.R.
KAT-19	M	20-25			X	M_1 U.L.
KAT-20	M	50-55			X	M_1 L.L.
KAT-21	M	50-55			X	M_1 U.L.
KAT-24	M	50-55			X	M_1 L.L.
KAT-25	M	35-40			X	M_1 U.R.

Collagen Extraction

The method for collagen extraction is based upon a modified Longin (1971) process in which the bone sample is demineralized using a weak solution of hydrochloric acid (HCl). Samples of bone were removed from the femoral mid-shaft using a Dremel tool with a fiberglass

reinforced cut-off wheel. Original bone samples weighed between 7 and 17 grams, depending on sample size and cortical thickness. Each sample was then cleaned using ultrasonication and surface brushing, and then dried overnight in an oven at 60°C. Dry samples were then ground down into similar-sized pieces using a mortar and pestle, with a total weight between 3 and 6 grams, and placed into a 50 millimeter plastic test tube. Any lipids, or fats, were removed by rinsing samples three times for 20 minutes in a 2:1 chloroform:methanol solution and then left to dry overnight in a fume hood. The samples were then placed in 10 millimeters, or until sample is covered, of a 0.5M HCl solution, which is changed daily until the bone is fully demineralized.

Samples were then rinsed in distilled water three times, followed by placing them in 10 millimeters of a weak, 0.1M, sodium hydroxide (NaOH) solution for 20 minute intervals until the solution was clear in color; this indicates that all humic acids have been removed from the sample. Once the NaOH solution had become clear the samples were rinsed with distilled water six times or until a neutral pH (7.0 ± 1.0) was reached. The samples were then placed in 10 millimeters of a 0.25M HCl solution for 10 minutes and then they were rinsed once more with distilled water so that a pH of 2.5 – 3.0 was obtained.

After these processes were completed the remaining demineralized collagen were placed in an oven at 90°C for approximately 16 hours to heat the sample to a water soluble state. The samples were then transferred to three-dram glass vials and placed back in the oven at 90°C until they were fully dried and had a glassy appearance. The vials were weighed and the collagen yield was determined. Collagen yield is a percentage of the collagen that is extracted from the

sample bone and was calculated through the following equation taken from the University of Central Florida Bioarchaeology Lab protocol:

$$collagen\ yield\ \% = \frac{weight\ of\ vial\ with\ treated\ sample\ (g) - weight\ of\ empty\ vial\ (g)}{weight\ of\ dry\ sample\ (g)} \times 100 \quad \textbf{(5)}$$

The last step was to transfer 0.55 – 0.66 milligrams of each sample into tin weigh cups for final processing through an elemental analyzer isotope ratio mass spectrometer (EA-IRMS). All collagen samples were sent to Colorado Plateau Stable Isotope Laboratory (CPSIL) at Northern Arizona University.

The collagen extraction protocol used for dentin is the same as that for bone with minor differences. The total initial weight of the dentin prior to treatment was 1 ±0.2 grams. Unlike the bone samples, the dentin samples did not go through lipid removal with the 2:1 chloroform:methanol solution. The dentin was also demineralized with a 0.5M HCl solution for roughly eight weeks. Also, the dentin samples were heated in the oven at 90°C but this step took about 24 hours as compared to the 16 hours for the bone samples. Lastly, the dentin samples were dried at 60°C rather than at 90°C.

Apatite Processing

The method used for processing the hydroxyapatite of the bone and enamel is modeled after Sullivan and Krueger (1983). First, the samples were cleaned using the same ultrasonication method. The teeth were then broken down using a mortar and pestle in order to separate the enamel from the dentin. Then the bone and enamel were ground into a powder and sifted through

a 180 micron sieve until a total weight of 20 – 30 milligrams for each sample was reached. The powdered samples were then placed into plastic microcentrifuge vials and immersed in a 2% diluted-bleach solution for 24 hours (for the enamel) and for 72 hours (for the bone). The amount of 2% bleach solution used for each sample was calculated with the following equation:

$$weight\ of\ sample\ (mg) * 0.04 = 2\%\ bleach\ solution\ needed\ (mL) \quad \textbf{(6)}$$

After the appropriate time had elapsed the samples were rinsed with distilled water five times and were then placed into a 1.0M acetic acid solution (using the same amount of solution as was calculated for the bleach solution) for four hours. The samples were then rinsed again with distilled water five times and placed into a freezer overnight. The bone and enamel samples were then placed in the condenser chamber of a freeze dryer for 24 hours followed 24-48 hours in the manifold or until all samples were completely dry. The vials were weighed and the apatite yield was determined. Apatite yield is a percentage of the apatite that was extracted from the sample bone and was calculated through the following equation taken from the University of Central Florida Bioarchaeology Lab protocol:

$$apatite\ yield\ \% = \frac{weight\ of\ vial\ with\ treated\ sample\ (mg) - weight\ of\ empty\ vial(mg)}{weight\ of\ dry\ sample\ (mg)} \times 100 \quad \textbf{(7)}$$

Lastly, 1.0 – 1.5 milligrams of each sample was weighed into new plastic microcentrifuge vials for final analysis via a temperature conversion elemental analyzer isotope ratio mass

spectrometer (TC/EA-IRMS). All apatite samples were sent to the Light Stable Isotope Mass Spec Lab in the Department of Geological Sciences at the University of Florida.

CHAPTER FOUR: RESULTS

The level of accuracy of the respective mass spectrometers from the stable isotope labs at the Northern Arizona University (CPSIL) and the University of Florida are summarized in Table 3. Bone collagen and dentin collagen were processed at the CPSIL and the reported precision of the EA-IRMS for the bone collagen was ±0.20 for carbon and ±0.08 for nitrogen. Additionally, three samples were run twice. The difference between the duplicate samples was averaged to determine the accuracy of the carbon and nitrogen data for the study sample; these results are provided in Table 4. The reported precision of the EA-IRMS for the dentin collagen was ±0.06 for carbon and ±0.04 for nitrogen. Additionally, two samples were run twice. The difference between the duplicate samples was averaged to determine the accuracy of the carbon and nitrogen data for the study sample; these results are provided in Table 4. Bone and enamel apatite were processed at the University of Florida and the reported precision of the TC/EA-IRMS was ±0.04 for carbon and ±0.07 for oxygen.

Table 3: Summary of Reported Accuracy

Lab	**Accuracy for $\delta^{13}C$**	**Accuracy for $\delta^{15}N$**	**Accuracy for $\delta^{18}O$**
Colorado Plateau Stable Isotope Lab (Bone collagen – NIST peach leaves)	±0.20	±0.08	-
Colorado Plateau Stable Isotope Lab (Dentin – NIST peach leaves)	±0.06	±0.04	-
Light Stable Isotope Mass Spec Lab (NBS-19 Standard)	±0.04	-	±0.07

Table 4: Summary of Calculated Accuracy

Sample (Bone collagen)	**Difference in $\delta^{13}C$ values**	**Difference in $\delta^{15}N$ values**	***Sample (Dentin)***	**Difference in $\delta^{13}C$ values**	**Difference in $\delta^{15}N$ values**
KAT-K2B	-0.03	-0.06	*KAT-K21*	0.08	-0.07
KAT-K11	-0.08	0.07	*KAT-K25*	0.07	0.01
KAT-K20	0.11	-0.09	-	-	-
Accuracy	**±0.00**	**±0.03**	***Accuracy***	**±0.08**	**±0.03**

Sample Preservation

As soon as an organism transitions from life to death a multitude of factors begin affecting the preservation of the remains. Both taphonomy and diagenesis, a "sub-field" of taphonomy, impact the integrity of skeletal material and how well it survives into the archaeological record (Christensen et al., 2014). The term diagenesis refers solely to those mechanisms that affect bone in its post-burial or final depositional environment (Lyman, 1994; Hedges et al., 1995). Or as more clearly defined, diagenesis is "any chemical, physical, or biological change to a bone after its initial deposition" (Christensen et al., 2014:125). The properties of diagenesis are most relevant for stable isotope studies of archaeological material, particularly for the analysis of collagen. This is due to one of the parameters of diagenesis being bone collagen loss resulting from the invasion of microorganisms in the surrounding soil matrix seeking out protein sources (Hedges, 2002).

Visually, all of the samples used in this study were well preserved. The bones and teeth were hard and resistant to breaking. They were also resistant to demineralization as evidenced by this process taking roughly six weeks (June 5, 2012 until July 20, 2012) for the bone samples and

roughly nine weeks (March 3, 2014 until May 7, 2014) for the dentin samples. Sample preservation is most concerned with collagen analyses as the organic portion of bone is more susceptible to degradation than the inorganic portion (Hedges, 2002). Ambrose (1990) and Ambrose and Norr (1992) discuss three ways to determine the level of preservation of a sample – the collagen yield, percent of carbon and nitrogen, and as already mentioned the C:N ratio.

Collagen comprises approximately 20 to 25% of the weight of fresh bone (Tuross et al., 1988; Schoeninger et al., 1989; van Klinken, 1999). Several acceptable collagen yields have been suggested by various authors and range from 1% to 5 or 6% (DeNiro, 1985; DeNiro and Weiner, 1988; Tuross et al., 1988; Schoeninger et al., 1989; Ambrose, 1990; Ambrose and Norr, 1992; van Klinken, 1999). Ambrose (1990) claims that collagen quality deteriorates after 3.5% but van Klinken (1999) accepts collagen yield values as low as 1%. Therefore the quality of the preservation for this study was determined by a collagen yield of greater than 2%. This criterion indicates that all of the samples used in this study exhibited exceptional preservation as all collagen yields are well above 2% (Tables 5 and 6).

Good collagen preservation is also determined by the percent weight of the carbon and nitrogen concentrations of the collagen (Ambrose, 1990; Ambrose and Norr, 1992). These values provide a clear indication of the sample preservation but are also the least used criteria, likely because of the wide ranges and slight overlap. Bone collagen has a carbon concentration of 15.3 – 47% and a nitrogen concentration of 5.5 – 17.3% (Ambrose, 1990; Ambrose and Norr, 1992). Again, the samples used in this study exhibit exceptional preservation as all carbon and nitrogen concentrations are within the accepted limits (Tables 5 and 6).

The final and most commonly referenced indicator of collagen preservation is the atomic C:N ratio. The accepted range for this value is 2.9 – 3.6 (DeNiro, 1985; DeNiro, 1987; Schoeninger et al., 1989; Ambrose, 1990; Ambrose and Norr, 1992; van Klinken, 1999). Katzenberg (2008) states that modern mass spectrometers calculate C:N ratios that are typically 1.16667 lighter than the atomic ratios described by DeNiro (1985) and Ambrose (1990). Thus, the atomic ratios received by the CPSIL are recalculated using the following equation:

$$C{:}N\ ratio = (14 \div 12) \times (\frac{C\%weight}{N\%weight}) \quad \textbf{(8)}$$

This criterion of collagen preservation indicates that the samples used in this study are of exceptional preservation (Tables 5 and 6).

Table 5: Summary of Collagen Preservation Values for Bone Collagen

Sample (Bone collagen)	**Collagen Yield (%)**	**%C Weight**	**%N Weight**	**Atomic C:N Ratio**
Accepted Values	> 2%	15.3 – 47	5.5 – 17.3	2.6 – 3.9
KAT-2B	18.29	46.13	16.88	3.19
KAT-3	16.16	44.92	16.48	3.18
KAT-4	19.05	47.41	17.64	3.14
KAT-5	15.75	46.02	17.04	3.15
KAT-6	17.05	48.45	18.00	3.14
KAT-8	17.89	45.02	16.80	3.13
KAT-9	16.56	46.87	17.52	3.12
KAT-10	17.35	41.02	15.23	3.14
KAT-11	18.83	47.51	17.84	3.11
KAT-11a	18.27	45.74	17.21	3.10
KAT-12	17.69	43.10	16.22	3.10
KAT-13	15.95	44.35	16.79	3.08
KAT-14a	18.01	44.10	16.66	3.09
KAT-15	17.95	43.43	16.54	3.06
KAT-16	17.39	44.16	16.85	3.06
KAT-17	18.39	45.79	17.57	3.04
KAT-18	16.14	45.11	17.37	3.03
KAT-19	19.83	44.47	17.18	3.02
KAT-20	18.15	47.15	18.27	3.01
KAT-24	17.53	48.91	17.88	3.19
KAT-25	16.98	45.09	16.44	3.20

Table 6: Summary of Collagen Preservation Values for Dentin

Sample (Dentin)	**Collagen Yield (%)**	**%C Weight**	**%N Weight**	**Atomic C:N Ratio**
Accepted Values	> 2%	15.3 – 47	5.5 – 17.3	2.6 – 3.9
KAT-K2B	13.94	43.99	16.10	3.19
KAT-K4	14.91	44.33	16.16	3.20
KAT-K5	16.77	43.59	15.89	3.20
KAT-K5A	16.92	43.22	15.73	3.21
KAT-K6	13.55	44.03	15.98	3.21
KAT-K10	14.02	43.46	15.81	3.21
KAT-K11	15.93	44.01	15.98	3.21
KAT-K12	16.45	43.75	15.97	3.20
KAT-K15	14.17	44.28	16.21	3.19
KAT-K17	15.07	43.54	15.86	3.20
KAT-K18	14.81	43.80	16.02	3.19
KAT-K19	15.53	43.18	15.77	3.19
KAT-K20	14.25	44.24	16.19	3.19
KAT-K21	14.30	41.66	15.19	3.20
KAT-K24	14.29	44.23	16.05	3.22
KAT-K25	13.96	44.23	16.28	3.17

Several bone samples exhibit carbon and nitrogen weight concentrations above the upper range limit of 47% and 17.3%, respectively. However, the other indicators of collagen preservation, e.g. the atomic C:N ratio, support the inclusion of these samples in the study.

The preservation of the inorganic portion of bone must also be considered. While, bone apatite is often preserved in bone that has suffered extensive collagen degradation, it is even more susceptible to the effects of diagenesis than bone collagen. This is due to the potential transfer of minerals from the surrounding sediment and groundwater (Hedges, 2002; Katzenberg, 2008; Yoder and Bartelink, 2010). The most reliable form of determining the preservation of bone apatite and the impact, if any, of diagenesis is through the use of Fourier transform infrared

spectroscopy (FTIR) (Yoder and Bartelink, 2010). This particular analysis was not performed on the study samples due to its unavailability. Instead the preservation will be estimated via the percent yield of apatite after pretreatment; enamel, with a much lower organic content, consists of 95-97% apatite while bone consists of 75-80% apatite (Katzenberg, 2008; Crowley and Wheatley, 2014). This criterion of apatite preservation indicates that the samples used in this study are of exceptional preservation (Table 7).

Table 7: Summary of Preservation Values for Bone Apatite and Enamel

Sample (Bone apatite)	**Apatite Yield (%)**	***Sample (Enamel)***	**Apatite Yield (%)**
KAT-K2B	78.50	*KAT-K2B*	94.07
KAT-K3	73.12	*KAT-K4*	93.40
KAT-K4	82.95	*KAT-K5*	94.20
KAT-K5	73.53	*KAT-K5A*	90.57
KAT-K6	74.39	*KAT-K6*	93.75
KAT-K8	67.46	*KAT-K10*	93.29
KAT-K9	60.27	*KAT-K11*	93.44
KAT-K10	68.21	*KAT-K12*	93.39
KAT-K11	85.40	*KAT-K15*	95.16
KAT-K11a	64.00	*KAT-K17*	93.80
KAT-K12	74.59	*KAT-K18*	94.91
KAT-K13	68.40	*KAT-K19*	92.36
KAT-K14a	68.59	*KAT-K20*	86.24
KAT-K15	71.70	*KAT-K21*	78.78
KAT-K16	70.87	*KAT-K24*	94.78
KAT-K17	79.50	*KAT-K25*	90.65
KAT-K18	74.32		
KAT-K19	70.39		
KAT-K20	70.36		
KAT-K21	63.13		
KAT-K24	72.97		
KAT-K25	74.49		

Stable Isotope Value Results

Stable isotope analyses were conducted on a total of 38 samples (Table 8) from a crypt under the northern nave of Vilnius Cathedral to determine diet and geographical location differences from childhood and adulthood where possible. Stable carbon and nitrogen isotope values were determined from bone collagen for 21 samples and from dentin for 16 samples. Stable carbon and oxygen isotope values were determined from bone apatite for 22 samples and from enamel for 16 samples.

Table 8: Results of the Stable Isotope Analyses

Sample ID	**Collagen**				**Apatite**			
	Bone		Dentin		Bone		Enamel	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{18}O_{VSMOW}$	$\delta^{13}C$	$\delta^{18}O_{VSMOW}$
KAT-K2B	-18.7	10.6	-18.7	12.1	-11.6	23.7	-11.6	22.8
KAT-K3	-20.0	13.5			-14.9	25.3		
KAT-K4	-20.0	11.0	-20.0	10.2	-14.9	26.9	-14.1	26.1
KAT-K5	-19.8	11.6	-19.2	11.5	-14.3	26.8	-13.4	26.2
KAT-K5A			-19.7	11.1			-14.2	24.9
KAT-K6	-19.9	10.8	-20.0	9.6	-14.5	26.6	-14.0	24.4
KAT-K8	-19.8	13.1			-13.8	26.1		
KAT-K9	-20.3	12.2			-15.8	26.6		
KAT-K10	-19.7	11.8	-19.3	11.7	-14.5	26.6	-12.8	24.9
KAT-K11	-19.6	13.1	-19.5	12.5	-14.5	26.8	-14.3	26.3
KAT-K11a	-20.0	12.0			-14.1	25.0		
KAT-K12	-19.8	11.2	-18.7	11.0	-14.0	25.2	-8.9	22.1
KAT-K13	-20.2	10.8			-13.6	24.1		
KAT-K14a	-19.7	11.5			-12.9	26.9		
KAT-K15	-19.9	12.9	-19.0	12.4	-15.4	28.0	-13.9	25.3
KAT-K16	-20.3	11.0			-14.8	26.4		
KAT-K17	-20.3	10.8	-20.3	10.1	-14.1	24.9	-14.5	22.4
KAT-K18	-19.4	12.4	-19.3	11.9	-13.5	26.5	-14.0	24.9
KAT-K19	-20.3	12.3	-20.3	11.7	-13.8	25.4	-14.7	23.0
KAT-K20	-19.4	12.6	-19.8	12.8	-14.5	26.6	-14.9	27.0
KAT-K21			-19.6	10.9	-15.1	27.1	-13.9	26.1
KAT-K24	-20.5	11.6	-19.4	12.3	-14.2	26.8	-13.4	26.0
KAT-K25	-20.1	11.1	-19.8	11.5	-14.4	26.6	-13.9	25.9

Bone Collagen

The $\delta^{13}C$ values range from -20.5‰ to -18.7‰ with a mean value of -19.9 ± 0.4 (1σ) (Fig. 8). The $\delta^{15}N$ values range from 10.6‰ to 13.5‰ with a mean value of 11.8‰ ± 0.9 (1σ) (Fig. 8). Several outliers were noted for this data set. Individual KAT-K2B has both the most depleted $\delta^{13}C$ value at -18.7‰, which is 1.2‰ higher than the average, and the most depleted $\delta^{15}N$ value at 10.6‰, which is 1.2‰ lower than the average. Individual KAT-K3 has the most enriched $\delta^{15}N$ value at 13.5‰, which is 1.7‰ higher than the average. Individual KAT-K24 has the most enriched $\delta^{13}C$ value at -20.5‰, which is 0.6‰ lower than the average.

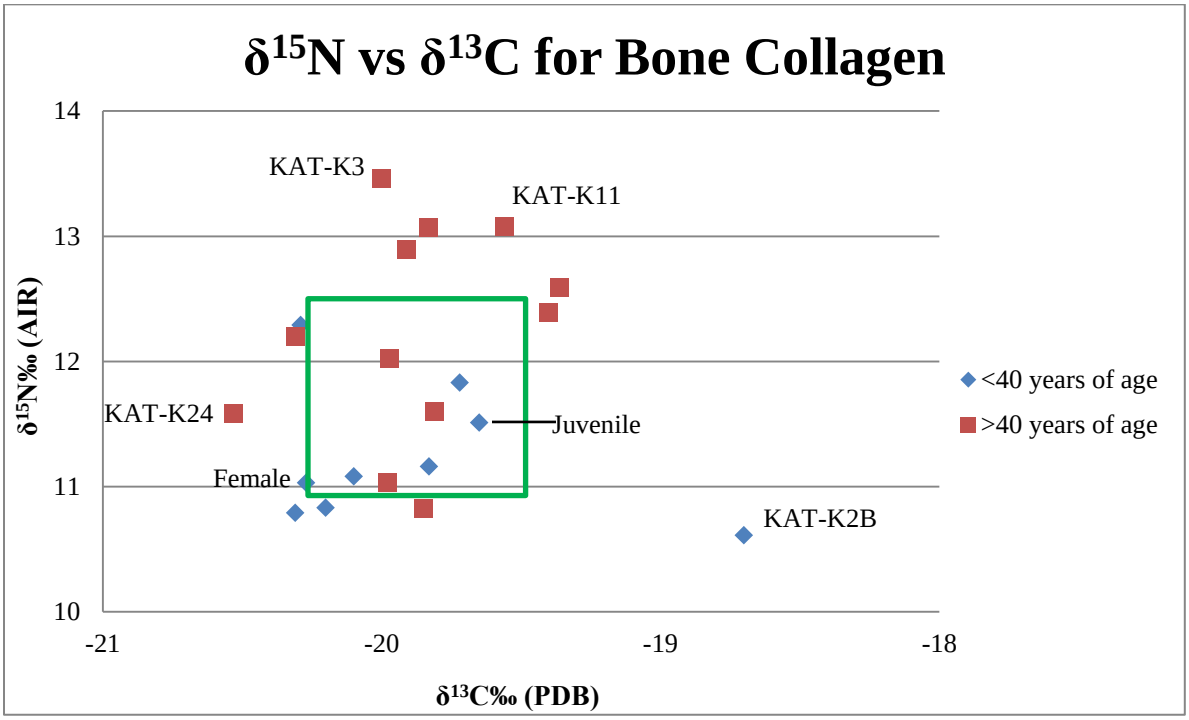

Figure 8: Graph of the stable carbon and nitrogen values for bone collagen; several outliers have been labeled. The green box represents the average $\delta^{15}N$ and $\delta^{13}C$ values ± 1σ.

Dentin

The $\delta^{13}C$ values range from -20.3‰ to -18.7‰ with a mean value of -19.5 ± 0.5 (1σ) (Fig. 9). The $\delta^{15}N$ values range from 9.6‰ to 12.8‰ with a mean value of 11.5‰ ± 0.9 (1σ) (Fig. 9). The values for the dentin collagen are widespread and do not appear to cluster around any certain value, however there are still several outliers. Individual KAT-K12 has the most depleted $\delta^{13}C$ value at -18.7‰, which is 0.8‰ higher than the average. Individual KAT-K20 has the most enriched $\delta^{15}N$ value at 12.8‰, which is 1.3‰ higher than the average. Individual KAT-K17 has most enriched $\delta^{13}C$ value at -20.3‰, which is 0.8‰ lower than the average. Individual KAT-K16 has the most depleted $\delta^{15}N$ value at 9.6‰, which is 1.9‰ lower than the average.

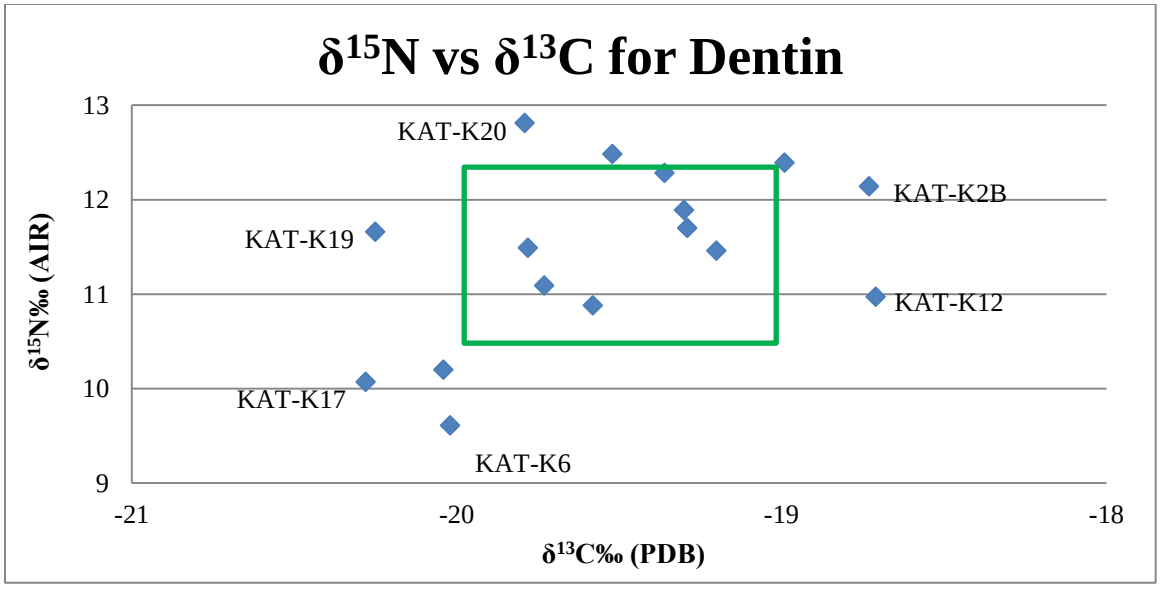

Figure 9: Graph of stable carbon and nitrogen values of dentin; several outliers have been labeled. The green box represents the average $\delta^{15}N$ and $\delta^{13}C$ values ± 1σ.

Bone Apatite

The $\delta^{13}C$ values range from -15.8‰ to -11.6‰ with a mean value of -14.2‰ ± 0.9 (1σ) (Fig. 10). The $\delta^{18}O$ values range from 23.7‰$_{VSMOW}$ (-7.0‰$_{VPDB}$) to 28.0‰$_{VSMOW}$ (-2.9‰$_{VPDB}$) with a mean value of 26.1‰$_{VSMOW}$ ± 1.1 (1σ) (-4.7‰$_{VPDB}$ ± 1.0 (1σ)) (Fig. 10). The $\delta^{18}O$ values were converted from Vienna Pee Dee Belemnite (VPDB) to Vienna Standard Mean Ocean Water (VSMOW) using the following equation (Coplen, 1988):

$$\delta^{18}O(VSMOW) = 30.91 + 1.03091 \times \delta^{18}O(VPDB) \quad \textbf{(9)}$$

Several outliers exist for the bone apatite values. Individual KAT-K2B has the most depleted $\delta^{13}C$ value (also seen in bone collagen) at -11.6‰, which is 2.6‰ higher than the average. This individual also has most depleted $\delta^{18}O$ at 23.7‰, which is 2.4‰ lower than the average. Individual KAT-K15 has the most enriched $\delta^{18}O$ value at 28.0‰, which is 1.9‰ higher than the average. Individual KAT-K9 has the most enriched $\delta^{13}C$ value at -15.8‰, which is 1.6‰ lower than the average.

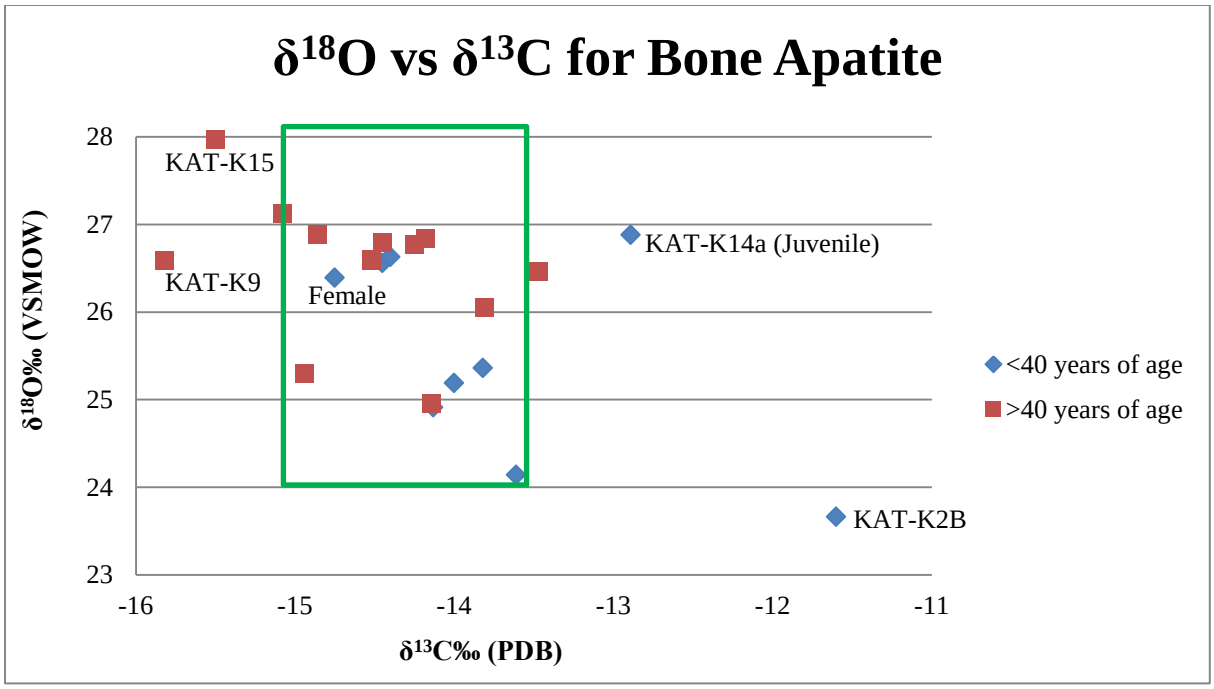

Figure 10: Graph of stable carbon and oxygen values for bone apatite; several outliers have been labeled. The green box represents the average $\delta^{18}O$ and $\delta^{13}C$ values ± 1σ.

Enamel

The $\delta^{13}C$ values range from -14.9‰ to -8.9‰ with a mean value of -13.5 ± 1.5 (1σ) (Fig. 11). The $\delta^{18}O$ values range from 22.1‰$_{VSMOW}$ (-8.6‰$_{VPDB}$) to 27.0‰$_{VSMOW}$ (-3.8‰$_{VPDB}$) with a mean value of 24.9‰$_{VSMOW}$ ± 1.5 (1σ) (-5.8‰$_{VPDB}$ ± 1.5 (1σ)) (Fig. 11). The $\delta^{18}O$ values for enamel were also converted from VPDB to VSMOW using Equation 6.

The values for the enamel apatite are clearly clustered around the means but there are several outliers. Individual KAT-K12 has the most depleted $\delta^{13}C$ value at -8.7‰, which is 4.8‰ higher than the average. This individual also has the most depleted $\delta^{18}O$ value at 22.1‰, which

is 2.8‰ lower than the average. Individual KAT-K20 has the most enriched $\delta^{13}C$ value at -14.9‰, which is 1.4‰ lower than the average, as well as the most enriched $\delta^{18}O$ value at 27.0‰, which is 2.1‰ higher than the average.

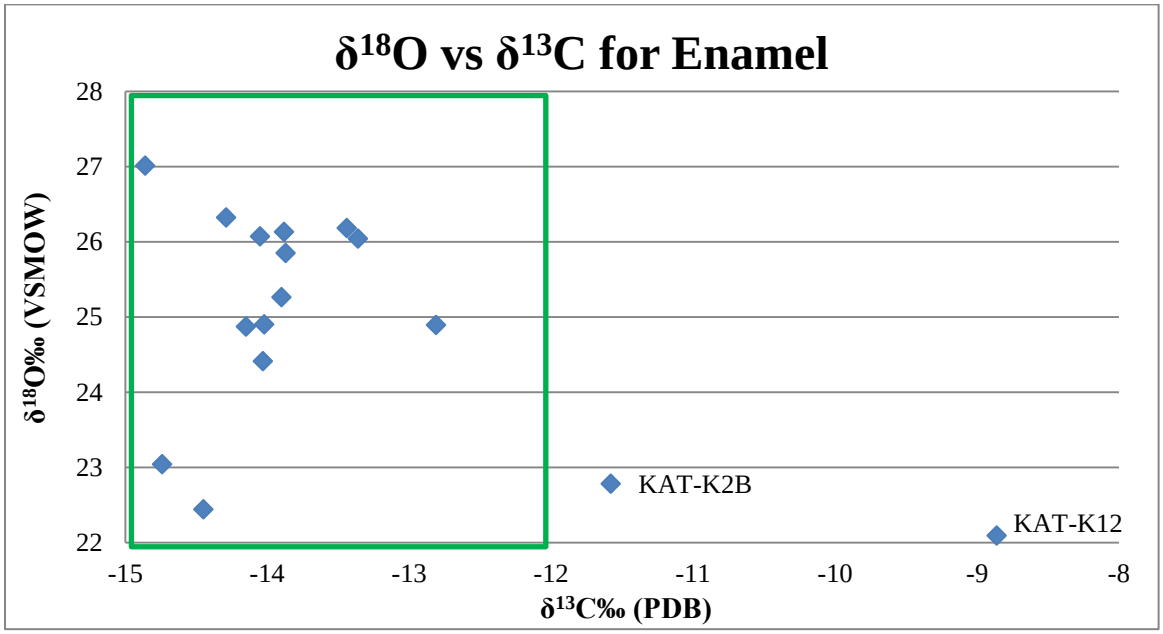

Figure 11: Graph of stable carbon and oxygen values for enamel; several outliers have been labeled. The green box represents the average $\delta^{18}O$ and $\delta^{13}C$ values ± 1σ.

Table 9: Results of Osteological Analysis of Study Samples (Conducted at Vilnius University)

Sample ID	Skeletal Pathology	Trauma
KAT-K3	Bechterew disease; osteoarthritis	Healed sharp force trauma on left parietal; healed fracture of right tibia and fibula
KAT-K4		
KAT-K5	Minor periostitis on right tibia	Healed blunt force trauma on right frontal eminence
KAT-K8	Degenerative joint disease – both shoulders, right elbow, both hips, both knees, and vertebral column	
KAT-K9	Osteomyelitis – right radius, right ulna, right femur, and left tibia; degenerative joint disease – both shoulders (marked), right wrist (slight), right knee (slight), and vertebral column	
KAT-K10	Minor periostitis on left tibia	Fractures of right 2nd and 3rd metatarsals
KAT-K11	Cribra orbitalia	Healed penetrating trauma on right parietal eminence; superficial healed blunt force trauma on left parietal and right frontal; healed fracture of nasal bones; healed fracture right ulna; healed fracture left fibula; healed trauma of left hand
KAT-K11A	Osteochondritis dissecans of right elbow	Healed nose trauma
KAT-K12	Periostitis of both tibia	Healed blunt force trauma on right parietal
KAT-K13		Healed Colles fracture of right radius
KAT-K15	Osteomyelitis of left tibia	
KAT-K16	Osteoperiostitis of left tibia	
KAT-K20	Periostitis of right tibia; degenerative joint disease of both knees (slight)	Healed fracture of right 5th metacarpal
KAT-K21	Periostitis of left tibia; degenerative joint disease – both wrists, both hips, and both knees	
KAT-K24	Osteoperiostitis of both tibia	
KAT-K25	Periostitis of both tibia	Healed sharp force trauma on left frontal bone, continuing into parietal; healed impression above left orbit

Comparing the Stable Isotope Values

Several comparisons can be made within the stable isotope values obtained from this study. Figure 12 highlights the differences between childhood and adulthood collagen values. Figure 13 shows this comparison between childhood and adulthood but uses the apatite values instead. Figures 14 and 15 compare the $\delta^{13}C$ values of collagen and carbonate for the tissues representing childhood and adulthood, respectively. Additionally, Table 10 provides the numerical difference between the carbonate and collagen values ($\Delta_{CA\text{-}CO}$), which current literature suggests to be +4.4‰ (Ambrose and Norr, 1993; Ambrose et al., 1997) or +7‰ (Lee-Thorp et al., 1989; Garvie-Lok, 2001). Figure 16 contrasts the $\delta^{18}O$ values for childhood and adulthood and Table 11 provides the numerical difference for a quicker view of which individuals may have migrated from outside Lithuania.

Isoscapes are vital when determining the meaning behind $\delta^{18}O$ values. These maps present oxygen values for regions all over the globe. Figure 17 depicts an oxygen isoscape of Europe. Due to the range of $\delta^{18}O$ values per color, the absolute value of the numerical difference between childhood and adulthood values will not be considered significant if the difference is greater than or equal to 0.93; individuals with values greater than 0.93 are considered to be foreigners. The isotopic composition of body and environmental water has a relationship defined by the following equation (Dupras and Schwarcz, 2001):

$$\delta^{18}O(CO_3 apatite) = 0.78\delta^{18}O(water) + 31.2 \quad \textbf{(10)}$$

Using this equation allows for the conversion of the $\delta^{18}O$ values of the bone and enamel apatite into $\delta^{18}O$ values for environmental water and thus allowing for comparison with the isoscape in Figure 17. Table 12 lists the $\delta^{18}O$ values from the bone and enamel apatite and the corresponding $\delta^{18}O$ environmental water values.

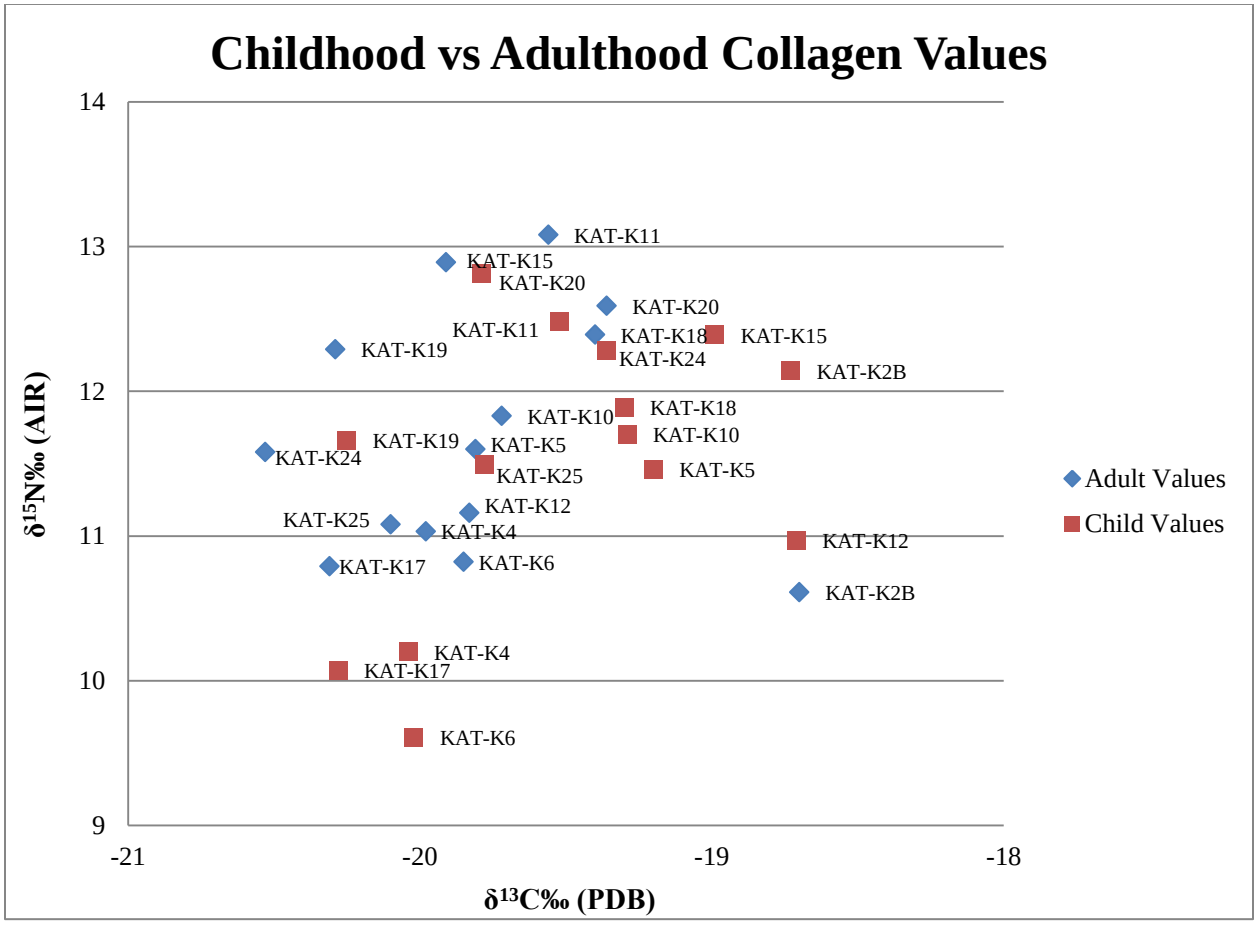

Figure 12: Graph of the collagen values for childhood (red squares) and adulthood (blue diamonds).

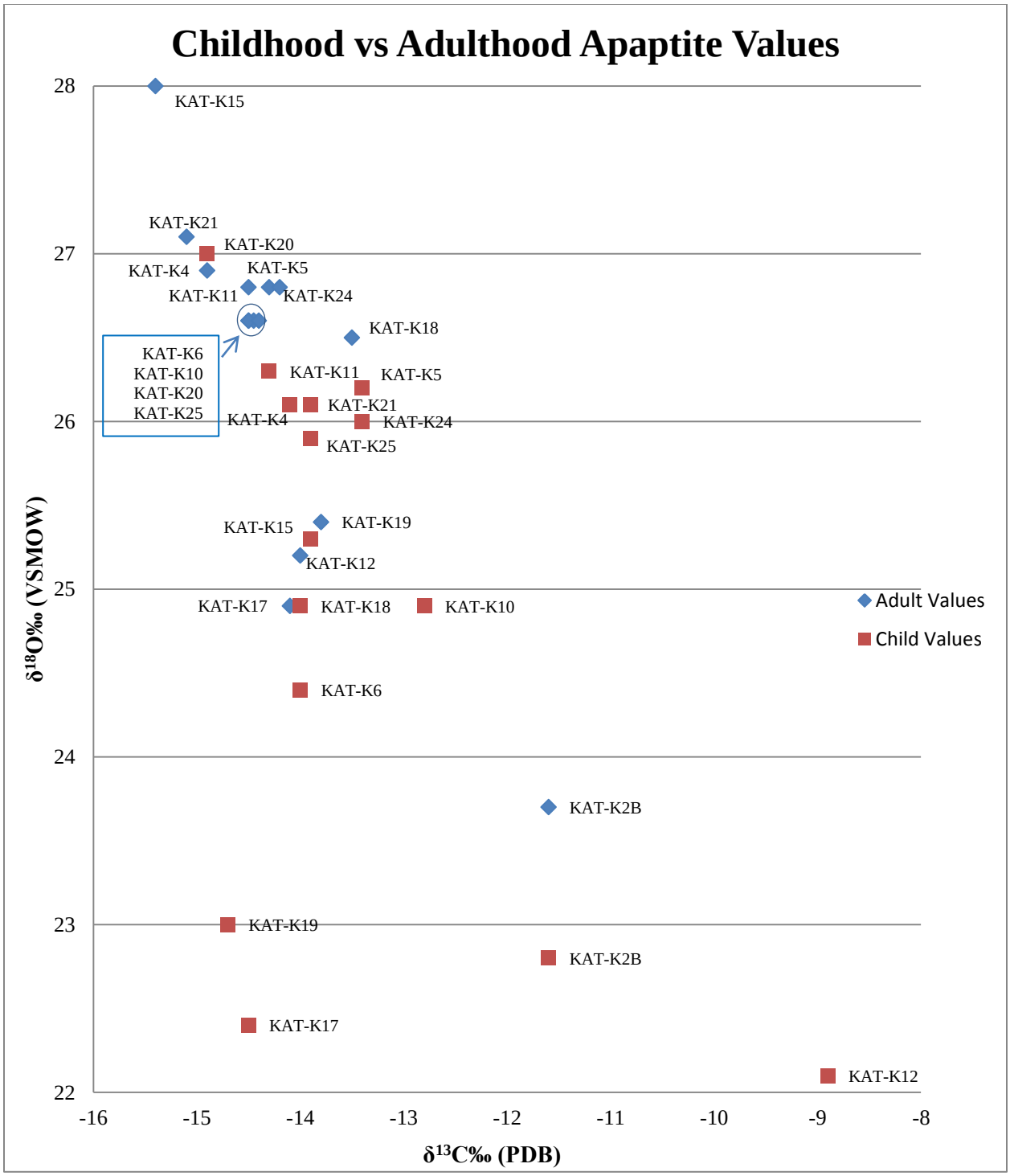

Figure 13: Graph of the apatite values for childhood (red squares) and adulthood (blue diamonds).

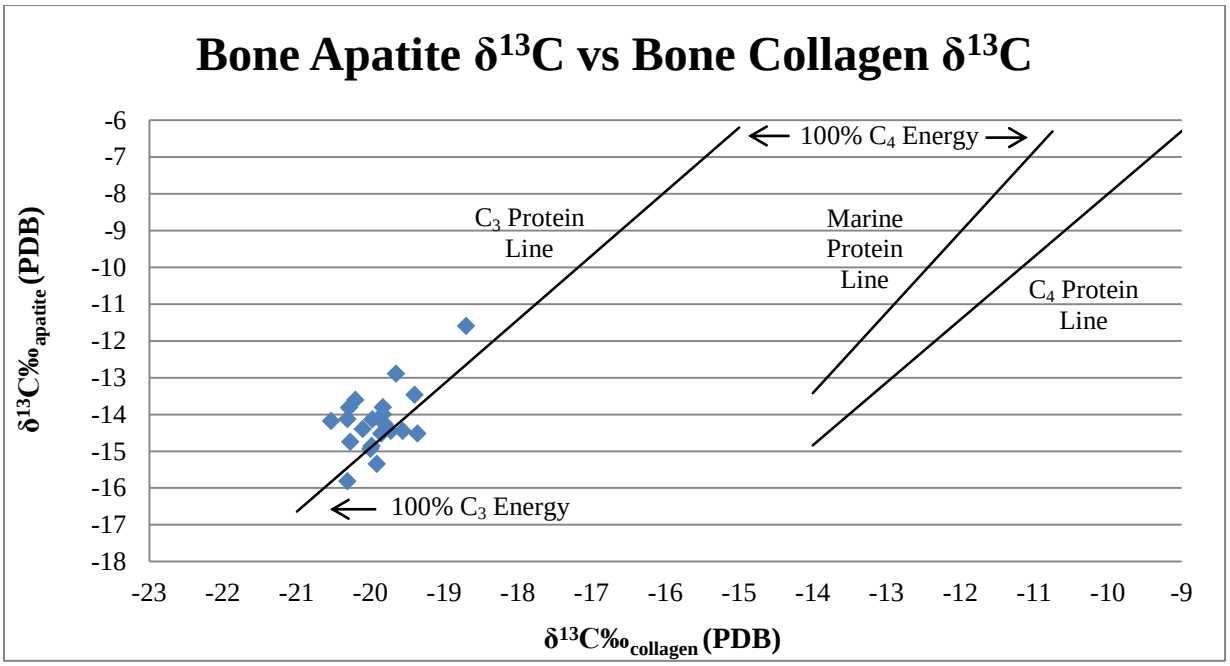

Figure 14: Graph of the $\delta^{13}C$ values for bone collagen and carbonate; several outliers have been labeled. Regression lines from Kellner and Schoeninger (2007). Refer to Table 9 for the numerical differences between the two tissues.

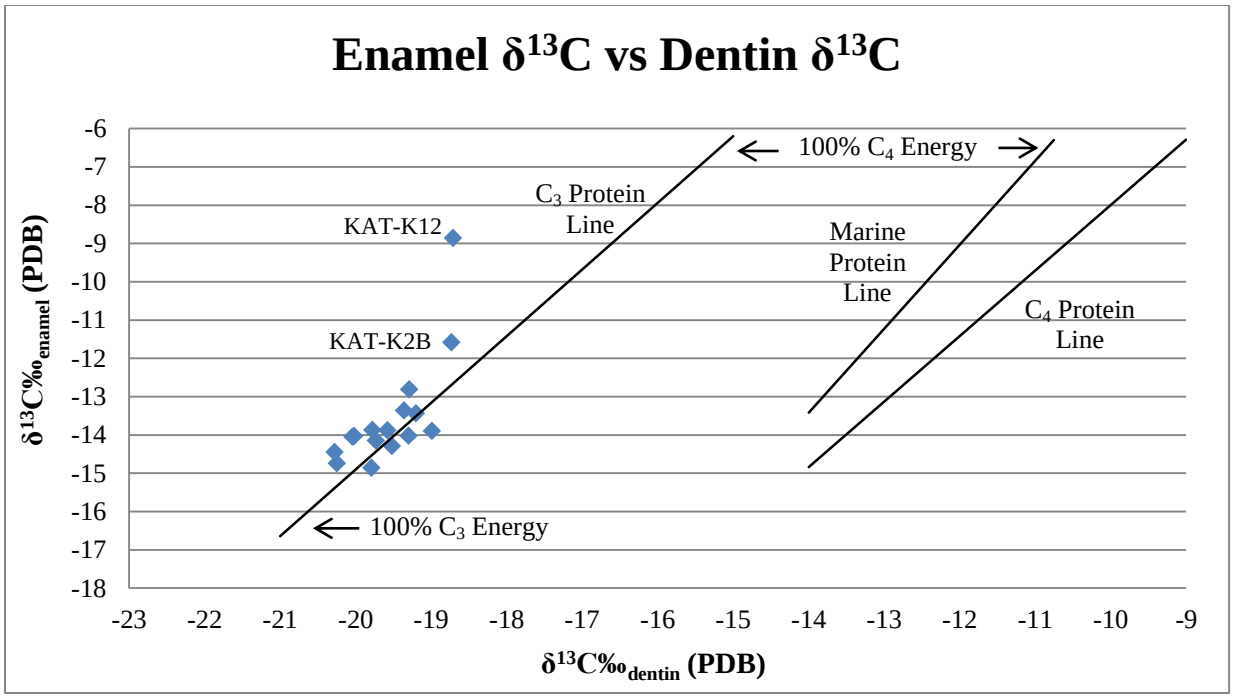

Figure 15: Graph of the $\delta^{13}C$ values for bone collagen and carbonate; several outliers have been labeled. Regression lines from Kellner and Schoeninger (2007). Refer to Table 9 for the numerical differences between the two tissues.

Table 10: Numerical Differences Between $\delta^{13}C$ Values for Carbonate and Collagen – Bone and Teeth

Sample ID (Bone)	**Δ_{CA-CO} (‰)**	***Sample ID (Teeth)***	**Δ_{CA-CO} (‰)**
KAT-K2B	7.10	*KAT-K2B*	7.15
KAT-K3	5.06	*KAT-K4*	5.99
KAT-K4	5.12	*KAT-K5*	5.76
KAT-K5	5.56	*KAT-K5A*	5.58
KAT-K6	5.33	*KAT-K6*	5.99
KAT-K8	6.02	*KAT-K10*	6.48
KAT-K9	4.49	*KAT-K11*	5.23
KAT-K10	5.27	*KAT-K12*	9.85
KAT-K11	5.11	*KAT-K15*	5.09
KAT-K11a	5.83	*KAT-K17*	5.83
KAT-K12	5.83	*KAT-K18*	5.28
KAT-K13	6.59	*KAT-K19*	5.51
KAT-K14a	6.76	*KAT-K20*	4.93
KAT-K15	4.56	*KAT-K21*	5.70
KAT-K16	5.52	*KAT-K24*	6.0
KAT-K17	6.18	*KAT-K25*	5.91
KAT-K18	5.93		
KAT-K19	6.47		
KAT-K20	4.84		
KAT-K24	6.35		
KAT-K25	5.70		
Average	**5.70 ± 0.72**		**6.02 ± 1.16**

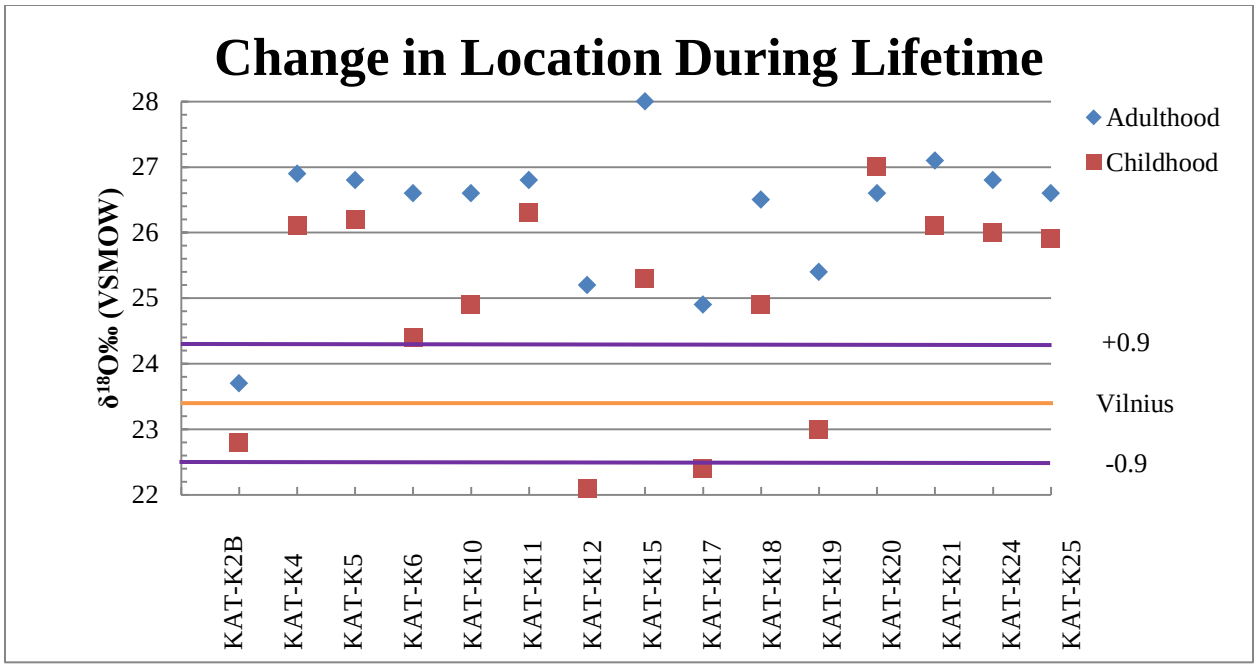

Figure 16: Graph of the $\delta^{18}O$ values for childhood (red squares) and adulthood (blue diamonds). The mean $\delta^{18}O$ annual precipitation value of Vilnius is represented by the orange line and the purple lines represent the ± 0.9 range. Refer to Table 10 for the absolute value of the numerical differences between these two tissues.

Table 11: Absolute Value of Numerical Differences between Childhood and Adulthood $\delta^{18}O$ Values*

Sample ID	$\lvert \Delta_{\text{Bone-Enamel}} \rvert$ **(‰)**
KAT-K2B	0.88
KAT-K4	0.81
KAT-K5	0.59
KAT-K6	**2.19**
KAT-K10	**1.67**
KAT-K11	0.47
KAT-K12	**3.10**
KAT-K15	**2.71**
KAT-K17	**2.47**
KAT-K18	**1.56**
KAT-K19	**2.32**
KAT-K20	0.42
KAT-K21	**0.99**
KAT-K24	0.80
KAT-K25	0.78

*those values indicative of individuals that changed location after 2 years of age and before 10 years prior to death are bolded

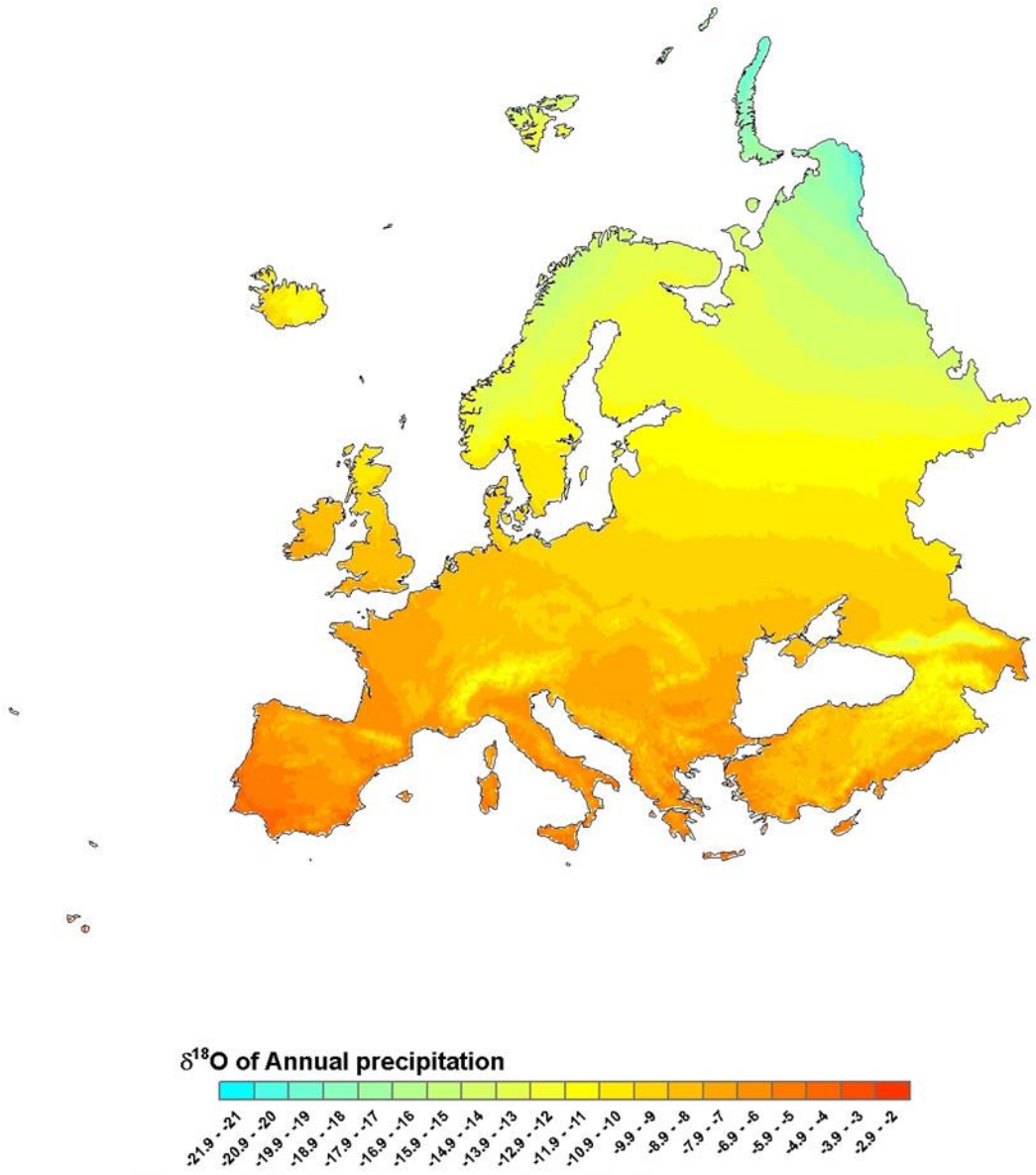

Figure 17: Isoscape map depicting oxygen values for the European continent; http://wateriso.utah.edu/waterisotopes/media/IsoMaps/jpegs/o_Euro/oma_Euro.jpg (Map public domain)

Using Equation 10 it is possible to determine that the average bone apatite $\delta^{18}O$ value for the study sample (26.1‰) correlates to an average water $\delta^{18}O$ value of -6.5‰. This value

represents a region much farther south than anticipated. It is possible that the map depicted in Figure 17 is inaccurate; however when utilizing a feature from http://waterisotopes.org it is calculated that the annual precipitation $\delta^{18}O‰_{VSMOW}$ value for Vilnius, Lithuania is -10.0‰. This suggests that a $\delta^{18}O_{apatite}$ value of 23.4‰ ±0.9 (22.5‰ to 24.3‰) correlates to the environmental water of Vilnius. If this is the case then the majority of the individuals in this study sample relocated to Vilnius during the last decade of their life and, due to turnover rates, this change would not be seen in the stable isotope values (Dupras and Schwarcz, 2001) (Table 12).

Table 12: Oxygen Values for Apatite ($\delta^{18}O_A$) and for Environmental Water ($\delta^{18}O_W$)

Sample ID (Bone)	**$\delta^{18}O‰_A$**	**$\delta^{18}O‰_W$**	***Sample ID (Tooth)***	**$\delta^{18}O‰_A$**	**$\delta^{18}O‰_W$**
KAT-K2B	23.7	-9.6	*KAT-K2B*	22.8	-10.8
KAT-K3	25.3	-7.6	*KAT-K4*	26.1	-6.5
KAT-K4	26.9	-5.5	*KAT-K5*	26.2	-6.4
KAT-K5	26.8	-5.6	*KAT-K5A*	24.9	-8.1
KAT-K6	26.6	-5.9	*KAT-K6*	24.4	-8.7
KAT-K8	26.1	-6.5	*KAT-K10*	24.9	-8.1
KAT-K9	26.6	-5.9	*KAT-K11*	26.3	-6.3
KAT-K10	26.6	-5.9	*KAT-K12*	22.1	-11.7
KAT-K11	26.8	-5.6	*KAT-K15*	25.3	-7.6
KAT-K11a	25.0	-7.9	*KAT-K17*	22.4	-11.3
KAT-K12	25.2	-7.7	*KAT-K18*	24.9	-8.1
KAT-K13	24.1	-9.1	*KAT-K19*	23.0	-10.5
KAT-K14a	26.9	-5.5	*KAT-K20*	27.0	-5.4
KAT-K15	28.0	-4.1	*KAT-K21*	26.1	-6.5
KAT-K16	26.4	-6.2	*KAT-K24*	26.0	-6.7
KAT-K17	24.9	-8.1	*KAT-K25*	25.9	-6.8
KAT-K18	26.5	-6.0			
KAT-K19	25.4	-7.4			
KAT-K20	26.6	-5.9			
KAT-K21	27.1	-5.3			
KAT-K24	26.8	-5.6			
KAT-K25	26.6	-5.9			
Average	**26.1**	**-6.5**		**24.9**	**-8.1**

CHAPTER FIVE: DISCUSSION

Childhood Values

Teeth develop in a regimented manner and once fully developed their chemical structure retains the footprint of their development due to the static nature of the tissues (Hillson, 2008). The permanent first molars begin forming at birth with the completion of the crown occurring prior to that of the root. The crown of the tooth is fully mineralized by about 2.5 years (Scheuer and Black, 2004). The root is fully developed by about 7 years (Scheuer and Black, 2004). These facts mean that stable isotope values derived from the apatite of a permanent first molar (this study) will inform on the average diet and geographical location of an individual from birth to 2.5 years. Stable isotope values derived from the collagen of a permanent first molar (this study) will inform on the average diet of an individual from 3.2 to 7 years of age (Scheuer and Black, 2004 pp. 175).

Overall the $\delta^{13}C$ values of the enamel apatite (mean of -13.5‰) support the conclusion that these juveniles were consuming a diet primarily of C_3 plants (Fig. 15). As noted previously, ^{13}C values from carbonate experience a larger fractionation value than what is seen in collagen. The typical fractionation between diet and carbonate ^{13}C values has been debated in the literature as either +12.0‰ (Lee-Thorp et al., 1989; Garvie-Lok, 2001) or +9.4‰ (Ambrose and Norr, 1993; Ambrose et al., 1997; Harrison and Katzenberg, 2003; Kellner and Schoeninger, 2007). The fractionation value of +9.4‰ is accepted for this study due to greater consensus within the literature on that value and the idea that digestive physiology rather than body size has a greater influence on the fractionation value observed in apatite (Kellner and Schoeninger, 2007). Thus,

the average $\delta^{13}C$ values of the diet can be estimated to be -22.09‰ which is representative of C_3 plants. This value is slightly depleted from the mean $\delta^{13}C$ value of C_3 plants and could indicate some consumption of C_4 plants, such as millet (Stančikaitė et al., 2008), during the end of crown development as deciduous teeth begin to erupt into the mouth at around one year (Scheuer and Black, 2004). There are several outliers in this data set (Fig. 11) but individual KAT-K12 has the most depleted $\delta^{13}C$ value at -8.9‰. The average $\delta^{13}C$ value for this individual's diet is 18.3‰, which suggests a diet with a greater portion of C_4-sourced lipids and carbohydrates (Fig. 15).

The $\delta^{13}C$ values of the dentin collagen (mean of -19.5‰) also support the conclusion of a diet made up predominantly of C_3-sourced protein (Fig. 15). The fractionation value between diet and collagen ^{13}C is +5‰. Thus, the average $\delta^{13}C$ values of the diet can be estimated to be -24.5‰. This value is more enriched than the values seen in the apatite and could be related to the extended growth time of the roots as well as this development occurring when all deciduous teeth have erupted (just under 2.5 years (Scheuer and Black, 2004)) and the individual has the ability to consume more complex foods. It is unlikely that there was any consumption of marine foods (Fig. 15) as most animals and plants occupying this type of niche have $\delta^{13}C$ values between -11.0‰ to -19.0‰ (Barret et al., 2008) which is well below the values of the study sample.

The $\delta^{15}N$ values of the dentin collagen (mean of 11.5) are suggestive of a diet in which protein consumption was almost exclusively from terrestrial fauna (Schoeninger et al., 1983). This value, while still within the range for terrestrially sourced protein (Table 1) is at the upper limits. The fractionation between diet and $\delta^{15}N$ values is accepted to be +3‰, thus the $\delta^{15}N$

values of the diet are estimated to be 8.45‰. These values could indicate that there was some incorporation of marine resources but the lack of enrichment seen in the $\delta^{13}C$ values suggests that consumption of marine foods is sporadic, if at all (Fig. 15). These values also discount any freshwater resource consumption as the $\delta^{13}C$ values of the enamel and dentin are not depleted enough (< -22‰) to suggest this type to resource in the diet (Dufour et al., 1999). Another cause for the observed nitrogen enrichment is from nutritional stress, possibly related to disease or trauma. Figure 18 shows the childhood nitrogen values in relation to any reported disease or trauma on the skeleton (Table 9).

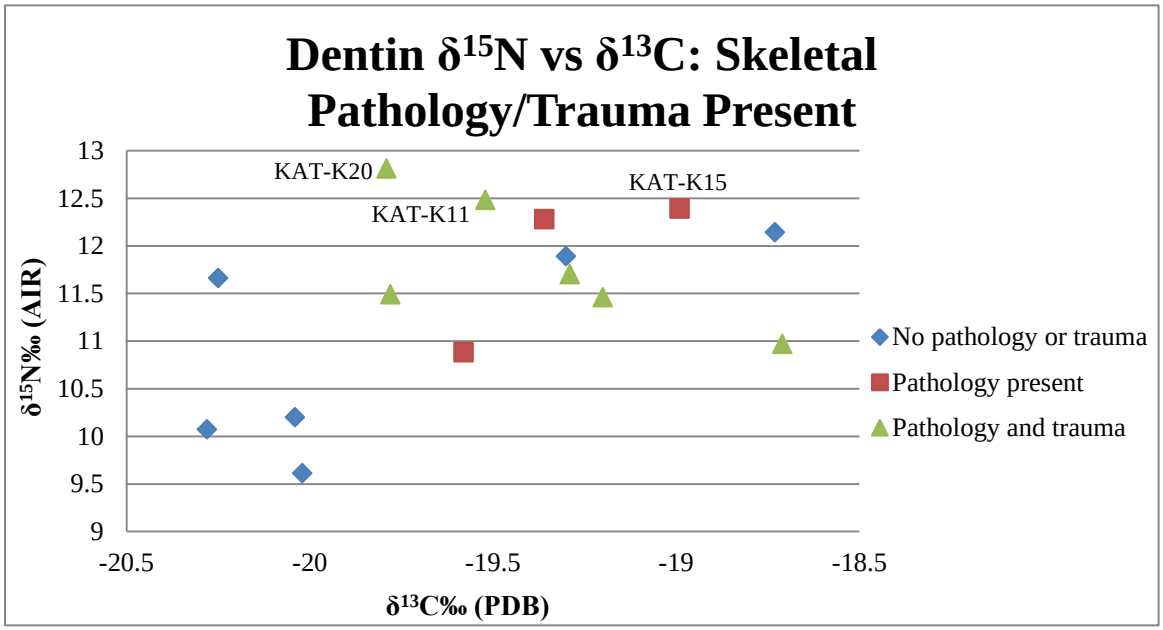

Figure 18: Graph showing relationship between childhood $\delta^{15}N$ values and the presence or absence of skeletal pathology and/or trauma as noted in the osteological examination done at Vilnius University; several outliers have been labeled.

It is acknowledged that the osteological examination was performed on the adult skeletons and not strictly applicable to the childhood nitrogen values. However, 50% of the analyzed individuals with no evidence of disease or trauma are well below those individuals with pathological bone. Additionally, the highest $\delta^{15}N$ values correlate to individuals with evidence of pathology or pathology and trauma. Individual KAT-K11 ($\delta^{15}N$ value of 12.48‰) has evidence of cribra orbitalia, a common symptom of iron deficiency (Ortner, 2003).

Some of the most interesting data comes from the $\delta^{18}O$ values from the enamel apatite. These values range from 22.09‰ to 27.01‰, with a mean value of 24.9‰. Utilizing the values from Figure 17 and Table 12, there appears to be five individuals (KAT-K2B, KAT-K6, KAT-K12, KAT-K17, and KAT-K19) that have $\delta^{18}O$ values consistent or close to those values given for Lithuania and some surrounding countries, such as Poland. This is unsurprising due to the close political relationship that Lithuania and Poland shared during this time period, i.e. the Polish-Lithuanian Commonwealth (1569 – 1648, AD) (Smith, 2002; Plakans, 2011). The remaining individuals have childhood values that would indicate ingestion of a water source that occurs further south, such as Greece, Italy, and Spain. It is also possible that these individuals could have been from Germany since after Lithuania converted to Christianity strong communities of both Russian and German migrants began to grow (Stancikaite et al., 2008).

Adulthood Values

The stable isotope data gathered from the bone apatite and collagen is considered to be representative of the average values for the last decade of life for each individual. However, KAT-K14a has been estimated to have died at 9.5 – 10.5 years of age and likely had a turnover

rate of bone at about 10-30% percent a year (Hedges et al., 2007). Thus, it is possible that the bone values for this individual may represent a timeframe of only three or five years which would coincide with the completion of the root development on the permanent first molar. Additionally, KAT-K16 and KAT-K17 died at around 20 years of age and the stable isotope values from their bones would be indicative of childhood values. However, all three of these individuals will still be discussed in this section for the ease of keeping all stable isotope values taken from bone in one section.

Overall, $\delta^{13}C$ values of the bone apatite (mean of -14.2‰) suggest a diet wherein the predominant energy intake is from C_3 plants (Fig. 14). After re-calculating this value to be an estimate of the average $\delta^{13}C$ value of the diet, the conclusion of a diet of C_3-sourced lipids and carbohydrates is still supported. This value is slightly lower than the $\delta^{13}C$ value calculated as an estimate of the average value of the diet during childhood. This could suggest that any incorporation of C_4 plants during the childhood years has been significantly reduced, if not completely excluded.

The $\delta^{13}C$ values of the bone collagen (-19.9‰) also suggest a diet predominately comprised of C_3-sourced protein. The estimated average value for the resources consumed is -24.9‰, which also supports the conclusion that dietary protein came from C_3 plants or sources that subsisted on C_3 plants. The $\delta^{13}C$ values of the bone collagen are neither enriched enough to suggest the incorporation of marine resources nor depleted enough to suggest the incorporation of freshwater resources in the diet. As was seen when comparing the dentin and enamel $\delta^{13}C$ values, the bone collagen carbon value is slightly more enriched than the apatite value. This is

likely the result of the carbon in collagen coming primarily from protein sources (Bayliss et al., 2004), thus the carbon in collagen is only representative of a fraction of the diet.

The $\delta^{15}N$ values of the bone collagen (mean of 11.8‰) are slightly enriched and could represent an increased proportion of aquatic resources in the diet. However, as mentioned previously nitrogen is heavily influenced by a variety of factors and one of the biggest impacts comes from the physiology of the individual being analyzed in which physiological stress will lead to an enrichment of ^{15}N. As evidenced by the results of the physical analysis of the skeletal remains (Table 9), all but five individuals (one individual was represented by a tooth only and not included in the physical analysis) displayed evidence for disease or trauma on the skeleton. It is highly likely that if 76% of the sample had evidence of disease and/or trauma, the remaining individuals could have been experiencing a physiological stress that affected soft tissue only. Additionally, those individuals who did not present with skeletal evidence of disease or trauma were mostly the younger individuals. This supports the assumption that there may have been an illness or damage that was only evident in soft tissue or that was relatively acute in nature, due to the fact that these younger individuals obviously died of something beyond old age. It is recognized that acute onset trauma or illness would likely not impact the $\delta^{15}N$ values of the bone collagen due to the averaging nature of the methods used for analysis but it could have been possible that these individuals were frailer than most of the population and may have suffered illnesses for a longer period of time. Of course this is all conjecture without further evidence and more fine-tuned tissue analysis.

Of the individuals with no evidence of trauma or disease, 57% of them have $\delta^{15}N$ values at the bottom of the range obtained from this study (Fig. 19). The top five nitrogen values belong to individual with evidence of a disease or of a disease and trauma. Individual KAT-K3 has the most enriched $\delta^{15}N$ value at 13.46‰. Additionally, this individual, a male of at least 55 years of age, appears to have suffered from Bechterew's disease or ankylosing spondylitis. This disease is most commonly marked by excessive bony growth underneath the longitudinal ligament running alongside the spinal column but can occur at any joint margin and results in decreased flexibility and pain in the affected areas (Ortner, 2003).

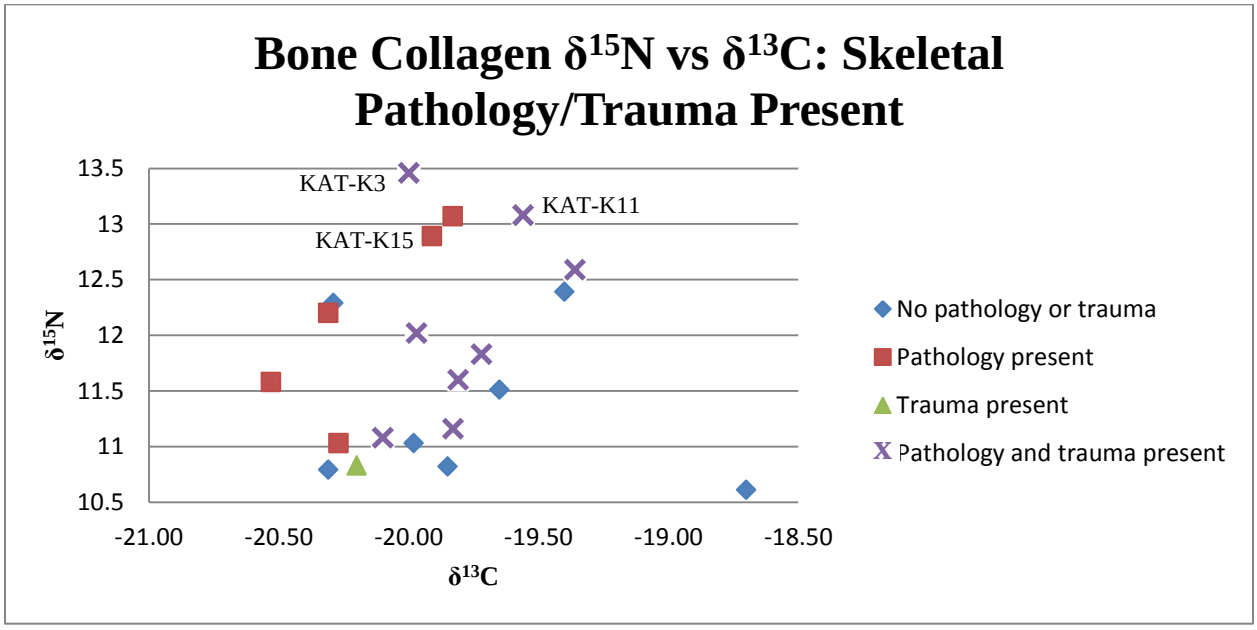

Figure 19: Graph showing relationship between adulthood $\delta^{15}N$ values and the presence of skeletal pathology and/or trauma observed during the morphological analysis conducted at Vilnius University; several outliers have been labeled. Note that the $\delta^{15}N$ values are highest in individuals with pathology and pathology and trauma.

Again, the $\delta^{18}O$ values offer some interesting clues as to the background and life histories of these individuals. The values range from 23.66‰ to 27.97‰, with a mean value of 26.12‰. All but one of the adult-related oxygen values are more enriched than the childhood values. Individual KAT-K20, an older male, has oxygen values that decreased from 27.0‰ to 26.6‰ from childhood to adulthood, respectively. This decrease is much less than the 0.93 difference suggested as representing a shift in location (Fig. 17). Eight individuals show significant differences between childhood and adulthood related oxygen values (Table 11), suggesting that these individuals changed location after the complete formation of their teeth, around two years, and before the last decade of life. Individuals KAT-K6, KAT-K10, KAT-K12, KAT-K15, KAT-K17, KAT-K18, KAT-K19, and KAT-K21 all have a difference of at least 0.93 between their childhood and adulthood values, indicating that their main water intake occurred in two different regions (Fig. 17). Individual KAT-K17 is one of the younger individuals included in this study at 18-20 years of age and appears to have migrated from north of Lithuania, likely Russia (Stancikaite et al., 2008) to somewhere further south of Lithuania, such as Poland or Belarus. All of these individuals experienced enrichment in their oxygen values which could suggest that prior to the last ten years of life these individuals were in region further south and/or closer to the sea than where they were when they were around two years of age. The oxygen values suggest that these individuals spend time in southern Europe, perhaps Spain or Italy.

Chenery, et al (2012) suggests that the $\delta^{18}O$ values ingested with food may have more of an impact on the oxygen concentration in carbonate than is recognized. If this is true, then it is possible that the enriched $\delta^{18}O$ values seen in the study sample (in relation to those values

plotted for Lithuania on the isoscape) may be the result of ingesting resources imported or traded from areas with naturally occurring enriched $\delta^{18}O$ values.

Individual Outliers

There are several individuals with stable isotope values classified as outlying (Table 13). Outlying values are determined to be those values that are greater than one standard deviation away from the average value (two standard deviations away for ^{18}O values). Each individual with an outlying stable isotope value will be discussed in further detail.

Table 13: Individuals with Outlying Stable Isotope Values

Sample ID	**$\delta^{15}N$ (Bone)**	**$\delta^{15}N$ (Dentin)**	**$\delta^{13}C_{collagen}$ (Bone)**	**$\delta^{13}C$ (Dentin)**	**$\delta^{13}C_{apatite}$ (Bone)**	**$\delta^{13}C$ (Enamel)**	**$\delta^{18}O$ (Bone)**	**$\delta^{18}O$ (Enamel)**
Average	**11.8‰**	**11.5‰**	**-19.9‰**	**-19.5‰**	**-14.2‰**	**-13.5‰**	**26.1‰**	**24.9‰**
KAT-K2B	10.6‰		-18.7‰	-18.7‰	-11.6‰	-11.6‰	23.7‰	
KAT-K3	13.5‰							
KAT-K4		10.2‰						
KAT-K6	10.8‰	9.6‰						
KAT-K8	13.1‰							
KAT-K9					-15.8‰			
KAT-K11	13.1‰	12.5‰						
KAT-K12				-18.7‰		-8.9‰		
KAT-K13	10.8‰							
KAT-K14a					-12.9‰			
KAT-K15	12.9‰			-19.0‰	-15.4‰			
KAT-K17	10.8‰	10.1‰		-20.3‰				
KAT-K19				-20.3‰				
KAT-K20		12.8‰	-19.4‰					

KAT-K2B has the greatest frequency of outlying values. This individual is a 30-35 year old male with no evidence of skeletal pathology or trauma. The carbon values are all enriched when compared with the average value, suggesting that this individual had a larger amount of

C_4 – based resources throughout their lifetime (Figs. 14 and 15). This individual's bone $\delta^{15}N$ value (10.6‰) is depleted when compared with the average value. This depletion makes sense due to a lack of observable nutritional stress on the skeleton. This does not indicate that they were in good health but a value of 10.6‰ falls within the terrestrial diet range (6‰ – 12‰) provided by Schoeninger et al. (1983), albeit on the higher side. Lastly, K2B's ^{18}O values are of interest in that only their adulthood value is considered to be an outlier. Figure 16 indicates that KAT-K2B is the only individual that lived in or around Vilnius their entire life. It is conceivable that this individual's values are a better representation of the urban Lithuanian diet than the other individuals of the study.

KAT-K3 is one of the older individuals as a 55+ year old male and has a greatly enriched $\delta^{15}N$ value at 13.5‰. This enrichment is likely due to the conditions of ankylosing spondylitis and osteoarthritis noted on the skeletal remains. Additionally, this individual has a healed sharp force trauma on the left parietal (trauma type unknown) as well as healed fractures to the right tibia and fibula (fracture type unknown). Trauma to the frontal or parietal bones is commonly associated with interpersonal violence (Lovell, 2008: Meyer et al., 2009; Šlaus et al., 2012). Sharp force traumas are usually the result of chopping, stabbing, or slashing and are therefore unlikely to be accidental. KAT-K3 is one of seven individuals with no childhood values but their adulthood ^{18}O value is outside the range for Vilnius. This is suggestive of a move to the city quite late in life, possibly from Germany, Ukraine, Hungary, or a similarly located country (Fig. 17).

KAT-K4 is a 45-50 year old male and has a depleted childhood $\delta^{15}N$ value of 10.2‰. This individual had no evidence of skeletal pathology or trauma, supporting this depleted value. Additionally, this individual has very similar $\delta^{18}O$ values from both their childhood and adulthood suggesting that they remained in approximately the same region. However, these values are outside the range for Vilnius and would indicate a move to the city late in life, possibly from Denmark, Poland, or Belarus (Fig. 17).

KAT-K6 is also one of the older males at 55+ years of age. Both his childhood (9.6‰) and adulthood (10.8‰) $\delta^{15}N$ values are depleted compared to the average. This individual has no evidence of skeletal pathology or trauma which would cause enrichment in the nitrogen values. KAT-K6 is one of the few individuals with ^{18}O values that fall within, or very close to, the range for Vilnius. However, their adulthood value is enriched by just over 2‰, suggesting time spent in regions other than Vilnius such as Poland, Belarus, or Denmark (Fig. 17).

KAT-K8 is a 50-55 year old male and has an enriched $\delta^{15}N$ value of 13.1‰. This individual showed skeletal evidence of degenerative joint disease in both his shoulders, right elbow, both hips, both knees, and his vertebral column. This disease has so far not been associated with nitrogen enrichment (Olsen et al., 2014), however nutritional stress is still a possibility due to the appetite suppression commonly associated with pain. KAT-K8 does not have any

childhood values for comparison but their adulthood $\delta^{18}O$ value falls outside of the range for Vilnius but could represent time spent in Poland or Denmark (Fig. 17).

KAT-K9 is a 40-50 year old male and has a depleted adulthood $\delta^{13}C_{apatite}$ value. This suggests that this individual's carbohydrate and lipid resources were comprised of a larger amount of C_3 – based plants. Despite skeletal evidence of osteomyelitis in the right radius, right ulna, right femur, and left tibia and degenerative joint disease in both shoulders (marked), right wrist (slight), right knee (slight), and the vertebral column, this individual does not have an outlying nitrogen value, however the value is still enriched at 12.2‰. KAT-K9 has no childhood values for comparison but the adulthood $\delta^{18}O$ value lies outside of the range for Vilnius but within that seen in Poland, Denmark, and Belarus (Fig. 17).

KAT-K11 is a 45-50 year old male and has both childhood and adulthood $\delta^{15}N$ values enriched over the average. This is understandable as this individual has skeletal evidence of nutritional stress in childhood (cribra orbitalia) as well as a healed penetrating trauma on right parietal eminence; a superficial healed blunt force trauma on left parietal and right frontal; a healed fracture of the nasal bones; a healed fracture of the right ulna; a healed fracture of the left fibula; and a healed trauma of the left hand. This constellation of healed trauma is considered to be a good indicator of interpersonal violence (Lovell, 2008: Meyer et al., 2009; Šlaus et al., 2012), particularly the cranial and facial injuries. This individual's

$\delta^{18}O$ values indicate a life lived outside of the region of Vilnius but possibly in areas such as Poland, Belarus or Germany (Fig. 17).

KAT-K12 is one of the younger males at 25-30 years of age and has childhood $\delta^{13}C$ values enriched from the average. Figure 14 indicates that this individual consumed a larger portion of C_4 – based energy resources. Additionally, this individual had evidence of periostitis on both tibia as well as a healed blunt force trauma on the right parietal. Here again, there is trauma located on the parietal bone. Further details of the injury are not known but blunt force trauma could result from falling to the ground but it could also be the result of a blunt weapon such as a club (Lovell, 2008). KAT-K12 has the largest range of ^{18}O values at 3.1‰. Their childhood $\delta^{18}O$ values lie just outside of the range for Vilnius suggesting that they spend their early years in this region. However, their adulthood would have been spent much further south, i.e. Italy or Bulgaria (Fig. 17).

KAT-K13 is also one of the younger males at 25-30 years of age and has a depleted adulthood $\delta^{15}N$ value. This individual has a healed Colle's fracture of the right radius. This type of fracture is one of the most common types and is usually associated with a fall where the individual attempts to catch themselves by straightening their arm out in front of them (Lovell, 2008). This individual does not have childhood values available for comparison but the adulthood $\delta^{18}O$ values are suggestive of time spent in Germany, Slovakia, Hungary, or similarly located countries (Fig. 17).

KAT-K14a is the one juvenile in the sample at 9.5-10.5 years of age and is only represented by a bone sample. Their $\delta^{13}C_{apatite}$ value is enriched which suggests that they consumed a larger quantity of C_4 – sourced energy foods than the average individual in the study sample. There was no evidence of skeletal pathology or trauma on the remains. Interestingly, K14a's $\delta^{18}O$ value is indicative of time spent in Poland, Denmark or Belarus rather than Vilnius.

KAT-K15 is a 40-45 year old male and has an enriched adulthood $\delta^{15}N$ value, a depleted adult $\delta^{13}C_{apatite}$ value, and an enriched childhood $\delta^{13}C_{dentin}$ value. The ^{13}C values suggest that as a child this individual consumed a larger amount of C_4 – sourced protein resources and as an adult they consumed a larger amount of C_3 – sourced energy resources. This individual has evidence of osteomyelitis on the left tibia. This skeletal pathology is known to cause enrichment in nitrogen (Katzenberg and Lovell, 1999; Olsen et al., 2014). This individual has the most enriched $\delta^{18}O$ value during their adulthood. The value could indicate time spent in southern Europe, perhaps Italy or even Spain (Fig. 17).

KAT-K16 is the one possible female in the sample, aged at 20+ years. None of the values for this individual are considered to be outliers but the sex of the individual warrants a discussion. There is evidence of osteoporosis on the left tibia. Her $\delta^{18}O$ value suggests that this individual may have spent time in Poland (Fig. 17). Without any available childhood values for comparison it is difficult to determine where this individual may have come from.

KAT-K17 is one of the younger males at 18-20 years of age and has a depleted adulthood $\delta^{15}N$ value and depleted childhood $\delta^{15}N$ and $\delta^{13}C$ values. These values indicate that this individual consumed a greater amount of C_3 – sourced protein resources as a child and either a lower quantity of protein resources during their lifetime, or that he did not suffer from long-term health problems. The osteological analysis revealed no evidence of skeletal pathology or trauma in this individual. KAT-K17 has a $\delta^{18}O$ value at the lowest range for Vilnius and could indicate that the individual spent their early years near or around the capital city, and potentially lived in Poland or Belarus prior to death.

KAT-K19 is also one of the younger males at 20-25 years of age and has a depleted childhood $\delta^{13}C$ value. This indicates that as a child this individual would have consumed a greater amount of C_3 – sourced protein sources. No evidence of skeletal pathology or trauma was noted for this individual. K19 is one of the few individuals with a childhood $\delta^{18}O$ value that falls within the range for Vilnius but their adulthood value suggests time spent outside of Lithuania, possibly in Germany or another central European country (Fig. 17).

KAT-K20 is a 50-55 year old male and has an enriched childhood $\delta^{15}N$ value and a depleted adulthood $\delta^{13}C_{collagen}$ value, suggesting greater access to protein sources or possibly a nutritional stress during childhood and a larger portion of C_3 – sourced protein resources as an adult. This individual has periostitis of the right tibia and degenerative joint disease of both knees (slight) as well as a healed fracture of the right 5^{th} metacarpal. Specific details as

to the type of fracture on the 5th metacarpal are unknown but in light of the other possible indicators of interpersonal violence seen thus far, it is possible that this individual suffered from a "boxer's fracture". This injury often occurs when an individual suffers an impact on a clenched fist, such as when throwing a punch (Lovell, 1999).

With 14 of the 23 represented individuals having outlying stable isotope values it is clear that this unique population was very diverse in their background and lifeways.

Inter-Population Comparisons

The focus will now turn to comparing the study sample stable isotope values with related samples to better understand how this sub-population fits into the landscape. Unfortunately, some of the only published stable oxygen isotope data for Lithuania dates from 43,300 to 13,720 BP and thus cannot be used for comparison (Arppe and Karhu, 2010). There will be some comparison with other published studies conducted in European countries that appear to have similar $\delta^{18}O$ values. Additionally, there will be several related studies focusing on the dietary aspects of past populations that will be used for comparison.

One of the aims of this research was to determine whether the study sample was comprised of high status members of society, which has been inferred from their burial placement. Recent research at the rural site of Alytus, Lithuania analyzed bone and tooth collagen from subadults and adults (Page, 2014; Whitmore, 2014). The samples used in these studies are from a slightly earlier timeframe, the late 14th to early 18th centuries, but their location in Lithuania makes them valuable comparative samples.

Page (2014) analyzed femoral and humeral samples from 70 subadults ranging in age from 38 weeks gestation to 16 years of age. Stable isotope analysis gave a femoral $\delta^{13}C$ value of -20.1‰ and $\delta^{15}N$ value of 11.1‰ and a humeral $\delta^{13}C$ value of -20.0‰ and $\delta^{15}N$ value of 11.1‰. Page determined that the subadults ate a diet primarily of C_3 plants and terrestrial protein sources with some possible incorporation of riverine sources (2014). The elevated ^{15}N average value was attributed to the breastfeeding subadults. In comparing these values to the dentin ^{13}C and ^{15}N values of the present study the average values have a difference of 0.50‰ and 0.35‰, respectively (Fig. 20). The similarity in the values of these two studies suggests that if the study sample did comprise a high status population, any difference in diet would occur in adulthood.

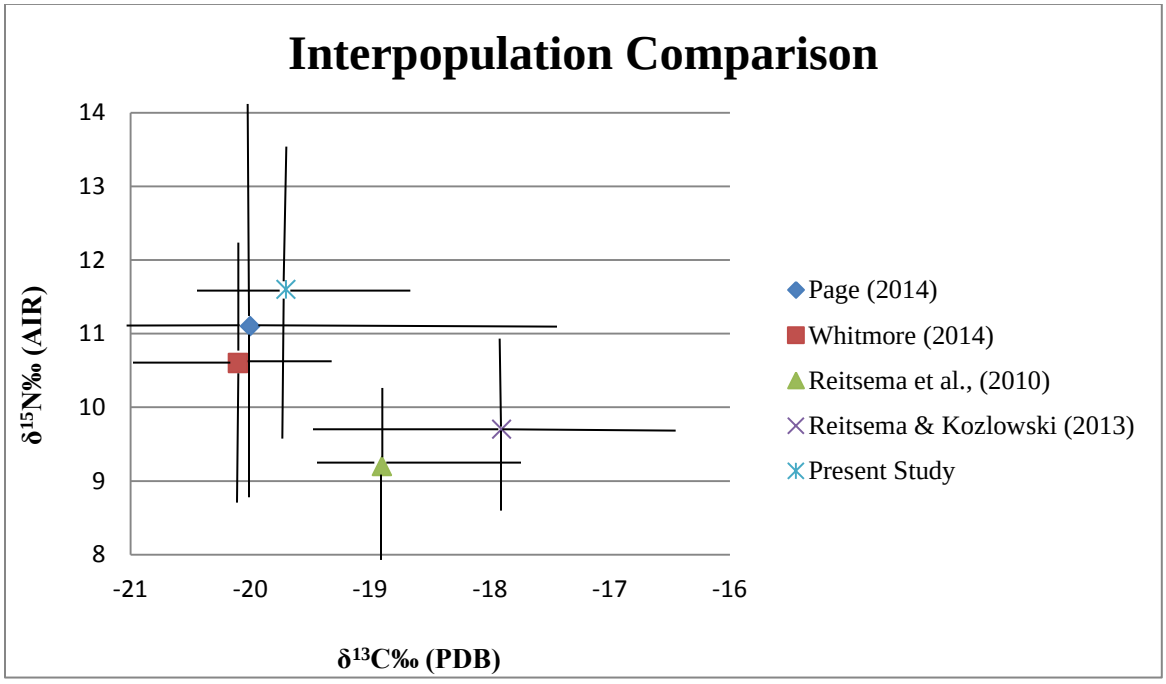

Figure 20: Chart depicting the average value and range for $\delta^{15}N$ and $\delta^{13}C$ of the comparative studies and the current study.

Whitmore (2014) analyzed 35 femoral samples and 38 dentin samples representing 39 individuals, 34 of which had both dentin and bone collagen. Stable isotope analysis gave a bone collagen $\delta^{13}C$ value of -20.1‰ and $\delta^{15}N$ value of 10.3‰ and a dentin collagen $\delta^{13}C$ value of -20.1‰ and $\delta^{15}N$ value of 10.7‰. Whitmore's conclusions supported those drawn by Page (2014) in that the individuals of Alytus subsisted primarily on C_3 plants and terrestrial animal protein sources with likely inclusion of riverine sources. In comparing the bone collagen values obtained by Whitmore to the ^{13}C and ^{15}N values of the present study the average values have a difference of 0.20‰ 1.51‰, respectively (Fig. 20). The large difference between the $\delta^{15}N$ values could suggest a greater meat consumption of the study sample or increased access to aquatic resources, although unlikely due to the model utilized in Figures 14 and 15 which appear to rule out marine resources. While Alytus is situated on a river, it could be that the majority of the resources taken from the river were exported. The large discrepancy in the nitrogen could also be from the disease and traumas that were analyzed on the present study. Whitmore (2014) has a sample that suffered from several diseases as well but those individuals with an illness still have similar values to those individuals without (2014).

Several studies out of Poland are also apt for comparison due to the close geographical location and the historically known connections between Poland and Lithuania. Rib samples were taken from 24 adult individuals believed to be peasants interred in a Polish cemetery that was used between the 11^{th} and 12^{th} centuries (Reitsema et al, 2010). The $\delta^{13}C$ had a mean value of -18.90‰ and the $\delta^{15}N$ average was 9.19‰. The authors conclude that the diet of these

individuals was likely predominantly C_3 plants with terrestrial protein sources with sporadic C_4 plant or marine resource consumption. Similar values and conclusions were obtained for another sample from Poland which was thought to be of a higher social status due to several burials consisting of females and jewelry (Reitsema and Kozlowski, 2013). In comparison with the present study the average ^{13}C and ^{15}N values have a difference of 0.98‰ and 2.60‰, respectively (Fig. 20). The differences are much more pronounced than with those seen in the Alytus samples and could be due to cultural differences in diet. It is likely that the study sample had a higher level of consumption for C_3 plants and a possible increased access to aquatic resources; again, this appears to be unlikely and the observed enrichment in nitrogen could be due to the presence of pathologies and traumas in the study sample (Table 9). This potential increased access to "higher quality" foods could suggest that the study sample is indeed of a high status or at least of a higher status in comparison with the Polish peasantry.

A study by Keenleyside et al. (2011) analyzed stable oxygen isotopes from teeth of 60 individuals ranging in age from 18 to over 50 years from the Kalfata necropolis of the ancient Greek colony Apollonia on the southwest coast of the Black Sea dating from the 4th and 3rd centuries BC. Despite the lack of similarity in timeframe and geographical location to the present study, the values obtained by Keenleyside and colleagues (2011) are similar to the present study. Additionally, the authors were able to determine the local values through the analysis of a modern sample. The mean $\delta^{18}O_{VSMOW}$ value is 24.12‰ (converted from a VPDB value of -5.8‰) and a range of 22.57‰ to 26.70‰ (converted from VPDB values of -7.3‰ and -3.3‰, respectively). All of these values are noted in the present study (Table 12) and could indicate that

some individuals migrated from the area around the Black Sea, e.g. Ukraine or Russia. This is an extremely likely situation due to the historically tumultuous political relationship between Lithuania and Russia that resulted in prolonged Russian occupation of Lithuanian government and country (Smith, 2002; Plakans, 2011).

Another study of interest by Fricke et al. (1995) reports the $\delta^{18}O$ values for several Norse and Inuit sites that are temporally comparable to the study sample. The oxygen values were derived from phosphate portion of the hydroxyapatite where the present analyzed the carbonate portion of bone. Iacumin et al. (1996) provide an equation to convert oxygen values from phosphate to carbonate:

$$\delta^{18}O(PO_4) = 0.998\,\delta^{18}O(CO_3 apatite) - 8.5 \quad \textbf{(11)}$$

Converting the average $\delta^{18}O(PO_4)$ value (18.1‰) for a medieval (1100 – 1600 AD) Danish site called Risby into a carbonate value that can be compared with the present study (26.7‰), it appears that some of the individuals in the study sample could be from this region. As mentioned previously, the average $\delta^{18}O$ adult value for the study sample is 26.1‰ which is only 0.6‰ lower than the average for this Danish site. The well-known religious tolerance in Lithuania resulted in numerous nationalities residing in Vilnius and throughout the country (Plakans, 2011) and this could easily extend to individuals from Denmark or anywhere along the Baltic coast.

Who Were They?

The research questions developed for this study revolve around the need to determine who these individuals were. Did they possess a social position of high status? If so, what position

did they hold? Who could they have been during their lifetime? Burial within a cathedral, not just a church, is reserved for people of high standing in society; whether they be Grand Dukes, secular nobility, or clergy leaders, it is safe to say that they were respected individuals (Ottaway, 1982; Gregersen et al., 2006; Perring, 2013; Jankauskas, personal comm.). Using the information made available for the individuals of this study, several options for societal positions present themselves: leaders in the clergy, secular high status individuals, or a type of religious-warrior.

Individuals with high social standing in the secular world, i.e. the nobility, are certainly a possibility. Both Ottaway (1982) and Gregersen et al. (2006) analyze skeletal remains recovered from cathedral crypts located under the nave (main aisle) and deemed to represent an individual of the secular nobility. The idea of noblemen and their families is particularly applicable to the juvenile, the female, and the younger males seen in this sample. It is likely that their death at such a young age would have prevented them from acquiring a high social standing on their merit, suggesting that the concept of ascribed status is at work in this situation. Additionally, Perring (2013) describes the reformation in the 1500s of the cathedral of York Minster in England. In her article she describes the emerging burial practices of secular nobility and other individuals of high status, e.g. lawyers, in crypts under the main aisle of the cathedral.

The possibility that this group of individuals belonged to the clergy and held some standing with in the Catholic Church has great potential. Individuals are often buried in a way that reflects their life, thus religious leaders buried in a cathedral makes plenty sense (Gill, 2001). Pawlikowska-Butterwick (2014) provides an in-depth discussion of the men of the clergy at Vilnius Cathedral during the second half of the 16th century. Many of the clergy held positions in

society outside of the church, such as serving at royal functions or acting as traveling diplomats. Of even greater interest, Pawlikowska-Butterwick writes about the origins of many of the clergy of the Vilnius Cathedral chapter. Many individuals came from Poland as well as from other areas of Lithuania, which at this time stretched much farther south and east of its current modern borders (Markman, 2011) and included Belarus, Ukraine, and a portion of Russia. There are even reports of clergymen involved at the Vilnius Cathedral who came from at that period, Sweden and Italy but with modern country borders would be from Finland and Croatia, respectively. In addition to a diverse geographical background, the clergy in Vilnius also held a diverse social background with clergy coming from the Polish Crown as well as from the peasantry (Pawlikowska-Butterwick, 2014). Towards the end of the 16th century and into the 17th century, the clergymen from the Grand Duchy of Lithuania were vastly outnumbered by foreign individuals with 55% of the clergy coming from Poland and 28% percent coming from elsewhere.

Another possible explanation for this unique group of individuals is something else entirely – knights, more specifically, Teutonic Knights or their derivation. This possibility stems primarily from the strangely high frequency of trauma seen in this group.

The Teutonic, or German, Order was founded in 1190 during the Crusades as a means of meeting the needs of German knights (Urban, 1994). In 1197 it was recognized as a religious-military force and while the order maintained several convents and hospitals, their prime focus was on warfare and the defense of Christianity. The conflict between the Teutonic Knights and the pagans of Lithuania was long (Urban, 1994; Markman, 2011; Knoll, 2014). Tensions rose,

resulting in armed conflict in 1409 through 1411; known as the "Great War" (Knoll, 2014). After the devastating defeat at Tannenberg (Markman, 2011; Knoll, 2014) the conflict became one of a diplomatic nature. Eventually the Knights began to lose the support of other Europeans. The Order began to dwindle and by the end of the 15th century they resided primarily within the borders of Germany.

Despite the hostilities between the Teutonic Knights and Lithuania the ideals of chivalry practiced by the Knights had a lasting effect on the nobility (Urban, 1994; Petrauskas, 2006), to the extent that in the 15th century Lithuania became a place "where one could go to learn knighthood" (Petrauskas, 2006 pp. 41). At the beginning of the 15th century traveling throughout Europe to gain knowledge of knighthood was encouraged of the nobility. However, it was not until the 16th century that this practice of traveling and acquiring an education became a customary experience in a young noblemen's life (Knoll, 2014). The noblemen who achieved knighthood participated in a variety of jousting and other tournaments to hone and display their skill. Becoming a knight was heralded with great ceremony and obtaining the status of knighthood was awarded a very prestigious rank among Lithuanian nobility (Knoll, 2014). Knighthood in Lithuania never gained much momentum, likely due to its exclusion to the nobility and a large gap in tradition that occurred during the 16th century. Nonetheless, the influence of the Teutonic Knights on the nobility is unquestionable (Knoll, 2014).

There are several possibilities for the social identity of the individuals buried in the Vilnius Cathedral. It is clear, however, that they were respected and high standing members of society. Were they religious, secular, or a combination in the form of knights? Perhaps each of

these identities is represented in this group. The high frequency of trauma suggestive of interpersonal violence (Table 9) certainly lends credence to idea of knights. The majority of the $\delta^{18}O$ values lying outside of the Vilnius area can be explained not only by the extensive borders of the Grand Duchy of Lithuania in comparison to its modern borders but also by the high percentages of foreign clergymen at the Vilnius Cathedral (Pawlikowska-Butterwick, 2014). The burial of younger individuals as well as a female lends support to the idea of a high status secular presence in the crypt burials. Without further information and possible future analyses, it is unlikely the true identity of this unique set of individuals will be definitively known.

CHAPTER SIX: CONCLUSION

The focus of this research was to create life histories for a unique sub-population found in a crypt under the Cathedral Basilica of Saints Stanislaus and Vladislaus in the capital city of Vilnius, Lithuania. The reconstruction of life activities was carried out by the analysis of bone collagen and apatite as well as dentin collagen and enamel apatite, representing adulthood and childhood values respectively. Not only were dietary practices able to be determined through the analysis of $\delta^{13}C$ and $\delta^{15}N$ values but the use of $\delta^{18}O$ values allowed for the estimation of geographical origin and potential migration.

Through analysis of the remains and comparison with previous research in Lithuania and in Poland, it was determined that the study sample consisted of potentially high status individuals. They likely subsisted on a diet of predominantly C_3 plants and terrestrial animal protein. Due to the enriched levels of ^{15}N, it is also likely that there was incorporation of aquatic resources. About 76% of the individuals analyzed in this study were suffering from disease or results of trauma at the time of death and these physiological stressors could account for the increased ^{15}N levels, although that type of enrichment is expected several permil higher when compared with the Alytus samples (Page, 2014; Whitmore, 2014).

One of the interesting results of this research was the ability to perform an analysis of $\delta^{18}O$ values from childhood and adulthood in an attempt to locate foreign individuals. Unfortunately, there is a complete lack of usable ^{18}O data for Lithuania and this makes the determination of foreigners and locales somewhat subjective without knowing precisely when

these individuals arrive in Lithuania or if they were still traveling during the last decade of their life.

Aside from future directions and research needs, there are several limitations of the present study. The absence of a site report and accompanying site map from the excavation of the remains for these individuals leads to a lack of useful context details. Where, precisely, were these individuals buried in the Cathedral? What were they in proximity to? Were they closely clustered in a clear group or groups? Was there anything linking them together? In which direction were they facing? How were they positioned? Was there any evidence of multiple inhumations? If so, are some individuals clearly from one time period? Additionally, there are no images of the burials or of the skeletal remains of these individuals. Another limitation is the small sample size but this is representative of those who were buried in the cathedral. Accompanying the small sample size is the fact that not every individual has a reconstructed life history due to several individuals lacking a first molar. The ability to analyze childhood and adulthood values for all of these individuals could have possible allowed for a more robust and definitive conclusion. This is particularly true for the one juvenile and suspected female who are represented only by a bone sample.

Future Directions

Several future directions and improvements are noted for this study. In reference to the oxygen values, the ability to incorporate microsampling into the research would allow for a more fine-grained analysis (Koon and Tuross, 2013) of shifts in geographical location. Microsampling allows the researcher to break the 10 year turnover rate down into smaller units and thus obtain

further and more nuanced information about the individual. Additionally, incremental analysis of the dentin allows for stable isotope values at predictable ages (Miller et al., 2015). This type of analysis avoids the averaging of several years of growth and information into one figure. It is also suggested that incremental analysis be performed on the enamel as well. Since teeth have a predictable growth rate, researchers should be able to correlate these two tissues so that information from collagen and apatite are available for the same growth periods.

Another need for this study is a baseline foodweb of terrestrial and marine plants and animals, where possible. Some foodwebs are available but many of these look at prehistoric archaeological assemblages and while there is value in this information, analysis of more modern samples would greatly improve the interpretations of the $\delta^{13}C$ and $\delta^{15}N$ values.

With the level of preservation seen in this sample, it is possible that an analysis of the mitochondrial DNA take place. This analysis would not only provide evidence of (maternal) familial relationship between any of the individuals, e.g. if KAT-K14a and KAT-K16 are perhaps siblings, but, depending on the type of DNA analyses available, the ability to more accurately plot these individuals on the geographic landscape is a potential outcome.

The value of the present research lies more in its ability to inform further research than to inform current researchers. Integrating this research into future research on the floral, faunal, and human landscape of historical or modern Lithuania will shine on a light on past lifeways. Additionally, it is the hope that this research will instigate further analysis of the stable oxygen isotope in Lithuania so as to discover what nationalities have called Lithuania home throughout history.

REFERENCES

Adams TS, Sterner RW. 2000. The Effect of Dietary Nitrogen Content on Trophic Level ^{15}N Enrichment. Limnology and Oceanography 45(3):601-607.

Ambrose SH. 1990. Preparation and Characterization of Bone and Tooth Collagen for Isotopic Analysis. Journal of Archaeological Science 17:431-451.

Ambrose SH, Norr L. 1992. On Stable Isotopic Data and Prehistoric Subsistence in the Soconusco Region. Current Anthropology 33(4):401-404.

Ambrose SH, Norr L. 1993. Experimental Evidence for the Relationship of the Carbon Isotope Ratios of Whole Diet and Dietary Protein to Those of Bone Collagen and Carbonate. In: Lambert JB, Grupe G, editors. Prehistoric Human Bone: Archaeology at the Molecular Level. New York: Springer.

Ambrose SH, Butler BM, Hanson DB, Hunter-Anderson RL, Krueger HW. 1997. Stable Isotopic Analysis of Human Diet in the Marianas Archipelago, Western Pacific. American Journal of Physical Anthropology 104:343–361.

Ambrose SH, Krigbaum J. 2003. Bone Chemistry and Bioarchaeology. Journal of Anthropological Archaeology 22:193-199.

Arppe L, Karhu JA. 2010. Oxygen Isotope Values of Precipitation and the Thermal Climate in Europe during the Middle to Late Weichselian Ice Age. Quaternary Science Reviews 29:1263-1275.

Barrett J, Johnstone C, Harland J, Van Neer W, Ervynck A, Makowiecki D, Heinrich D, Hufthammer AK, Enghoff IB, Amundsen C, Christiansen JS, Jones AKG, Locker A, Hamilton-Dyer S, Jonsson L, Lougas L, Roberts C, Richards MP. 2008. Detecting the Medieval Cod Trade: a New Method and First Results. Journal of Archaeological Science 35:850–861.

Bayliss A, Popescu ES, Beavan-Athfield N, Ramsey CB, Cook GT, Locker A. 2004. The Potential Significance of Dietary Offsets for the Interpretation of Radiocarbon Dates: an Archaeologically Significant Example from Medieval Norwich. Journal of Archaeological Science 31:563-575.

Centre for the Co-Operation with the Economies in Transition. 1996. Review of Agricultural Polices: Lithuania. Paris: Organisation for Economic Co-Operation and Development

Chenery C, Müldner G, Evans J, Eckardt H, Lewis M. 2010. Strontium and Stable Isotope Evidence for Diet and Mobility in Roman Gloucester, UK. Journal of Archaeological Science 37:150-163.

Chenery CA, Pashley V, Lamb AL, Sloane HJ, Evans JA. 2012. The Oxygen Isotope Relationship between the Phosphate and Structural Carbonate Fractions of Human Bioapatite. Rapid Communications in Mass Spectrometry 26:309-319.

Christensen AM, Passalacqua NV, Bartelink EJ. 2014. Forensic Taphonomy: Current Methods and Practice. San Diego: Elsevier.

Cormie AB, Schwarcz HP. 1996. Effects of Climate on Deer Bone $\delta^{15}N$ and $\delta^{13}C$: Lack of Precipitation Effects on $\delta^{15}N$ for Animals Consuming Low Amounts of C_4 Plants. Geochimica et Cosmochimica Acta 60(21):4161-4166.

Crowley BE, Wheatley PV. 2014. To Bleach or Not to Bleach? Comparing Treatment Methods for Isolating Biogenic Carbonate. Chemical Geology 381:234-242.

Dansgaard W. 1964. Stable Isotopes in Precipitation. Tellus 16:436-468.

Darling WG. 2004. Hydrological Factors in the Interpretation of Stable Isotopic Proxy Data Present and Past: a European Perspective. Quaternary Science Reviews 23:743-770.

Daux V, Lecuyer C, Heran M, Amiot R, Simon L, Fourel F, Martineau F, Lynnerup N, Reychler H, Escarguel G. 2008. Oxygen Isotope Fractionation Between Human Phosphate and Water Revisited. 55:1138-1147.

DeNiro MJ. 1985. Postmortem Preservation and Alteration of In Vivo Bone Collagen Isotope Ratios in Relation to Paleodietary Reconstruction. Nature 317:806-809.

DeNiro MJ. 1987. Stable Isotopy and Archaeology. American Scientist 75:182-191.

DeNiro MJ, Weiner S. 1988. Chemical, Enzymatic and Spectroscopic Characterization of "Collagen" and Other Organic Fractions from Prehistoric Bones. Geochim Cosmochim Acta 52:2197-2206.

Dufour E, Herve B, Mariotti A, 1999. Paleodietary implications of isotopic variability in Eurasian lacustrine fish. Journal of Archaeological Science 26:617–627.

Dupras TL, Schwarcz HP. 2001. Strangers in a Strange Land: Stable Isotope Evidence for Human Migration in the Dakhleh Oasis, Egypt. Journal of Archaeological Science 28:1199-1208.

French RA. 1970. The Three-Field of Sixteenth-Century Lithuania. The Agricultural History Review 18:106-125.

Fricke HC, O'Neil JR, Lynnerup N. 1995. Oxygen Isotope Composition of Human Tooth Enamel from Medieval Greenland: Linking Climate and Society. Geology 23(10):869-872.

Fry B. 2006. Stable Isotope Ecology. New York: Springer.

Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2005. Nitrogen Balance and $\delta^{15}N$: Why You're Not What You Eat During Nutritional Stress. Rapid Communications in Mass Spectrometry 19:2497-2506.

Garvie-Lok S. 2001. Loaves and Fishes: A Stable Isotope Reconstruction of Diet in Medieval Greece. PhD Thesis, Department of Anthropology, University of Calgary.

Gill MJ. 2001. Death and the Cardinal: The Two Bodies of Guillaume d'Estouteville. Renaissance Quarterly 54:347-388.

Gregersen M, Boldsen J, Bjørn H, Boel LW, Fromholt P. 2006. Examination and Identification of a Danish 17th – Century Nobleman, Laurids Ebbesen. Forensic Science, Medicine, and Pathology 2(1):51-58.

Harrison RG, Katzenberg MA. 2003. Paleodiet Studies Using Stable Carbon Isotopes from Bone Apatite and Collagen: Examples from Southern Ontario and San Nicolas Island, California. Journal of Anthropological Archaeology 22:227-224.

Hedges REM, Millard AR, Pike AWG. 1995. Measurements and Relationships of Diagenetic Alteration of Bone from Three Archaeological Sites. Journal of Archaeological Science 22:201-209.

Hedges REM. 2002. Bone Diagenesis: An Overview of Processes. Archaeometry 44(3):319-328.

Hedges REM, Stevens RE, Koch PL. 2005. Isotopes in Bones and Teeth. In: Leng MJ, editor. Isotopes in Palaeoenvironmental Research. Netherlands: Springer. p. 117-145.

Hedges REM, Reynard LM. 2007. Nitrogen Isotopes and the Trophic Level of Humans in Archaeology. Journal of Archaeological Science 34:1240-1251.

Hedges REM, Clement JG, Thomas DL, O'Connell TC. 2007. Collagen Turnover in the Adult Femoral Mid-Shaft: Modeled From Anthropogenic Radiocarbon Tracer Measurements. American Journal of Physical Anthropology 133:808-816.

Hillson S. 2005. Teeth. New York: Cambridge University Press. p. 146-229.

Hillson S. 2008. Dental Pathology. In: Katzenberg MA, Saunders SR, editors. Biological Anthropology of the Human Skeleton, Second Edition. New Jersey: John Wiley and Sons Inc.

Hillson S. 2014. Tooth Development in Human Evolution and Bioarchaeology. New York: Cambridge University Press.

Hollinger JO. 2005. Bone Dynamics. In: Lieberman JR, Friedlaender GE, editors. Biology and Clinical Applications. New Jersey: Humana Press Inc.

Iacumin P, Bocherens H, Mariotti A, Longinelli A. 1996. Oxygen Isotope Analyses of Co-Existing Carbonate and Phosphate in Biogenic Apatite: a way to Monitor Diagenetic Alteration of Bone Phosphate? Earth and Planetary Science Letters 142:1–6.

Katzenberg MA, Lovell NC. 1999. Stable Isotope Variation in Pathological Bone. International Journal of Osteoarchaeology 9:316-324.

Katzenberg MA. 2000. 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. New York: Wiley-Liss Inc.

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 Jersey: John Wiley & Sons Inc.

Keenleyside A, Schwarcz HP, Panayotova K. 2011. Oxygen Isotopic Evidence of Residence and Migration in a Greek Colonial Population on the Black Sea. Journal of Archaeological Science 38:2658-2666.

Kellner CM, Schoeninger MJ. 2007. A Simple Carbon Isotope Model for Reconstructing Prehistoric Human Diet. American Journal of Physical Anthropology 133:1112-1127.

Knoll PW. 2014. Working for the King (and Queen): Krakovian Scholars in Royal Service in Late Medieval Poland. The Polish Review 59(2):3-18.

Koon H, Tuross N. 2013. The Dutch Whalers: A Test of a Human Migration in the Oxygen, Carbon and Nitrogen Isotopes of Cortical Bone Collagen. World Archaeology 45(3):360-372.

Lane T. 2002. Lithuania: Stepping Westward. In: Smith DJ, Pabriks A, Purs A, Lane T, editors. The Baltic States: Estonia, Latvia and Lithuania. London and New York: Routledge.

Larson CS. 2002. Bioarchaeology: The Lives and Lifestyles of Past People. Journal of Archaeological Research 10:119-166.

Lee-Thorp JA, Sealy JC, van der Merwe NJ. 1989. Stable Carbon Isotope Ratio Differences between Bone Collagen and Bone Apatite, and their Relationship to Diet. Journal of Archaeological Science 16:585-599.

Le Huray JD, Schutkowski H. 2005. Diet and Social Status during the La Tène Period in Bohemia: Carbon and Nitrogen Stable Isotope Analysis of Bone Collagen from Kutná Hora-Karlov and Radovesice. Journal of Anthropological Archaeology 24:135-147.

Longin R. 1971. New Method of Collagen Extraction for Radiocarbon Dating. Nature 230:241-242.

Lovell NC. 2008. Analysis and Interpretation of Skeletal Trauma. In: Katzenberg MA, Saunders SR, editors. Biological Anthropology of the Human Skeleton, 2nd ed. New Jersey: John Wiley and Sons Inc.

Luz B, Kolodny Y, Horowitz M. 1984. Fractionation of Oxygen Isotopes between Mammalian Bone-Phosphate and Environmental Drinking Water. Geochimica et Cosmochimica Acta 48:1689-1693.

Lyman RL. 1994. Vertebrate Taphonomy. Cambridge: University Press.

Markman K. 2011. Tactics of Manipulation: A Revisionist Study of Gediminas and the Threat of Teutonic Invasion, 1315-1342. A Journal of Medieval and Renaissance Studies 42:115-133.

Mays SA. 1997. Carbon Stable Isotope Ratios in Mediaeval and Later Human Skeletons from Northern England. Journal of Archaeological Science 24:561-567.

Mays S, Beavan N. 2012. An Investigation of Diet in Early Anglo-Saxon England using Carbon and Nitrogen Stable Isotope Analysis of Human Bone Collagen. Journal of Archaeological Science 39:867-874.

Mekota A, Gisela G, Sandra U, Ullrich C. 2006. Serial Analysis of Stable Nitrogen and Carbon Isotopes in Hair: Monitoring Starvation and Recovery Phases in Patients Suffering Anorexia Nervosa. Rapid Communications in Mass Spectrometry 20(10):1604-1610.

Meyer C, Brandt G, Haak W, Ganslmeier RA, Meller H, Alt KW. 2009. The Eulau Eulogy: Bioarchaeological Interpretation of Lethal Violence in Corded Ware Multiple Burials from Saxony-Anhalt, Germany. Journal of Anthropological Archaeology 28:412-423.

Moorrees CFA, Fanning EA, Hunt EE. 1963. Age Variation of Formation Stages for Ten Permanent Teeth. Journal of Dental Research 42(6):1490-1502.

Norman K, Claude P, Herbert L, Matthias P. 2008. Prognostic Impact of Disease-Related Malnutrition. Clinical Nutrition 27(1):5-15.

O'Connor K. 2006. Culture and Customs of Baltic States. Connecticut: Greenwood Press.

Olsen KC, White CD, Longstaffe FJ, von Heyking K, McGlynn G, Grupe G, Rühli. 2014. Intraskeletal Isotopic Compositions ($\delta^{13}C$, $\delta^{15}N$) of Bone Collagen: Nonpathological and Pathological Variation. American Journal of Physical Anthropology 153:598-604.

Ortner DJ. 2003. Identification of Pathological Conditions in Human Skeletal Remains. San Diego: Academic Press.

Ottaway P. 1982. A Burial fromm the South Aisle of Winchester Cathedral. The Archaeological Journal 139:124-135.

Page KE. 2014. Bioarchaeological Assessment of Diet and Changes in Femoral and Humeral Stable Isotopic Values Among Subadults at Medieval Alytus, Lithuania. M.A. Thesis, Department of Anthropology, University of Central Florida.

Pawlikowska-Butterwick W. 2014. A 'Foreign' Elite? The Territorial Origins of the Canons and Prelates of the Cathedral Chapter of Vilna in the Second Half of the Sixteenth Century. The Slavonic and East European Review 92(1):44-80.

Perring SM. 2013. Reformation of the English Cathedral Landscape: Negotiating Change in York Minister Close c. 1500-1642. World Archaeology 45(1):186-205.

Petrauskas R. 2006. Knighthood in the Grand Duchy of Lithuania from the Late Fourteenth to the Early Sixteenth Centuries. Lithuanian Historical Studies 11:39-66.

Plakans A. 2011. A Concise History of the Baltic States. New York: Cambridge University Press.

Reitsema LJ, Crews DE, Polcyn M. 2010. Preliminary Evidence for Medieval Polish Diet from Carbon and Nitrogen Stable Isotopes. Journal of Archaeological Science 37:1413-1423.

Reitsema LJ, Kozłowski T. 2013. Diet and Society in Poland before the State: Stable Isotope Evidence from a Wielbark Population (2^{nd} c. AD). Anthropological Review 7691):1-22.

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

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(4604):1381-1383.

Schoeninger MJ, Moore KM, Murray ML, Kingston JD. 1989. Detection of Bone Preservation in Archaeological and Fossil Samples. Applied Geochemistry 4:281-292.

Schoeninger MJ, Moore K. 1992. Bone Stable Isotope Studies in Archaeology. Journal of World Prehistory 6(2):247-296.

Šlaus M, Novak M, Bedić Ž, Strinović D. 2012. Bone Fractures as Indicators of Intentional Violence in the Eastern Adriatic from the Antique to the Late Medieval Period (2^{nd} – 16^{th} Century AD). American Journal of Physical Anthropology 149:26-28.

Smith BH. 1991. Standards of Human Tooth Formation and Dental Age Assessment. In: Kelley MA, Larsen CS, editors. Advances in Dental Anthropology. New York: Wiley-Liss Inc.

Smith DJ, Pabriks A, Purs A, Lane T. 2002. The Baltic States: Estonia, Latvia and Lithuania. London: Routledge.

Stančikaitė M, Kisielienė D, Mažeika J, Blaževičius P. 2008. Environmental Conditions and Human Interference during the 6th and 13th–15th Centuries A.D. at Vilnius Lower Castle, East Lithuania. Vegetation History and Archaeobotany 17:S239-S250.

Sullivan CH, Krueger HW. 1983. Carbon Isotope Analysis of Separate Chemical Phases in Modern and Fossil Bone. Nature 292 333–335.

The World Factbook (Internet). (CIA) Central Intelligence Agency; cited 2015 July 21. Available from: https://www.cia.gov/library/publications/the-world-factbook/geos/lh.html

Tuross N, Fogel ML, Hare PE. 1988. Variability in the Preservation of the Isotopic Composition of Collagen from Fossil Bone. Geochim Cosmochim Acta 52:929-935.

van Klinken GJ. 1999. Bone Collagen Quality Indicators for Paleodietary and Radiocarbon Measurements. J Archaeol Sci 26:687-695.

White CD, Spence MW, Stuart-Williams HLQ, Schwarcz HP. 1998. Oxygen Isotopes and the Identification of Geographical Origins: The Valley of Oaxaca versus the Valley of Mexico. Journal of Archaeological Science 25:643-655.

White CD, Longstaffe FJ, Law KR. 2004. Exploring the Effects of Environment, Physiology and Diet on Oxygen Isotope Ratios in Ancient Nubian Bones and Teeth. Journal of Archaeological Sciences 31:233-250.

Whitmore KM. 2014. Diet at Medieval Alytus, Lithuania: Stable Carbon and Nitrogen Isotope Analysis of Bone and Dentin Collagen. M.A. Thesis, Department of Anthropology, University of Central Florida.

Yoder CJ, Bartelink EJ. 2010. Effects of Different Sample Preparation Methods on Stable Carbon and Oxygen Isotope Values of Bone Apatite: a Comparison of Two Treatment Protocols. Archaeometry 52(1):115-130.