Stable Isotope Evidence for the Geographic Origins and Military Movement of Napoleonic Soldiers During the March From Moscow in 1812

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STABLE ISOTOPE EVIDENCE FOR THE GEOGRAPHIC ORIGINS AND MILITARY MOVEMENT OF NAPOLEONIC SOLDIERS DURING THE MARCH FROM MOSCOW IN 1812

by

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A thesis submitted in partial fulfillment of the requirements for the Honors in the Major Program in Anthropology in the College of Sciences and in The Burnett Honors College at the University of Central Florida Orlando, Florida

Spring Term 2015

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ABSTRACT

In 2001, 3269 unidentified individuals were found in a mass grave on the Northern part of Vilnius, Lithuania. Artifactual context indicates that these individuals were likely soldiers that were a part of Napoleon’s Grand Army. Stable oxygen isotope analysis was performed on bone apatite from 9 femoral bone samples to determine whether or not these individuals were Lithuanian locals and to test ratio variation. If individuals were foreigners, then geographical origins were approximated utilizing percentages of C4 plants from Holder (2013) and δ18O values that were extracted from bone apatite. The carbonate oxygen isotope compositions (δ18Ocarbonate) of bone apatite from the femoral samples (–4.4‰ to –6.2‰) indicate that these individuals were from central and western Europe (–4.0‰ to –6.9‰). It is significant that none of the individuals have values consistent with the area around Lithuania (–10.0‰ to –11.9‰), because it means that they all were non-local. It is also indicative that the Lithuanians were not burying their citizens in the grave and therefore strongly support that these individuals were Napoleonic soldiers.

Additionally, although C4 percentages in the diet ranged from 17.8% to 31.7%, which overlaps with eastern European consumption patterns (approximately 15% to 25% of C4 plants) (Reitsema et al., 2010), the slight shift towards a higher C4 percentage is more representative of a central and western European diet. These results are significant because they provide stable isotopic evidence that these individuals were Napoleon’s soldiers whom participated in the Russian campaign of 1812.
DEDICATIONS

In memory of the thousands of victims who died during the march from Moscow and their families.

For my mentors, Dr. Tosha Dupras, Dr. J. Marla Toyne, and Dr. Graham Worthy.

And especially, for my family and friends for their unlimited supply of support and love.
ACKNOWLEDGMENTS

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CHAPTER ONE: INTRODUCTION

Anthropologists use stable isotopes to reconstruct human behavior such as food consumption and migration patterns of ancient and contemporary societies. “The results of such analyses make a significant contribution to the reconstruction of past human life” (Katzenberg, 2008:413). Although isotopic analyses can be performed on any tissue, bone and teeth are most common. Stable oxygen isotope analysis, specifically, is recognized for the ability to assist in interpreting mobility and geographic origins (Dupras and Schwarcz, 2001)—the focus of this study. These techniques have become a recent trend within bioarchaeology, and one area of investigations pertains to historical military sites, such as Snake Hill in Fort Erie, Ontario (Sledzik and Sandberg, 2002). This is especially important for the Baltic country of Lithuania, where there is a focus on building and reconstructing their national identity since gaining independence from Russia in 1991. The role of Lithuania during the Napoleonic Wars (1803-1815) is part of this history, particularly Napoleon’s march to Moscow in 1812.

In 2001 during the initial phases of a construction project, a mass grave was discovered in Vilnius, Lithuania. It was quickly discovered that the individuals in this grave were likely Napoleonic soldiers who had fallen victim to disease and starvation during their retreat from an unsuccessful campaign in Moscow during 1812. A literature review concerning these skeletal remains reveals a limited amount of detailed information about these individuals. Only salvage archaeological data (Signoli et al., 2004), dental studies (Palubeckaité et al., 2006; Palubeckaité-Miliauskienė, 2007), and diet and nutritional stress analyses (Holder, 2013) have been published.
thus far. The aim of this pilot study is to provide further insight into the lives of these individuals by using stable oxygen ($^{18}$O/$^{16}$O) and carbon isotope ($^{13}$C/$^{12}$C) analyses to address questions concerning geographic origins (where they grew up during childhood), military movements, and the diet of the unidentified soldiers.

Fueled by the French Revolution, the Napoleonic Wars (1803-1815) were a series of military conflicts between France and opposing European nations. Napoleon conquered most of Europe and developed the Continental System, which included the following countries: France, Austria, Prussia, Switzerland, Italy, Westphalia, Poland, Belgium, Holland, Germany, Spain, Portugal, Lithuania, Poland, Croatia, Illyria, and Russia for a brief time (Bell, 2006; Connelly, 2006). The primary goal of this system was to isolate Great Britain economically, France’s ultimate rival, and force them to succumb to Napoleon’s control.

Control over Russia was the last piece to Napoleon’s plan to completely disrupt Great Britain’s economic system. Initially, Czar Alexander I signed the Treaty of Tilsit with France agreeing to cooperate with Napoleon’s strategy to starve and bankrupt Great Britain. Although peace was agreed between the two superpowers, the Continental System was detrimental to Russia’s economy. Eventually, Czar Alexander I severed the treaty and resumed trading with Great Britain. The Czar further exacerbated the friction for three reasons: (1) he refused to allow Napoleon to marry his sister; (2) threatened to invade Poland; and (3) continued to undermine the Continental System. Napoleon responded by creating a Prussian state to force Russia to negotiate, but to no avail (Bell, 2006). Out of desperation and the belief that war was inevitable, Napoleon implemented the notorious Russian campaign in 1812 to capture Moscow.
At deployment on June 1812, Napoleon assembled a large army consisting of approximately 675,000 soldiers that would swell to over 900,000 (Bell, 2006), an unprecedented accomplishment at the time. Conscripted soldiers from conquered adjacent European countries contributed to less than half of the Grand Army. Figure 1 shows a map of Europe ruled under Napoleon in 1812 and Figure 2 illustrates the number of enlisted soldiers and the countries that they represented.

Figure 1. Map of territories allied with Napoleon during 1812

Source: “Europe in 1812. Political situation before Napoleon's Russian Campaign.” by Alexander Altenhof (Link) Used under CC BY-SA 3.0
Figure 2. Pie chart showing number of enlisted soldiers in the Grand Army. Image created from data found in Connelly (2006)

As the French army advanced to Moscow, they passed through Vilnius, Lithuania on June 28, 1812, which was Russian territory at the time, and successfully captured it with little revolt. The army was well received by the citizens of the town, along with the Polish, because it was believed that Napoleon’s campaign against Russia was aimed to liberate them. Within twenty-four hours, however, the French army was looting and plundering which decreased the people’s desire to help them. During their time in Lithuania, the soldiers dug trenches in the Northern part of the city, where they were most vulnerable to attack. After successfully recuperating, the Grand Army left Vilnius to continue following the Russian forces into Russian territory.

Throughout the march, the Russians continuously retreated and used scorched-earth tactics to prevent the Grand Army from finding supplies, food, and shelter. When Napoleon reached Moscow on September 14, 1812 (Connelly, 2006), he found the city deserted and burnt
down. Although there was mass starvation among the soldiers, Napoleon arrogantly stayed in Moscow for 35 days determined to wait for Alexander I’s surrender. Due to the lack of supplies and the oncoming threat of the Russian winter, Napoleon was forced to retreat in defeat on October 19, 1812 (Bell, 2006) (see Fig.3 for retreat route). When they departed, the ruthless Russian winter was upon them with “temperatures dropping to 16 degrees below freezing” (Connelly, 2006), and soldiers suffered from frostbite, lice, and vermin (Bell, 2006).

![Figure 3](image_url)

**Figure 3.** Map of Europe with Napoleon's route of retreat from Moscow to Vilnius. Image created from data found in Connelly (2006)

At the time of the withdrawal, the Grand Army was only comprised of approximately 100,000 soldiers, a mere fraction of when the campaign first started. Napoleon was blocked one of the Russian infantries from going south, where there were more fertile lands, and the army
was forced to return the way they came. The army was not prepared for the Russian winter and numerous soldiers and horses died from the cold, disease, and starvation. When they finally reached Berezina River (located in modern day Belarus, southeast of Lithuania), only 50,000 of the 100,000 soldiers who left Moscow survived (Connelly, 2006). Figure 4 shows one of Minard’s famous maps that incorporates the number of soldiers dwindling with time and movement throughout the campaign.

![Minard's map of soldiers lost throughout the campaign](http://www.datavis.ca/gallery/minard/minard.odt.jpg)

**Figure 4.** Minard's map of soldiers lost throughout the campaign

Source: [http://www.datavis.ca/gallery/minard/minard.odt.jpg](http://www.datavis.ca/gallery/minard/minard.odt.jpg) (public domain image)

After arriving in Vilnius on December 1812, Napoleon returned to Paris to inform his administration of the situation that occurred in Russia and left the rest of the army in Lithuania. Although hope left with their leader, food was abundant for the remaining soldiers and “there
were flour and meat to feed 100,000 men for 40 days” (Nicolson, 1985:168). The famished soldiers took advantage and ravished every resource they could find. “Many starving men had savagely torn into the food and drink depots, many gorging themselves to death” (Asprey, 2001:281).

The unorganized chaos created a great panic among the Lithuanians and the locals ceased to accommodate the army. Consequently, most soldiers died from hypothermia, malnutrition, exhaustion, starvation, and disease—mainly from typhus, also known as “war-fever” (Lobell, 2002). Factors that caused many deaths were rapid temperature changes, lack of food and water, and disease (Bell, 2006). At first the deceased soldiers were burnt, but the smell was so horrid that the locals decided to bury the bodies in mass burials, created from the trenches that, ironically, the Grand Army had dug months before.

The purpose of this research is to examine the geographic origins or military movement patterns of the soldiers who died in Vilnius, Lithuania. Seventy-eight femoral bone samples were originally collected from the mass grave and 9 of them were utilized in this pilot research project. Stable oxygen and carbon isotope analyses of bone apatite were used to approximately determine regional locations around Europe where these individuals may have originated. This study is a continuation of a stable isotopic project (Holder, 2013) that focused on the dietary variation and nutritional stress within these individuals.

Additionally, this study aims to examine whether or not the individuals within the mass grave were the soldiers of Napoleon’s Grand Army and test the variation of the sample through stable oxygen and carbon isotope analyses. It is expected that there will be a wide range of
isotopic variation but limited to the central and western parts of Europe. Assuming that each European region and individual is isotopically distinct, following 5 hypotheses were tested through this research:

1. It is hypothesized that the younger individuals (20-27 years old) will have $\delta^{18}$O values that will reflect the location of their adolescence.

2. It is hypothesized that the $\delta^{18}$O values of the younger individuals will reflect the geographical areas of Europe (i.e., France, Italy, etc.) from which Napoleon was known to have recruited soldiers.

3. Assuming that older individuals (>28 years old) were military career soldiers, it is hypothesized that the older individuals will have $\delta^{18}$O values that reflect movement during their recent years in military service.

4. If the previous hypothesis (#3) is supported, then it is speculated that $\delta^{18}$O values will be the average of their participation in previous campaigns—expected to be approximately (-7.00‰ to -7.99‰). These prior campaigns could have been organized by Napoleon (e.g., Egyptian campaign), from their country of origin, or another nation.

5. If an individual’s $\delta^{18}$O values do not fall within the isotopic average mentioned above, then it might be an indicator that the individual was not a career soldier, but a recent recruit to the Russian campaign. Therefore, the $\delta^{18}$O values would represent the country in which he was conscripted from during the draft.

This research is important because it will provide scientific evidence that will either support or refute the assumption that the individuals within the grave were Napoleonic soldiers. Given that Napoleonic artifacts were found in the grave, if the study demonstrates that the individuals
were non-local to Lithuania, then it is most likely that they were Napoleon’s soldiers. Since Napoleon’s Grand Army consisted of people from all over Europe, stable oxygen isotope analysis will also allow insight into the diversity of nationalities within the army, and vise-versa, if the study supports the hypothesis that the individuals were local, then they might have been civilians or the Lithuanian regiment portion of the Grand Army who were carelessly thrown into the grave. In addition, this study will add anthropological understanding of how war in Europe was organized and conducted during the 19th century.
CHAPTER TWO: ISOTOPIQUE THEORY AND METHODOLOGY

The purpose of this chapter is to provide the framework for the stable isotopic theory and methodology in the context of examining the geographic origins or military movement patterns. It begins with a brief description of stable isotopes in general and then more specifically about those isotopes used in this study: oxygen and carbon. Previous applications of both isotopes are then discussed in greater detail to provide context to how they are going to be applied in the current study. The section concludes with a short discussion about the tissues utilized in stable isotope analysis and bone turnover rates.

What are isotopes?

The term ‘isotope’ was coined by Professor Frederick Soddy, from the University of Glasgow, to mean ‘in equal position’ (Meier-Augenstein, 2010). Isotopes are alternate forms of an element atom that have the same number of protons but differ in neutrons. This results in atomic mass variation and is indicated by a superscript located to the left of an element abbreviation. Stable isotopes are unique in that they are not radioactive and do not decay over time.

Stable isotopes’ resistant property against degeneration is beneficial for increasing the opportunity to analyze the bone chemistry within skeletal materials. It has been utilized in numerous anthropological, as well as interdisciplinary, applications to explore questions about ecology and human behavior. Various isotopes reveal different information depending on the nature of what is being investigated. Carbon and nitrogen isotope compositions of skeletal material reflect information about diet, nutrition, metabolic states, physiology, and paleoecology.
(e.g., DeNiro and Epstein, 1978; Buikstra and Milner, 1991; Holder, 2013). Oxygen and strontium isotope ratios are applied to investigate geographic origins, migration patterns, and paleoclimates (e.g., Dupras and Schwarcz, 2001; Ugan et al., 2012; Oelze et al., 2012). Table 2 shows the stable isotopes used in this study and their corresponding natural abundances.

Table 1. Natural Abundances of Selected Isotopes.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Natural Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{12}$C</td>
<td>98.90</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>0.015</td>
</tr>
<tr>
<td>$^{16}$O</td>
<td>99.76</td>
</tr>
<tr>
<td>$^{17}$O</td>
<td>0.04</td>
</tr>
<tr>
<td>$^{18}$O</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Atomic mass differences in stable isotopes affect their chemical behavior and lead to isotope fractionation, or the process that alters the ratios in the environment (Brown and Brown, 2011). As Katzenberg (2008:416) summarizes, “isotope fractionation is the basis for stable isotope variation in biological and geochemical systems”. There are two different types of isotopic effects that cause fractionation—kinetic and thermodynamic isotopic effects—however, to stay within the scope of this study, only thermodynamic isotopic effects will be discussed. For more information about kinetic isotopic effects reference Meier-Augenstein (2010).

Thermodynamic isotope effect is when there are changes in the physiochemical properties that are caused by kinetic isotope effects (e.g., difference in mass), “such as infrared
absorption, vapour pressure, and boiling points” (Meier-Augenstein, 2010:12). In the example of the water cycle, $^{18}$O atoms contain a heavier mass and evaporate at a slower rate than $^{16}$O atoms because they require more thermal energy. However, during condensation $^{18}$O atoms will move faster toward the Earth’s surface than the $^{16}$O atoms because gravity will accelerate the heavier massed particles. This difference in reaction rates cause oxygen isotope fractionation and is one of the ways to track climatic changes and identify geographical areas.

**How stable isotopes are measured and reported**

Stable isotopes are measured by gas isotope ratio mass spectrometers (IRMS), which consists of four parts: an inlet, ion source, mass analyzer, and ion collector (Fig. 5). First, the prepared samples are converted into a gas (i.e., CO$_2$ for oxygen) and ionized by the ion source. The charged particles are then moved through the flight tube and magnets separate them by mass. Subsequently, the particles are collected in the detector, measured, and then the ratios are compared to the universal standard of that element.

![Diagram of Mass Spectrometer and its components](image-url)

**Figure 5.** Diagram of Mass Spectrometer and its components

Source: “A full diagram of a mass spectrometer” by Prof. Delmar Larsen ([Link](link)) Used under CC BY-NC-SA 3.0
The ratios are denoted as delta (δ) values in parts per mil, or ‰ (Equation 1).

$$\delta (\text{‰}) = \frac{R(\text{sample}) - R(\text{standard})}{R(\text{standard})} \times 1000$$

(1)

Vienna Pee Dee Belemnite (VPDB) is commonly used as the standard for oxygen isotopes, but can be converted to the Vienna Standard Mean of Ocean Water (VSMOW) standard (Equation 2), so that values can be compared directly to the literature (Meier-Augenstein, 2010:21).

$$\text{VSMOW} = 1.03091 \times \delta^{18}\text{O}_{\text{VPDB}} + 30.91$$

(2)

After calculating the delta values from stable oxygen isotope ratios that were extracted from the bone, they are matched to regions that contain identical values on isoscape maps, or geographical maps that show spatial variation of isotopic values across the landscape. These maps are generated by databases, such as the Global Network of Isotopes in Precipitation (GNIP), which compile monthly isotopic data from water stations all over the world. Average annual water isoscape maps were utilized for this study and were taken from the University of Utah’s IsoMAP database. Although modern day isoscapes maps will not be completely accurate of the climatology during 1812, it is suitable enough to provide general estimations for the purpose of this study.

**Stable isotopes used in this study**

Incorporating multiple stable isotopes is beneficial because it reveals different avenues of information and can be combined to generate a deeper understanding of what is being investigated. For this study, stable oxygen and carbon isotopes were used to investigate geographic origins and diet, respectively. Understanding what these soldiers consumed during
their lifetime can be an invaluable tool to assist in the process of elimination of locations and narrow down where they might have lived during their adolescence or adulthood before they died in Vilnius.

**Oxygen isotopes.** In this study, $^{18}$O and $^{16}$O are utilized because they the most naturally abundant oxygen isotopes. They make up 21% of the Earth’s atmosphere and 88% of the world’s oceans (Meier-Augenstein, 2010). Water sources (i.e., rivers, streams, springs, and lakes) have unique oxygen isotopic signatures due to fractionation. Oxygen values increase when temperature and humidity levels increase and values decrease when there is an increase in from the sea, altitude, and precipitation (Dansgaard, 1964; Katzenberg, 2008; Dupras and Schwarcz, 2001). The effect of fractionation on oxygen isotopes is the key asset to this study because each geographical area has its unique combination of climatological and geological conditions. By working backwards, the results can produce information about potential geographical areas that these soldiers might have come from.

The various oxygen isotopic ratios from bone samples can be utilized for identifying migrants as well as determining their mobility patterns or place of origin. There are three main sources in which an organism, including humans, consumes oxygen: (1) water, (2) atmospheric oxygen, and (3) food. According to (Daux et al., 2008) since humans are omnivores we mainly get our water from places like streams and lakes, rather than plants or other food resources. Fractional loss, such as sweating and exhaling CO$_2$ gas, causes “variations in the balance between these fluxes [and] control the relationship between body water and the $\delta^{18}$O of the local water” (White et al., 1998:645). Equilibrium is produced between the water that is being consumed and what is being discarded. Therefore, oxygen isotopic ratios in human tissues
directly relate to the water source that an individual ingests throughout their lifetime, a ratio of approximately 1:1 (White et al., 1998).

**Carbon Isotopes.** Carbon isotopes are used to reconstruct consumption patterns of C₃ and C₄ plants in the diet. C₃ plants use the 3-phosphoglyceraldehyde (3-PGA) pathway of CO₂ fixation and consist of herbaceous vegetation, such as fruit and grains like wheat and barley. The δ¹³C values of these plants often range from -22‰ to -33‰, with an approximate average of -26‰ (Dupras and Tocheri, 2007; Schoeninger, 1995). C₄ plants, on the other hand, use the dicarboxylic acid pathway also known as the Hatch-Slack cycle, which allows for plants to increase the production of glucose in more arid climatic conditions. Examples of these types of plants include sugar cane, millet, maize, and sorghum, which have δ¹³C values ranging from -9.00‰ to -16.00‰ with an average of about -12.00‰ (Dupras and Tocheri, 2007; Schoeninger, 1995).

Diets that preferential use C₃ or C₄ plants can be easily revealed due to “the non-overlapping ranges of C₃ and C₄ plants” (Katzenberg, 2008). According to Dupras and Schwarcz (2001), an individual whose diet is solely C₃ plants will have δ¹³C values of their tissues approximately -19‰, while a strict C₄ plant diet will have values of approximately -5‰. Diets that incorporate both types of plants will have a value between the extreme ranges. Additionally, δ¹³C values in bone are enriched by 5‰ over the diet, “which is the basis of the expression, ‘you are what you eat +5‰’” (Katzenberg, 2008:424). When paired with oxygen values, it can be used to help narrow down regional geographical origins if there are large variations in the diet.
**Previous applications of oxygen isotopes**

Stable oxygen isotopes have been used in many disciplines such as geochemistry, bioarchaeology, and forensics (human and animal). Geochemistry, the science that uses chemistry to explain Earth’s geological systems, is imperative for the success of studying migration patterns in bioarchaeology and identifying provenience in forensic cases. Geochemists study and document the spatial variation of isotopes all over the world and create isoscapes maps of various stable isotopes (e.g., oxygen). The literature provides a plethora of stable oxygen and hydrogen isotopic research that biological anthropologists can utilize as geographical references to identify water values of regions worldwide.

After understanding how stable oxygen isotopes work in nature and how they are distributed throughout the landscape, biologists began to incorporate this knowledge to research the geographic origins and mobility patterns of animals. One significant discovery showed that the fractionation relationship between blood water and $\delta^{18}$O values from bone were constant (Longinelli, 1984). This indicates that there is a one to one ratio of consumed water to blood to bone and therefore the $\delta^{18}$O ratios that are extracted from the bone are directly representative of the water that was ingested.

Animal studies have also used stable oxygen isotopes as a thermometer to reconstruct climatic changes and investigate evolutionary changes. Secord et al. (2012) used this technique to examine the evolution and body size changes of early horses, and that in addition, animal skeletal remains can be used to recreate paleoclimates by analyzing bone phosphate from horse teeth (Bryant et al., 1996). Although the study of animals began producing scientific discoveries, they were not a useful proxy for the study of humans because there are many differences
between species. For example, fractionation between water and bone tissues contrasts between species. Nevertheless, after fully understanding the correlation of bone tissues and oxygen isotopes, the focus then finally shifted to a more complicated species—*Homo sapiens sapiens.*

The shift in focus of stable isotopic studies from animals to humans perpetuated an increase in interest for interdisciplinary collaboration between biochemistry and bioarchaeology. Bioarchaeology is an interdisciplinary science that primarily uses oxygen isotope values to aid in identifying the possible provenance of an individual. This discipline draws upon disciplines, such as paleoceanography, to reconstruct past environments (e.g., (Koch, 1998)), which is vital for detecting changes that might alter the interpretation of bioarchaeological data. Combining biological anthropology with archaeological context also contributes to answering anthropological questions. For example, stable oxygen isotope analysis in archaeology is utilized to investigate mobility and residence patterns of ancient populations (e.g., Ugan et al., 2012; Oelze et al., 2012; Dupras and Schwarcz, 2001).

Additionally, an example of utilizing bioarchaeology in a modern and legal setting is forensic anthropology. This sub-discipline uses the same isotopic techniques and archaeological methods for investigating crime scenes. In forensic contexts, oxygen isotope values extracted from remaining tissues (bone, hair, fingernails, or teeth) can provide helpful geographic information. It can direct investigations to specific locations where databases of DNA, fingerprints, or missing persons will most likely produce matches to the victims (Meier-Augenstein, 2010). Over the last decade, stable oxygen isotopic analyses, especially of hair, have grown exponentially in forensic science, anthropology, and other sciences (Coplen and Qi,
2012). The use of hair allows for the determination of time-dependent geographic region-of-origin and can be especially useful in forensic cases.

**Tissues utilized in stable isotope analysis**

Stable isotope analysis can be conducted on any body tissue (e.g., hair and fingernails), but in bioarchaeological studies teeth and bone are more commonly used. Bone is approximately 70% inorganic (bone apatite) and 30% organic (collagen). Bone apatite is a calcium-phosphate mineral that closely resembles hydroxyapatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}_2)]\), which is made up of phosphate \((\text{PO}_4)\), the main anionic portion of bone mineral, and carbonate \((\text{CO}_3)\), a minor constituent of bone mineral (Dupras and Schwarcz, 2001; Metcalfe et al., 2009). Although stable isotope analysis can be performed on both constituents of apatite, this project will focus on the analysis of oxygen in carbonate. Bone phosphate is stronger and more durable against digenesis, but utilizing carbonate in stable isotope analysis is more practical. It is beneficial for four reasons: (1) analysis is easier, (2) it is less expensive because phosphate requires rocket fuel to break the bonds, (3) more precise, and (4) for oxygen analysis, it is determined concurrently with \(\delta^{13}\text{C}\) values (Bryant et al., 1996).

Collagen represents ingested protein, while carbonate represents the overall dietary components of carbohydrates, fats, and protein (Garvie-Lok et al., 2004). Most studies rely on analyses of collagen for stable carbon and nitrogen isotope values, but in the case where the bone is in poor condition, bone mineral can be utilized as a substitute. It was debated whether this method was valid because of the belief that the inconsistency in results was caused by diagenesis, or the degradation of the bone (Garvie-Lok et al., 2004; Meier-Augenstein, 2010). Research conducted by Koch (1998) suggests that bone carbonate \(\delta^{18}\text{O}\) values were not
compromised by diagenetic changes in modern skeletal remains, but raised questions about consequences of diagenesis in long buried remains. It is well known that carbonate laid in dental enamel, a static bone tissue, is permanent and does not degrade over time unlike regular bone tissues (Hillson, 1996). C-O bonds were thought as susceptible to diagenesis because the bonds were not as strong as those found in PO₄ bonds. Although PO₄ bonds are stronger, factors such as recrystallization and microbial attacks can still dislodge these bonds. Eventually, the issue of diagenesis was overcome by the development of appropriate pretreatment procedures and methods for the identification of alteration (Ambrose and Krigbaum, 2003). Relevant to this study, Katzenberg (2008) mentions two main benefits from using carbon from bone apatite: (1) it allows stable isotope studies to be applied to older materials, and (2) carbon values records slightly different dietary information than collagen.

**Bone remodeling.** Bone turnover, or bone remodeling, is a natural process of the removal of old bone tissue by osteoclasts and replaced with newly deposited bone via osteoblasts. This is an essential factor in identifying location using stable oxygen isotope analysis because it determines how far into the past the data can represent. Bone remodels constantly throughout out an individual’s lifetime and therefore can be utilized to detect migration during adulthood.

It is widely accepted that the approximate bone turnover rate for the entire skeleton is 10% per year, which is equivalent to 10 years (Manolagas, 2000). Meier-Augenstein (2010), however, argues that since bone-remodeling rates differ from bone to bone, having a selection of bone will be able to provide a more accurate time record for the reconstruction of life history trajectories or geographic life histories. Relevant to this study, he also states, based on studies conducted by Hedges et al. (2007), that “femurs appear to remodel completely only every 20-25
years” (Meier-Augenstein, 2010:23). The study also demonstrated that bone turnover rates vary dependent upon age and sex (Hedges et al., 2007). During adolescence, males of ages 16-18 have a faster turnover rate (7.3 years) than females of ages 14-16 (16.7 years). Bone turnover rates swap after the cessation of growth during adulthood and adult males from ages 23-27 have a slower turnover rate (33 years) than females of ages 18-20 (24.4 years).

During the excavation of the mass grave in Vilnius, the archaeologists determined age by 3 criteria: (1) chronology of epiphysis fusion, (2) age changes of pubic symphysis, and (3) cranial suture closure. Additionally, sex was determined by (1) the morphology of the pelvic bones, and (2) the morphology of the skull as complementary (Signoli et al., 2004). Applying the bone turnover rates of adolescent males (7.3 years), it is expected that the approximate minimum age for a deceased male to have $\delta^{18}O$ values reflective of his adolescent years is 23.3 years old and the maximum age around 25 years old. This is important because the majority of individuals in this sample are within the age range of 20 to 28 years old. It should be noted, however, it is likely that the speed of bone turnover constantly fluctuated due to the strenuous physical activities performed in the army. Contributing factors to variation in bone turn-over rates may include: (1) an increase in exercise and injury (2) disease and infection, and (3) malnutrition and chronic starvation.
CHAPTER THREE: MATERIALS AND METHODS

Over the years, Lithuania has witnessed various wars, disasters, and social cataclysms that produced numerous mass graves, and thus have notoriously earned the reputation as the European Graveyard (pers. comm with Dr. Jankauskas). In 2001, a mass grave of Napoleonic soldiers was discovered in the capital of Vilnius, and has since become a UNESCO heritage site. The grave consisted of 3269 individuals who were victims of the retreat from Moscow in 1812. The following chapter describes the archaeological site, research studies that were conducted on this group, and the sample used in this study.

Site and Sample: Vilnius, Lithuania

In 2001, a mass grave was discovered by construction workers in the Northern Town section of Vilnius. Originally, it was hypothesized that these individuals were either victims of the Soviet KGB, the NKVD, or of the Nazi oppression from the 1940s (Lobell, 2002). Bioarchaeologists, Dr. Poskiene and Dr. Jankauskas, from the University of Vilnius concluded that the Imperial Eagle and numbers of regiments on the artifacts found in the grave (buttons, fabrics, metals, and coins) dated to the Napoleonic era. This was one of two trenches that were discovered and it is speculated that there might be a third one more to the north. Figures 6 and 7 are examples of artifacts that were found.
In addition to the material culture, Palubeckaite et al. (2006) state that the minimum number of individuals (MNI), including males and females, in the mass grave was 3269. Artifacts found in the grave suggest that Napoleon’s Grand Army, also known as the “20-Country Army” (Signoli et al., 2004), consisted of individuals from diverse European origins (e.g., France, Austria, and Croatia (Lobell, 2002)). Employing stable oxygen isotope analysis to this sample is a unique method to provide solid scientific evidence for the argument of diversity, thereby complementing the material culture found in the mass grave.

Two teams of anthropologists (French and Lithuanian) had only one month to conduct salvage archaeology and clear the site for construction. Weather, time, and resources were major constraints but the rescue of the site was a success. The French team focused on qualitative information by utilizing methods used in forensic anthropology while the Lithuanian team focused on quantitative material by gathering anthropological observations. A trench
approximately 10m wide by 40m was dug along the grave. The concentration of bodies was 7 corpses per meter squared. Figure 8 is a photograph of the excavation of the mass grave.

![Image](image.png)

**Figure 8.** Photo of the mass grave in Vilnius, Lithuania

Source: Adapted from Lobell (2002) ([Link](#)).

The positions of the individual skeletons were varied and disorderly, which indicated that they were dumped and not buried “nicely” before rigor mortis began. There were also 4 horses that were exhumed from the grave along with many artifacts. The majority of the individuals that could be sexed (n=1883) were males with ages ranging from 20-25 years old and very few 16-17 year olds. Females were also present in the grave and they were probably wives that tagged along to provide supplies, medical service, laundry, tobacco, wine, and sexual services. Jankauskas (pers. Comm.) notes that the majority of the males (20-25 years old) were short in stature, which could have been consequential of being children of stressed parents and nutritional deficiencies during the French Revolution.
Dental analysis of their teeth showed that many soldiers suffered from a lack of dental hygiene. There are examples of mild to moderate linear enamel hypoplasia caused by non-specific stresses during childhood. There is also evidence of caries in the 1st and 2nd molars, which are caused by highly cariogenic foods such as sugar, wine, and other sweets, with rates that are consistent with some of late medieval populations in Europe (Palubeckaité et al., 2006). Nevertheless, the low level of dental calculus, abscesses, tooth loss, and linear enamel hypoplasia suggest that these individuals were young, and relatively healthy men (Palubeckaité et al., 2006). Although toothbrushes were known during the 18th century, soldiers only had brushes for cleaning their guns, shoes, and clothing, but not for their teeth (Magueron, 1897-1906). Additionally, there are also nicotine stains and specific wear patterns on the teeth that provide evidence of tobacco and habitual pipe smoking.

The general skeletal physiology of the individuals reveal evidence of post-mortem fractures that were most likely caused by the looting of bodies for clothing and other resources. “Most often found were perimortal spiral and comminuted fractures of long bones (humeri were prevailing, 45 right, 39 left) from forceful bending, or from blows with blunt objects” (Signoli et al., 2004:226). Some individuals also had “rider facets” on the femurs, which suggested an occupation such as cavalry. Other pathological conditions include “march foot” also known as Schumann’s disease or fatigue fractures, which are caused by fast paced marching. From personal journals written by officers in the army, it is known that the Grand Army marched at 70-80 paces per minute, and this speed is acknowledged to cause these types of fractures.

Infectious diseases common during this time period include syphilis, dysentery, pneumonia, and typhus (Raoult et al., 2006). Syphilis was widespread in Europe during the
1800s and was treated with mercury, which could have led to poisoning and ultimately to Mad Hatter’s disease. Within historical records it has been documented that typhus, or war fever, caused 600 casualties as soon as soldiers stepped foot in Vilnius in June 1812. Body lice cause typhus and it is said that louse-borne diseases caused more deaths than that of weapons. Approximately 29% of the individuals in the mass grave had typhus (Jankauskas, pers. comm). Another parasitic transmitted disease was schistosimasis, which was caused by *Schistosoma haematobium* and of all the locations of Napoleon’s campaigns, this parasite is only found in Egypt. Regiments 13, 18, 19, 25, 61, and 85 were part of Napoleon’s campaign to Egypt from 1798-1802, where all but two (13 and 25) were not represented in the mass grave (pers. communication with Dr. Jankauskas; Signoli et al., 2004). Individuals who had this parasitic disease had been in Egypt and migrated to Europe to participate in the march to Moscow.

In the sample used for this research there are seven males, one potential male, and one female. Ages range from 22.5 to 40 years of age. Only 4 of the 9 individuals have signs of pathological conditions, which are shown in Table 2. Dr. Rimantas Jankauskas at Vilnius University conducted all demographic and pathological analyses. Samples, collected by Dr. Tosha Dupras and student assistants from Vilnius University, were procured from the femoral shaft using a Dremel tool with a fiberglass reinforced cutoff wheel. All samples were taken from the right femur when possible and were previously cleaned by Holder (2013).
Table 2. Individuals sampled for this project

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sex</th>
<th>Median Age</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-K1</td>
<td>M</td>
<td>30</td>
<td>N/A</td>
</tr>
<tr>
<td>NAP-K6</td>
<td>M</td>
<td>22.5</td>
<td>N/A</td>
</tr>
<tr>
<td>NAP-K7</td>
<td>PM</td>
<td>37.5</td>
<td>Enthesopathy pit in the left clavicle</td>
</tr>
<tr>
<td>NAP-K9</td>
<td>M</td>
<td>22.5</td>
<td>N/A</td>
</tr>
<tr>
<td>NAP-K17</td>
<td>F</td>
<td>27.5</td>
<td>N/A</td>
</tr>
<tr>
<td>NAP-K19</td>
<td>M</td>
<td>40</td>
<td>Louse-borne pathogen in tooth 33</td>
</tr>
<tr>
<td>NAP-K23</td>
<td>M</td>
<td>22.5</td>
<td>Periostitis in right tibia</td>
</tr>
<tr>
<td>NAP-K25</td>
<td>M</td>
<td>20</td>
<td>N/A</td>
</tr>
<tr>
<td>NAP-K26</td>
<td>M</td>
<td>27.5</td>
<td>Louse-borne pathogen in tooth 35</td>
</tr>
</tbody>
</table>

*PM = possible male

Methods

Bone Carbonate Sample Preparation. The following sample preparation protocol is based on Metcalfe et al. (2009) and Garvie-Lok et al. (2004). The 9 samples were ground by mortar and pestle into >180 microns sized pieces and weighed out to ~20-30 mg of dry bone. The weighed samples were then placed into a 2ml plastic capped tube. Subsequently, the samples were treated for 72 hours with bleach in a ratio of 0.04 ml per mg of sample, and then rinsed 5 times with deionized water.

Afterwards, the samples were treated with 0.1 M acidic acid in a ratio of 0.04 ml per mg of sample, and left at room temperature for four hours. The samples were then rinsed five times with de-ionized water. Following the acid treatment, the vials were covered with a modified Kimwipe® and rubber band, and placed in the freezer overnight. The next day, the samples were placed in the freeze dryer and frozen for 24 hours at -65° C. Following this, the samples were then placed under continuous vacuum for 24 to 48 hours.
**Analysis.** All samples were sent to the University of Florida, in Gainesville, to undergo mass spectrometry. The isotope lab at the University of Florida reported the $\delta^{18}O$ and $\delta^{13}C$ values. The oxygen isotope ratios were reported in VPDB values and then were converted to VSMOW. The oxygen values of each individual were compared to geographic European oxygen isotopic values found on water isoscape maps of Europe and Africa that were generously provided by the University of Utah.
CHAPTER FOUR: RESULTS

This purpose of this chapter is to present the results of the stable isotope analysis and evaluate the preservation of the sample. Oxygen values were originally reported against the VPDB standard and then were converted to VSMOW. Carbon isotopes values from bone apatite were compared to the values that were presented in a previous study of these same individuals (Holder, 2013). Differences between oxygen values and carbon values as well as the comparison of carbon values from bone apatite and collagen are also reported.

Preservation

After death, the body goes through the process of soft tissue decomposition and bone can be subject to diagenesis. Microbial agents and recrystallization of bone are capable of damaging the bone to where preservation can be compromised. Bone apatite yield, the proportion of extracted apatite to dry bone, is one of the ways to evaluate apatite preservation, which is calculated by utilizing Equation (1.3):

\[
\text{apatite yield (\%)} = \frac{\text{apatite weight (g)}}{\text{sample dry weight (g)}} \times 100
\]  

(1.3)

According to Schoeninger (1995), the average collagen yield in bones should be approximately 20\% to 25\% by weight. Consequently, the remaining weight of approximately 75\% -80\%, is apatite. The average apatite yield for these samples is 72.1\% \pm 2.8\% with values ranging from 67.7\% to 77.4\%. Table 3 presents the summary of apatite yields for each sample.
Table 3. A summary of bone apatite yield

<table>
<thead>
<tr>
<th>Sample</th>
<th>Apatite Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-K1</td>
<td>70.33</td>
</tr>
<tr>
<td>NAP-K6</td>
<td>74.53</td>
</tr>
<tr>
<td>NAP-K7</td>
<td>69.51</td>
</tr>
<tr>
<td>NAP-K9</td>
<td>72.39</td>
</tr>
<tr>
<td>NAP-K17</td>
<td>77.41</td>
</tr>
<tr>
<td>NAP-K19</td>
<td>72.50</td>
</tr>
<tr>
<td>NAP-23</td>
<td>70.56</td>
</tr>
<tr>
<td>NAP-K25</td>
<td>74.33</td>
</tr>
<tr>
<td>NAP-K26</td>
<td>67.70</td>
</tr>
</tbody>
</table>

In addition, the collagen preservation results of these individuals from Holder (2013) are presented in table 4. It should be noted that collagen preservation cannot be a proxy for apatite preservation because diagenesis can vary in both tissues. For this study, collagen results are used to complement the apatite values and demonstrate that there is overall good preservation.

Analyses to test for diagenetic changes indicated that the samples from the mass grave were well preserved. Table 4 lists the individuals in the sample along with their corresponding raw isotope ratios, %C, %N, collagen yield, and C:N ratios. The average collagen yield is 13.5% ± 6.6 with values ranging from 7.07% to 18.90%. All were found to be within the acceptable range above 2% (DeNiro and Weiner, 1988), which was used as the threshold in Holder’s (2013) study. The average %C is 44.6% ± 1.4% and 17.1% ± .43% for %N. All the individuals were in
the adequate range for %C (15% to 47%) and %N (5% to 17%) as defined by Ambrose (1990). According to Holder (2013) there is no relationship between %C and δ\(^{13}\)C as well as %N and δ\(^{15}\)N. The average of atomic C:N ratios (3.05 ± .02) are also located between the range of good preservation of 2.9 and 3.6 as was suggested by DeNiro (1985) and by Katzenberg (2008).

**Table 4.** Raw data for carbon, nitrogen, oxygen isotope ratios, %C, %N, Collagen Yield, and C:N ratios for each individual. Derived from Holder (2013)

<table>
<thead>
<tr>
<th>Sample</th>
<th>δ(^{13})C</th>
<th>δ(^{15})N</th>
<th>δ(^{18})O</th>
<th>%C</th>
<th>%N</th>
<th>Collagen Yield (%)</th>
<th>Atomic C:N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-K1</td>
<td>-16.96</td>
<td>9.37</td>
<td>-4.83</td>
<td>45.40</td>
<td>17.30</td>
<td>7.07</td>
<td>3.06</td>
</tr>
<tr>
<td>NAP-K6</td>
<td>-19.19</td>
<td>12.26</td>
<td>-5.44</td>
<td>42.79</td>
<td>16.34</td>
<td>3.53</td>
<td>3.05</td>
</tr>
<tr>
<td>NAP-K7</td>
<td>-18.47</td>
<td>10.93</td>
<td>-5.08</td>
<td>45.02</td>
<td>17.20</td>
<td>17.64</td>
<td>3.05</td>
</tr>
<tr>
<td>NAP-K9</td>
<td>-17.43</td>
<td>11.56</td>
<td>-4.36</td>
<td>44.93</td>
<td>17.13</td>
<td>18.04</td>
<td>3.06</td>
</tr>
<tr>
<td>NAP-K17</td>
<td>-18.91</td>
<td>10.60</td>
<td>-5.78</td>
<td>42.70</td>
<td>16.53</td>
<td>3.95</td>
<td>3.01</td>
</tr>
<tr>
<td>NAP-K19</td>
<td>-17.77</td>
<td>9.22</td>
<td>-5.64</td>
<td>43.74</td>
<td>16.96</td>
<td>17.20</td>
<td>3.01</td>
</tr>
<tr>
<td>NAP-23</td>
<td>-18.65</td>
<td>9.990</td>
<td>-5.62</td>
<td>46.93</td>
<td>17.79</td>
<td>18.90</td>
<td>3.08</td>
</tr>
<tr>
<td>NAP-K26</td>
<td>-19.22</td>
<td>8.68</td>
<td>-6.21</td>
<td>45.24</td>
<td>17.21</td>
<td>18.81</td>
<td>3.07</td>
</tr>
</tbody>
</table>
Interpretation of Geographic Locations

Stable oxygen isotope analysis was conducted on 9 of 78 Napoleonic soldiers who died in Vilnius, Lithuania during the Russian campaign. Since it is known that Napoleon accumulated soldiers from the countries that he conquered, the Grand Army consisted of a diversity of nationalities. This study aims to identify (1) variation in the sample these soldiers were conscripted and (2) if possible, approximate regions.

Table 5 presents a summary of the sample and their corresponding isotope values of oxygen and carbon. The δ¹⁸O values plotted against δ¹³C are presented in, which has a moderate, but not significant linear relationship (R² = 0.62319). Stable oxygen isotope values range from -4.3‰ to -6.2‰ with a mean of -5.3‰ ± 0.6‰ (1σ). Stable carbon isotope values from bone apatite values range from -10.9‰ to -13.6‰ with a mean of -12.2‰ ± 0.73‰ (1σ). Additionally, the δ¹³Capatite values are plotted against δ¹³Ccollagen values in Figure 10, which also demonstrates a moderate but not significant linear relationship (R² = 0.64951). The δ¹³Ccollagen values range from -19.2‰ to -16.9‰ with a mean of -18.3‰ ± 0.8‰ (1σ).

Table 5. Summary of stable oxygen and carbon results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Apatite/Collagen δ¹³C (‰, vs VPDB)</th>
<th>δ¹⁸O (‰, vs VPDB)</th>
<th>δ¹⁸O (‰, vs VSMOW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-K1</td>
<td>-10.97/-16.96</td>
<td>-4.83</td>
<td>25.83</td>
</tr>
<tr>
<td>NAP-K6</td>
<td>-12.03/-19.19</td>
<td>-5.44</td>
<td>25.30</td>
</tr>
<tr>
<td>NAP-K7</td>
<td>-12.59/-18.47</td>
<td>-5.08</td>
<td>25.67</td>
</tr>
<tr>
<td>NAP-K9</td>
<td>-11.57/-17.43</td>
<td>-4.36</td>
<td>26.42</td>
</tr>
<tr>
<td>NAP-K17</td>
<td>-12.49/-18.91</td>
<td>-5.78</td>
<td>24.95</td>
</tr>
<tr>
<td>NAP-K19</td>
<td>-12.18/-17.77</td>
<td>-5.64</td>
<td>25.10</td>
</tr>
<tr>
<td>NAP-K23</td>
<td>-12.64/-18.65</td>
<td>-5.62</td>
<td>25.12</td>
</tr>
<tr>
<td>NAP-K25</td>
<td>-12.09/-18.14</td>
<td>-4.92</td>
<td>25.84</td>
</tr>
<tr>
<td>NAP-K26</td>
<td>-13.60/-19.22</td>
<td>-6.21</td>
<td>24.51</td>
</tr>
</tbody>
</table>
**Figure 9.** Graph showing linear relationship between $\delta^{18}O$ (VPDB) and $\delta^{13}C$ values from bone apatite

\[ y = 0.6108x + 2.1557 \]
\[ R^2 = 0.6232 \]

**Figure 10.** Graph showing linear relationship between $\delta^{13}C$ values from collagen and bone apatite

\[ y = 0.8698x - 7.6578 \]
\[ R^2 = 0.6495 \]
In Holder’s (2013) study, the equation created by (White and Schwarcz, 1989), see Equation 1.3, to calculate the percentage of C₄ plants within the diet was altered to accommodate the individuals in the sample. The original values were δₓ = sample, δₓdc = -5, δₓ₃ = -26 and δₓ₄ = 9.

\[
\text{Percentage } C₄ = \frac{(δₓ - δₓ₃ + δₓdc)}{(δₓ₄ - δₓ₃)} \times 100
\]  

(1.3)

The δₓ₄ value was changed to -12.5 because it is representative of the average δ¹³C for C₄ plants in a number of populations (Van der Merwe, 1982) and “maize was not a staple C₄ plant consumed in Europe during the 1800s” (Holder, 2013:53). Additionally, the δₓ₃ value was changed to -26.5‰ and δₓdc to -5.1, which resulted in the following altered equation:

\[
\text{Percentage } C₄ = \frac{(δₓ - (26.5) + (-5.1))}{(-12.5 - (-26.5))} \times 100
\]  

(1.4)

Studies on the of abundance of C₃ and C₄ plants caused by climate change such as Wang and Greenberg (2007) and Collatz et al. (1998), generated two general patterns characterize the relationship between δ¹³C and δ¹⁸O values. The first trend is when δ¹³C values were low so were the δ¹⁸O values, suggesting that C₃ plants were more favorable towards the cooler climate. Alternatively, the second trend is when δ¹³C are high, so are the δ¹⁸O values, indicating that C₄ plants favored warmer climates. There is an additional trend of when δ¹³C values are low and δ¹⁸O values are high, then it most likely suggests that C₃ plants prefer wetter areas.

Table 6 shows δ¹⁸O values and ages of each individual with their corresponding percentages of C₄ plants in the diet, which ranged from 17.8% to 28.3% with a mean of 22.1% ± 5.7% (1σ). There is no visible connection between age and the consumption of C₄ plants and there is a weak linear relationship between C₄ plants and δ¹⁸O values that can be seen in Table 6.
as well as in Figure 11. Six of the 9 individuals consumed <25% C₄ plants, meanwhile 3 individuals consumed between 25% and 32% of C₄ plants.

Table 6. Summary of δ¹⁸O values with each individual’s age and C₄ percentage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C₄ percentage (collagen)</th>
<th>δ¹⁸O (%o, vs VPDB)</th>
<th>Approx. age of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-K1</td>
<td>31.72</td>
<td>-4.83</td>
<td>30</td>
</tr>
<tr>
<td>NAP-K17</td>
<td>17.80</td>
<td>-5.78</td>
<td>27.5</td>
</tr>
<tr>
<td>NAP-K19</td>
<td>25.93</td>
<td>-5.64</td>
<td>40</td>
</tr>
<tr>
<td>NAP-K23</td>
<td>19.66</td>
<td>-5.62</td>
<td>22.5</td>
</tr>
<tr>
<td>NAP-K25</td>
<td>23.31</td>
<td>-4.92</td>
<td>20</td>
</tr>
<tr>
<td>NAP-K26</td>
<td>15.57</td>
<td>-6.21</td>
<td>27.5</td>
</tr>
<tr>
<td>NAP-K6</td>
<td>15.81</td>
<td>-5.44</td>
<td>22.5</td>
</tr>
<tr>
<td>NAP-K7</td>
<td>20.93</td>
<td>-5.08</td>
<td>37.5</td>
</tr>
<tr>
<td>NAP-K9</td>
<td>28.37</td>
<td>-4.36</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Figure 11. Graph presenting the linear relationship between δ¹⁸O and C₄ percentages

\[ y = 0.0742x - 6.9607 \]

\[ R^2 = 0.5438 \]
CHAPTER FIVE: DISCUSSION

This chapter provides detailed analysis of the results and utilizes the historical context, models of bone turnover rates, and water isoscape maps to approximately determine where these individuals may have originated. Possible identification of geographic origins and by using δ¹⁸O values and narrowing down by analyzing dietary trends are discussed and interpreted.

Utilizing water isoscape maps, the δ¹⁸O values indicate that the majority of the individuals from this sample are likely from central and western Europe (-4.3‰ to -6.2‰), which corresponds well with where Napoleon conscripted his soldiers. This is significant because it indicates that none of the individuals were local from Lithuania (-10‰ to -11.9‰). The following are several possible provenances where the majority of these soldiers might have come from: the southern parts of France, Spain, and Portugal, as well as along the west coast of Italy. There are 3 individuals, however, that have enriched oxygen values that correspond with the Southern-most tip of Spain (-3‰ to -3.9‰) (see Fig.12 for Europe isoscape map).
Figure 12. δ¹⁸O isoscape map of Europe with values reported in VPDB

Source: http://wateriso.utah.edu/waterisotopes/media/IsoMaps/jpegs/o_Euro/oma_Euro.jpg

Based on findings by Hedges et al. (2007), values of 4 individuals (NAP-K25, NAP-23, NAP-K6, and NAP-K17) most likely reflect water sources solely from their childhood. Values of 2 individuals (NAP-K9 and NAP-K26) most likely reflect a mix of water sources from their childhood and military service. The δ¹⁸O values of 3 individuals (NAP-K1, NAP-K7, and NAP-
K19) most likely reflect a mix of water sources but more representative of their military career.

See Table 7 for the summary of results for each individual.

Table 7. Approximate age of δ18O value representation based on bone turnover rates for each individual

<table>
<thead>
<tr>
<th>Sample</th>
<th>Approx. age of death</th>
<th>Bone Turnover Range (Age)</th>
<th>Time Representation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAP-K25</td>
<td>20</td>
<td>16 – 23 (7.3 years)</td>
<td>Childhood/Adolescence</td>
</tr>
<tr>
<td>NAP-K23</td>
<td>22.5</td>
<td>16 – 23 (7.3 years)</td>
<td>Childhood/Adolescence</td>
</tr>
<tr>
<td>NAP-K6</td>
<td>22.5</td>
<td>16 – 23 (7.3 years)</td>
<td>Childhood/Adolescence</td>
</tr>
<tr>
<td>NAP-K9</td>
<td>27.5</td>
<td>23 – 56 (33 years)</td>
<td>Adolescence and Military Career</td>
</tr>
<tr>
<td>NAP-K26</td>
<td>27.5</td>
<td>23 – 56 (33 years)</td>
<td>Adolescence and Military Career</td>
</tr>
<tr>
<td>NAP-K1</td>
<td>30</td>
<td>23 – 56 (33 years)</td>
<td>Mainly Military Career</td>
</tr>
<tr>
<td>NAP-K7</td>
<td>37.5</td>
<td>23 – 56 (33 years)</td>
<td>Mainly Military Career</td>
</tr>
<tr>
<td>NAP-K19</td>
<td>40</td>
<td>23 – 56 (33 years)</td>
<td>Mainly Military Career</td>
</tr>
<tr>
<td>NAP-K17 (Female)</td>
<td>27.5</td>
<td>14 – 30.7 (16.4 years)</td>
<td>Adolescence</td>
</tr>
</tbody>
</table>

Carbon isotope analysis on bone collagen of the individuals in this sample was conducted by Holder (2013) to study nutritional stress and diet. It is known that variation of carbon values is smaller in Europe due to a much more limited environmental diversity (Holder, 2013) and that there is a higher concentration of C4 plants in southern Europe. Millet was predominately the C4 plant in Europe and was often consumed as porridge or unleavened bread, which were the main foodstuff for the poor. All of the individuals were consuming C3 plants, but there were a few that had a moderate concentration of C4 plants in their diet (25%–31%). Wheat and barley were consumed in forms of breads, soups, and porridge in the Grand Army, which would explain the high C3 plant content. C4 plants acted as a supplement and were not part of the main diet. Therefore, it explains why the majority of the individuals did not have a high percentage of C4 plants in their diet, with the exception of a few individuals. In Holder’s study (2013) the highest
percentage of C₄ plants seen in the diet was 50% to 60%. It is hypothesized that the older soldiers with a higher C₄ percentage might have been stationed or lived in southern Europe for a relatively long time period, as millet was more common there. As there is no correlation between age or sex for access to C₄ foods, it might be a possibility like Holder (2013:60) mentions, that “the variation values could be partially attributed to differences between soldiers and non-soldiers”.

NAP-K19 is one of the two most interesting individuals because he is the outlier in this sample, in terms of age and oxygen isotope values. This 40-year-old male has the most negative oxygen value (-5.6‰), assuming the ratio represents a colder climate, and had a relatively high C₄ intake (25.9%) compared to the rest of the individuals in the sample. The relationship between carbon and oxygen values is that as δ¹³C values become less negative, representative of C₄ plants, δ¹⁸O values also become less negative. C₄ plants grow in hotter and dryer climates, which is the relationship that we see with this sample. As the oxygen values represent warmer weather and an increase in the consumption of C₄ plants.

Given the aforementioned information, a process of elimination can be implemented to identify the location of origins for individual NAP-K19. First, it can be ruled out that the negative oxygen value for this particular individual is not representative of temperature or humidity levels because the percentage of C₄ plants is too high for a cold climate (Wang and Greenberg, 2007; Collatz et al., 1998). Other fractionation factors that can lower the value are an increase in latitude, altitude, and distance from the sea. Investigating the water isoscape of Europe, the two possible locations that this individual can be from considering the criteria above
would be in (1) the Pyrenees Mountains of southern France or (2) the Alps in the northern part of Italy.

There are two possibilities that could explain the oxygen values of this individual. The first one being that this individual could have participated in the Spanish War of Independence, also known as the Peninsular War, that occurred 9 years prior (1803). This war was fought in the icy peaks of the Pyrenees Mountains between Spain, Great Britain, and Portugal against Napoleonic France. Although this might be a possibility, the war only lasted for three years, which may not be enough time for the values to be reflected in the bone tissue.

The second possibility, and the most likely, the $\delta^{18}$O values represent the location of where he lived during his early adulthood. Conducting stable oxygen isotopes on tooth enamel would provide information about where this individual lived during their childhood and would either support the values reported for this study or refute it. Since tooth enamel is a static bone tissue, once it is laid down, it does not change, which preserves the original water isotope values within it (Hillson, 1996). This will allow us to further pin point locations in Europe of where these individuals might have grown up and reveal more about their lives.

NAP-K17 is another interesting individual because she is the only female represented in this sample. She died at approximately 28 years old and based on her oxygen values, 3 conclusions can be based by her values: (1) she was not local, (2) her values were similar to the rest of the individuals and may have come from the same area, and (3) likely possible that she was from southern France. Therefore, a possible conclusion is that she may have been either a wife or servicewoman that provided services, such as laundry or sexual favors, for the soldiers. It
was very common to have civilians accompany the army and so this individual would have followed them to Vilnius, where she either stayed or followed through the entire campaign to Moscow and then died in Lithuania. Either scenario may be correct, but there is no way to know for certain.

Additionally, there is an individual (NAP-K1) that has δ^{18}O and δ^{18}C values that are consistent with the Southern most tip of Spain (-4‰ to -4.9‰). Due to the elongated bone turnover rates for adult males (upper range of 33.3 years) (Hedges et al., 2007), the values could reflect the European region where he spent time during his adolescence. Although the argument could be made that the individual could be from southern Spain, there is a possibility that he could have potentially participated in Napoleon’s Egyptian Campaign (1798-1802). Although NAP-K1 (approximately 30 years old at death) would have been too young to participate in the entire campaign (16 years old), he could have participated in 1800 when he was 18 years old. This potential career soldier has δ^{18}O values of -4.8‰ that could possibly correspond with the delta region of northeast Egypt (-4‰ to -4.9‰) (see Fig.13), but also other areas of Europe where C₄ plants were present.
During the Egyptian campaign (1789-1802), Napoleon landed in Alexandria, traveled through the delta region to Cairo, and then back north to Syria. Soldiers who participated in this campaign were at risk of obtaining schistosimasis (pers. communication with Dr. Jankauskas). This parasitic disease has been discovered in the grave, therefore supporting the likelihood that at least someone was in Egypt or was from there. NAP-K9 has $\delta^{18}$O and $\delta^{13}$C values that are appropriate for the delta region. NAP-K1 has a $\delta^{18}$O value of -4.8‰ and $\delta^{13}$C value of -10.9‰ with $C_4$ plants making up 31.7% of his diet. The carbon values indicate a mixed diet, but still...
high in $C_4$ plant consumption, which is characteristic of the delta region. In a figure presented by Batanouny et al. (1988), the irrigated Nile delta region was approximately composed of 65% $C_4$ plants and 35% of $C_3$ plants. Batanoumy et al. (1988:546) notes that, “in areas under irrigation, e.g. the oases, the Nile delta, the Nile valley and the Fayoum depression, summer annuals appear in the cultivated fields. These are mainly $C_4$ plants”. Additionally, although $C_4$ plants can be found further south towards Sudan (Peters and Vogel, 2005), Napoleon stayed along the North coast of Africa during his campaign (Connelly, 2006). Therefore, there is a possibility that this individual partook in the Egyptian campaign.

Nevertheless, it is important to note that individuals have a choice in what they consume and there are two alternative scenarios to explain the individual’s values, assuming he participated in the campaign. (1) One situation would be that since Napoleon would need to have passed through southern Europe, where there was an abundance of $C_4$ plants, the army could have stocked up on these resources and consumed it with the $C_3$ plant diet in Egypt. (2) However, since the campaign lasted 4 years (1798-1802), it is mostly likely that the diet represents the resources that the army pillaged during their march through Egypt into Syria and not the food supply brought from Europe. $C_4$ plants also could have been brought up from southern Africa, like parts of Sudan, or from the Egyptian Oases, to the North, which supports the latter scenario.
CHAPTER SIX: CONCLUSION

To investigate the diversity of Napoleon’s Army, this study contributes bioarchaeological data regarding the soldiers’ militaristic migration and possible variation in geographic origins. Through the integration of analyses of material culture, human skeletal remains, and biochemistry, this research clearly furthers and advocates the importance of interdisciplinary studies. This study also complements Holder’s (2013) stable nitrogen and carbon isotope research from the same individuals. Identifying possible geographic origins can help to explain the consumption patterns of C₄ plants and possibly of marine resources.

The most important discovery is that none of the individuals had values consistent with Lithuania or surrounding areas. Stable oxygen isotope analysis demonstrates that the majority of these individuals are from central Europe, especially around southern France, Spain, Portugal, and parts of Italy. Based on historical context, these areas are where Napoleon conscripted his soldiers to build the Grand Army to attack Russia in 1812. Interesting finds within this sample included: (1) an individual that may have participated in the Egyptian campaign; (2) a possible French female who accompanied the Grand Army to Vilnius; and (3) the oldest male individual who might have lived in the southern mountains of Europe before joining the army.

From the stable carbon isotope analysis, values showed that there was somewhat of a linear relationship between the oxygen and carbon ratios, and also with the C₄ plant percentages in the diet. The majority of the individuals in this study had a mixed diet of C₃ and C₄ plants. Analyzing the amount of C₄ percentages with the oxygen values was able to eliminate potential geographical locations of where these individuals might have lived during their adolescence.
Although these are generalized conclusions, this study has revealed more about the lives of these Napoleonic soldiers. More research on bone turnover rates, tooth enamel analysis, and DNA analysis may be able to shed some light on how to better interpret $\delta^{18}O$ values of the individuals in this study.

**Future Considerations**

Although there are many historical interpretations regarding the soldiers that participated in the Russian campaign, having more archaeological resources can significantly add to the literature. Access to the catalog of artifacts found and their provenance relative to the individuals can supplement contextual information. Material culture such as campaign buttons, shakos, and uniform fragments can conceivably inform us of their regiments, division, and rank. Furthermore, access to field notes and excavation survey drawings, especially location of the graves relative to the city, are important complements to the study.

In addition to archaeological context, research of the relationship between osteons, the main structural unit of compact bone, and bone turnover rates could provide further information for this sample. The bone turnover rate is one of the significant parts to determine when in time the oxygen values represent. Since rates vary between sex and age, knowing approximately how many years have past since the beginning of the turnover cycle will allow for a more accurate timeline. Additionally, a more ambitious project might be to conduct DNA analysis and perhaps link these soldiers to living relatives. Furthermore, it can provide information about the physical characteristics of these soldiers, such as skin tone, hair, and eye color.
Conducting other stable isotopic studies such as strontium could also be beneficial to help eliminate certain locations. Strontium analysis is limited because substrates could be the same throughout the landscape, but can be significantly useful to identify differences in the substrates between continents. Strontium, along with stable oxygen isotope analysis on teeth, can answer the question of the two individuals who have oxygen values that are consistent with that of the delta region of Egypt and the southern tip of Spain. The differences between the substrates of Egypt versus Spain could indicate whether they were part of the Egyptian campaign or grew up in southern Spain.

This research is important and timely because it will contribute to a larger ambitious project that aims to aid in the development of Lithuania’s national identity. It also allows for the fostering international ties between the United States, Lithuania, and every European country these soldiers were connected to. This interdisciplinary study will be able to contribute to the expansion of anthropological, biochemical, and geological knowledge of European political and military history during the Napoleonic wars.
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