INTERPRETING DIET AND NUTRITIONAL STRESS IN NAPOLEON'S GRAND ARMY USING STABLE CARBON AND NITROGEN ISOTOPE ANALYSIS

by

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ABSTRACT

A mass grave containing the remains of at least 3269 Napoleonic soldiers from 1812 was discovered in Vilnius, Lithuania in 2001. Stable carbon and nitrogen isotopic analyses were performed on collagen from 78 femoral samples to explore dietary variation within the sample. Starvation and nutritional distress were also evaluated through the examination of nitrogen enrichment in bone collagen. Clinical studies have demonstrated that enriched nitrogen isotope values can indicate periods of nutritional stress and starvation; however, this is the first study to examine its use as a diagnostic indicator of starvation in a historical population using bone tissue. According to the carbon isotope data, a vast majority of the soldiers in this sample consumed predominantly C_3 plants with only five individuals consuming more than 50% of their plant dietary protein in the form of C_4 plants, with $\delta^{13}C$ ratios for this sample ranging from -19.22‰ to -11.76‰ and a mean of -17.18‰. This conforms to expectations given that a majority of human plant consumption in Europe is C_3 pathway plants. Twenty-nine individuals exhibit enriched $\delta^{15}N$ values, with $\delta^{15}N$ values for this sample ranging from 7.14‰ to 13.63‰ and a mean of 10.49‰. The combination of historical and isotopic evidence suggests prolonged nutritional stress may be the cause of enrichment in this sample. The results of this study demonstrate the applicability of this method on archaeological samples as a line of evidence in the investigation of starvation and famine.

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CHAPTER ONE: INTRODUCTION

Archaeologists have applied a number of methods to investigate diet: trace element analysis, analysis of osteological pathological conditions, midden analysis and faunal analysis are commonly used. One of the most common methods is stable isotope analysis. Stable isotopic analysis of bone directly measures long-term diet of past populations, which can provide valuable information and context concerning past life-ways (Katzenberg 2008). A newer area of investigation using stable isotopic analysis is the investigation of nutritional stress (Fuller et al. 2005, Hatch et al. 2006, Mekota et al. 2006). This is of particular relevance in the study of soldiers during times of war when nutritional resources may be scarce. "A skeletal series from a battlefield offers the opportunity to study the effects of field conditions and physical stress…in a specially selected subset of the general population in which age, sex, and other physical characteristics are relatively consistent as a result of induction standards" (Owsley 1997:8). Osteological investigations from historic military sites are a recent trend in bioarchaeology (Sledzik and Sandberg 2002). Stable isotope analysis of these samples is almost non-existent. In this study, I applied stable isotope analysis to a group of soldiers that perished in the retreat from Moscow during the Russian Campaign of the Napoleonic Wars in 1812 for the purpose of interpreting diet and nutritional stress in this sample.

The Napoleonic Wars were a series of military conflicts between Napoleon's French Empire and other European nations that took place from 1803 to 1815 (Esdaile 2008). They began in May of 1803 when Britain declared war on France. Alliances with different countries led to the deployment of Napoleonic troops throughout Europe including Poland, Prussia,

Austria, Portugal and Spain during the early 1800s (Esdaile 2008). Prior to the inception of war with Britain, campaigns took place in Italy and Egypt during the late 1700s.

In 1812, Napoleon's Grand Army invaded Russia in an attempt to prevent the invasion of Poland by Russian Emperor Alexander I. The Russian campaign began with approximately 675,000 men with less than half being French (Nicolson 1985). Figure 1 depicts the organization of the Grand Army in the Russian Campaign and the countries these soldiers represented. Napoleon's Grand Army retreated from Moscow in October of 1812, 35 days after entering the city when the Russian czar did not surrender. This was due to a lack of supplies in the capital largely abandoned and burnt down by retreating Russian soldiers.

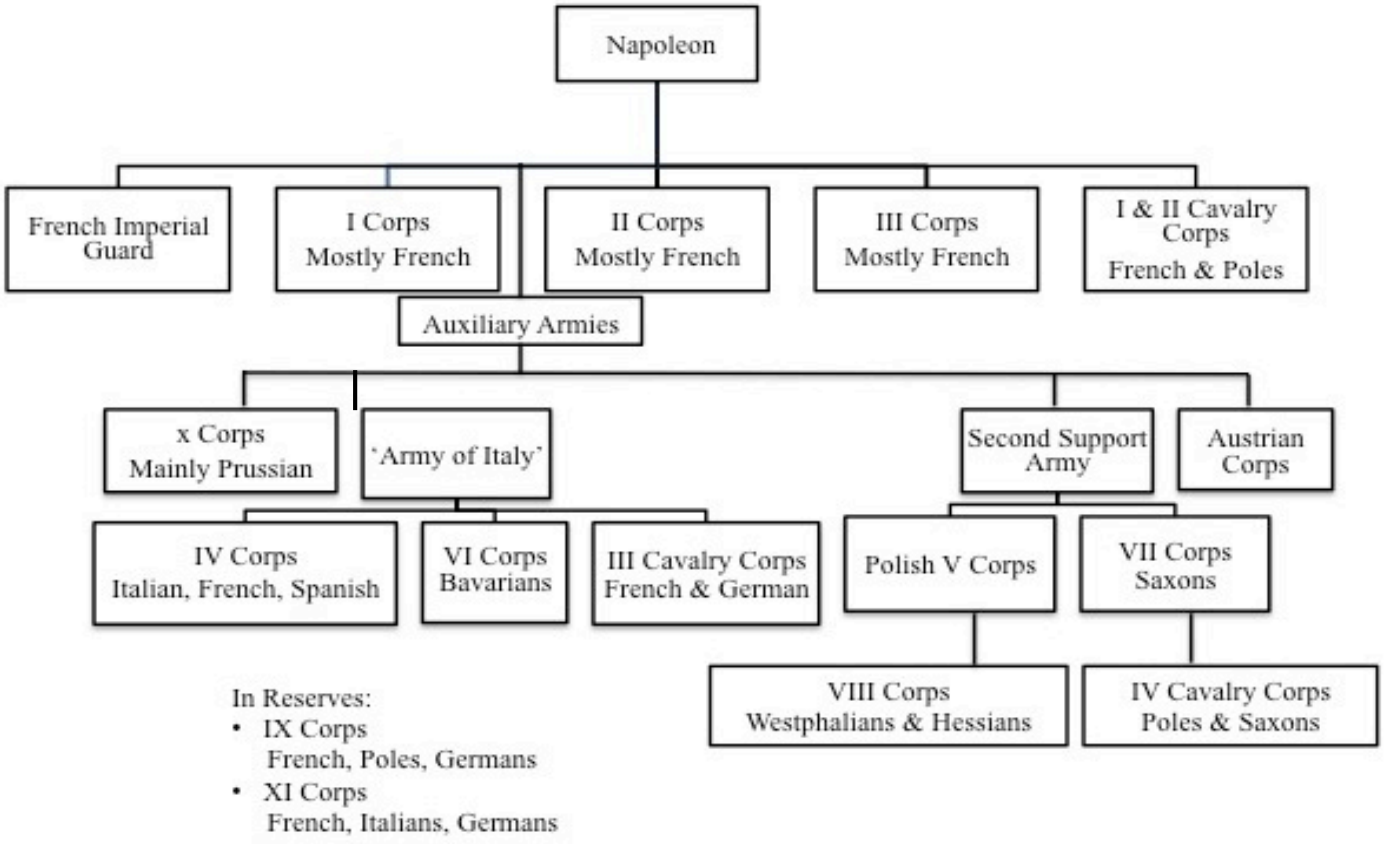

Figure 1. Flow chart showing the organization of Napoleon's Grand Army and the countries represented during the Russian campaign. Adapted from Nicolson (1985).

At the time of their exit, the French Army was comprised of over 100,000 infantry, cavalry and guns with a convoy of 40,000 carriages, wagons, women and children (Nicolson 1985). After a battle at Maloyaroslavets, Russia with Russian troops, the French Army retreated to Smolensk, Russia. Nicolson argues for three causes for the retreat to Smolensk: "the mounting harassment by the Cossacks and partisans, the condition of the horses, and the physical weakening of the troops by hunger and cold" (1985:125). By the time the Grand Army reached Smolensk, their troops dwindled to 41,000 soldiers (Nicolson 1985). Upon arrival in Smolensk, the Grand Army was promised food and rest. However, the city of Smolensk was in ruins from a previous battle and did not contain enough supplies to feed 40,000 starving soldiers. After a few days in Smolensk, the army continued west to Orsha and Studentka, Russia where they crossed the Beresina River, before arriving in Vilnius, Lithuania. Figure 2 shows a map of Europe with Napoleon's route of retreat from Moscow to Vilnius enlarged in greater detail.

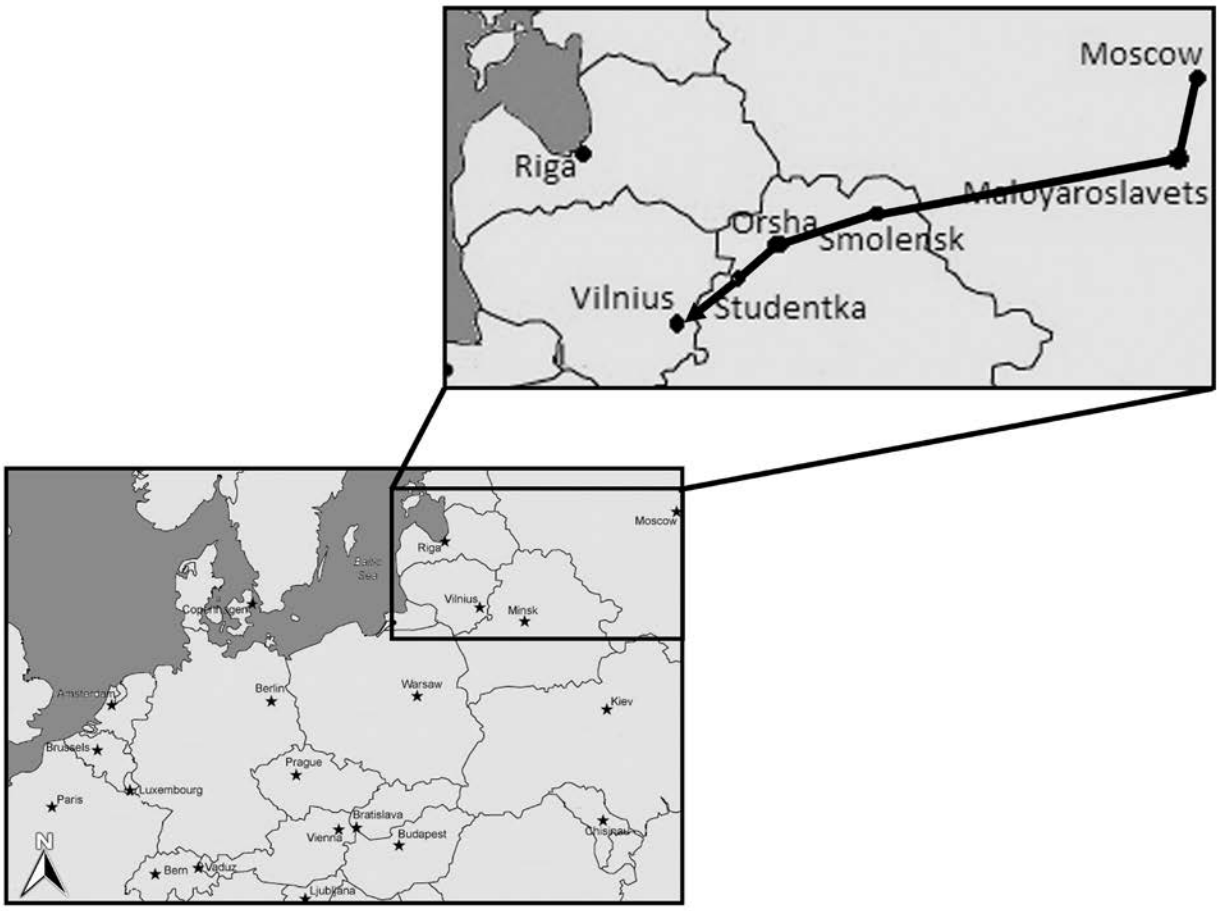

Figure 2. Map of Europe with Napoleonic retreat from Moscow to Vilnius highlighted. Map is not to scale.

Napoleon's soldiers arrived in Vilnius in December 1812. Vilnius contained enough "flour and meat to feed 100,000 men for forty days" (Nicolson 1985:168). However, there was no organization in food distribution and famine during the long march led to chaos. Historical records indicate an estimated 20,000 soldiers died of hypothermia, starvation and typhus in Lithuania during the retreat (Lobell 2002, Austin 2000). Initially, remains were burned, however, the stench and large numbers of the dead and dying led to disposal of bodies in mass graves.

Conditions in war, such as extreme environmental conditions and physical exertion while marching and during battle, are a major source of stress on the body and sufficient quality and quantity of food is necessary for adequate mental and physical performance (Hill et al. 2011). Dietary protein plays an important role in military nutrition. Inadequate protein consumption can lead to increased morbidity and mortality, atrophy of skeletal muscle, increase in infection problems and diminished wound healing (Hill et al. 2011). The Napoleonic Wars lasted almost than 15 years from 1803 to 1815. Dietary staples for Napoleonic soldiers were bread, meat, wine and beer (Forrest 2002). A common ration consisted of 'soup in the morning, at midday, soup again, ten ounces of meat and a ration of bread, with half a pot of beer; and in the evening half a pot of beer and vegetables' (Forrest 2002:139). During this time of war, historical documents attest to the fact that these soldiers suffered "from a lack of warmth and sleep, food and drink, boots and clothing. Too often supply trains were delayed, pay failed to come through, and men were left forage for their own sustenance" (Forrest 2002:1-2).

Following a failed invasion of Moscow in 1812, thousands of soldiers died due to starvation, hypothermia and typhus during their march back to France (Austin 2000). Russia's scorched earth policy left the towns along the route of the French retreat with little to no resources. Hunger, fatigue and cold plagued the retreat through a harsh Russian winter. As soldiers suffered and became more desperate, they turned to their dead and dying horses as a source of food (Austin 2000). In a letter to his family, a French soldier recounts how he managed to thwart starvation:

> The army has been without bread on its march, but it did have large numbers of horses which had died from exhaustion, and I can assure you that a slab of horsemeat, sliced and cooked in a pan with a little fat or butter makes a very reasonable meal. At Wiazma we enjoyed a very good cat stew; five of us consumed three fine cats, and they were excellent. (Forrest 2002, p 152)

Vilnius was one of the first towns that was not burned down and largely abandoned. Historical accounts recount the ghastly scenes of death at Vilnius shortly after arrival. Sir Robert Wilson of the Russian Army wrote in his journal:

> The hospital of St Bazile presented the most awful and hideous sight. 7,500 bodies were piled like pigs of lead over one another in the corridors; carcasses were strewn about in every part; and all the broken windows and walls were stuffed with feet, legs, arms, hands, trunks and heads to fit the apertures and keep out the cold air from the living. The putrefaction of thawing flesh emitted the most cadaverous smell. (Nicolson 1985, p 169)

The purpose of this research is to explore dietary variation and starvation within Napoleon Bonaparte's Grand Army following the Russian Invasion of 1812. Seventy-eight femur samples collected from a mass grave of approximately 3269 individuals in Vilnius and used in this research. This study addresses the following questions: what do isotopic values reveal about dietary patterns of Napoleon's Grand Army? Is it possible to determine whether these soldiers were under prolonged nutritional stress through stable nitrogen isotope analysis? Stable carbon and nitrogen isotope analyses were used to determine general dietary characteristics and variation within the sample. Starvation and nutritional distress were investigated through the examination of nitrogen enrichment in bone collagen. This research utilizes stable isotope analysis to advance the knowledge of the human past through the examination of a historic sample. This study is the first of its kind in that it uses stable isotope analysis as a line of evidence in an investigation of nutritional stress in an archaeological population. A comparative approach was taken in order to investigate similarities and differences between the current sample and other historic populations.

CHAPTER TWO: LITERATURE REVIEW

The purpose of this chapter is to present a theoretical and methodological framework for stable isotope analysis in the context of dietary reconstruction and the evaluation of nutritional stress. It begins with a brief background on stable isotopes, focusing on those isotopes used in this study: carbon and nitrogen. Clinical studies and animal studies investigating nutritional stress and studies in European populations are discussed in greater detail to construct a context for the current study. This section ends with a short discussion on the limitations of isotopic analysis in archaeological investigations.

What are isotopes and how do they work?

Isotopes are one of two or more alternative forms of an element (i.e. carbon) that have differing numbers of neutrons, resulting in varying atomic weights (Tykot 2006). Stable isotopes are isotopes that do not decay over time. Table 1 shows the stable isotope forms of carbon and nitrogen, the elements used in this study, and their abundances in the natural environment. The variation in the mass of these isotopes relative to one another, results in varying chemical and physical characteristics (Katzenberg 2008). For example, atmospheric carbon dioxide (CO_2) supplies carbon to terrestrial plants in two isotopic forms: $^{13}CO_2$ and $^{12}CO_2$ (Schoeninger 1995). Both forms are photosynthesized by plants and metabolized into carbohydrates, proteins and lipids (Tykot 2006). The plants and their metabolites "are consumed by organisms that then appropriate or convert, or both, these compounds into their bodily tissues" (Tykot 2006:132). Therefore, "you are what you eat" model can be used to reconstruct diet for human tissues. The lighter weight forms of elements react faster in photosynthetic reactions resulting in enriched levels of ^{12}C. This is known as isotope fractionation. When plants are consumed by animals, the

reverse process of fractionation occurs, leading to enriched ^{13}C isotopes in the body tissues of the consumer. Isotope fractionation "is the basis for stable isotope variation in biological and geochemical systems" (Katzenberg 2008:416).

Table 1. Carbon and Nitrogen Isotopes and their Relative Terrestrial Abundances

Element	Isotope	Abundance (%)
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37

Derived from Table 13.1 Katzenberg 2008.

How stable isotopes are measured and reported

Stable isotope ratios are measured by gas isotope ratio mass spectrometers (IRMS), which typically consist of four sections: an inlet, ion source, mass analyzer (and magnet) and ion collector (Katzenberg 2008). Figure 3 shows the components of a mass spectrometer and the path of the ion beams. First, the prepared sample is converted into a gas and ionized by the ion source. The charged molecules flow into the flight tube, where a magnet is used to separate the ionized molecules based on mass. Once separate, the ion beams continue to the ion collector (a faraday collector) where they are measured and the two most plentiful isotopes in the sample are compared to an international standard.

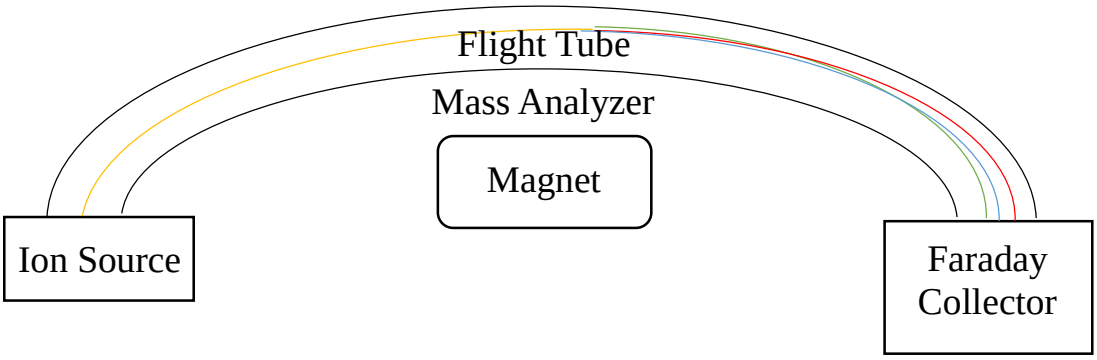

Figure 3. Diagram of a mass spectrometer showing its components. The colored lines represent the ionized gas that becomes separated by the magnet in the flight tube.

Isotope ratios are denoted as delta (δ) values in parts per mil (‰) comparative to international standards since differences in isotope ratios between samples are typically very small (Tykot 2006). These values are calculated using Equation 1.1:

$$\delta\ (\text{in } ‰) = \frac{R_{(\text{sample})} - R_{(\text{standard})}}{R_{(\text{standard})}} \times 1000 \qquad (1.1)$$

where R = the ratio of heavier isotopes to lighter isotopes. Equation 1.2 shows the equation for carbon:

$$\delta^{13}C\ (\text{in } ‰) = \frac{{}^{13}C/{}^{12}C_{\text{sample}} - {}^{13}C/{}^{12}C_{\text{standard}}}{{}^{13}C/{}^{12}C_{\text{standard}}} \times 1000 \qquad (1.2)$$

For nitrogen, Equation 1.3 is used to calculate the delta value:

$$\delta^{15}N\ (\text{in } ‰) = \frac{{}^{15}N/{}^{14}N_{\text{sample}} - {}^{15}N/{}^{14}N_{\text{standard}}}{{}^{15}N/{}^{14}N_{\text{standard}}} \times 1000 \qquad (1.3)$$

Atmospheric nitrogen (AIR) is commonly used as the standard for $\delta^{15}N$ and Vienna Pee Dee Belemnite (VPDB) is commonly used as the standard for $\delta^{13}C$. The value for AIR is 0.003676 and the VPDB value is 0.0112372.

Isotopes used in this study and previous applications

Chemists and physicists began studying stable isotopes in the early 1900s (Katzenberg 2008). Throughout the mid twentieth century, isotopic studies were common in the fields of chemistry, biology and geochemistry. The first element to be studied in an archaeological context was carbon, followed by nitrogen, oxygen, sulfur and most recently strontium (Katzenberg 2008). Radiocarbon dating in the 1960s and 70s using carbon isotope ratios showed differing ^{13}C values for human skeletal remains (van der Merwe 1982). This was attributed to differences in diet. In the late 1970s and throughout the 1980s, anthropologists investigated the correlation between diet and stable carbon and nitrogen isotope ratios (DeNiro and Epstein 1978, DeNiro and Epstein 1981) and trophic level and nitrogen isotope values (Schoeninger and DeNiro 1984, Ambrose and DeNiro 1987). These studies are the basis for present dietary isotopic studies.

Carbon isotopes. Carbon isotope analysis can be used to study differences in consumption of plants with different photosynthetic pathways (i.e., C_3 and C_4). During photosynthesis, less ^{13}C is incorporated into plants than the lighter isotope ^{12}C. As a result, plants are enriched in ^{12}C comparative to CO_2 in air, which provides carbon to plants. C_4 plants incorporate more ^{13}C in the photosynthetic process than their temperate counterparts, C_3 plants (Katzenberg 2008). Subsequently, C_4 plants exhibit enriched $\delta^{13}C$ values over C_3 plants. Prior to industrialization, it is estimated air exhibited a $\delta^{13}C$ value of approximately 7‰ (Katzenberg 2008). C_4 plants such

as maize, millet and sorghum exhibit $\delta^{13}C$ values around -12‰, while C_3 herbaceous vegetation exhibits values around -26‰ (Schoeninger 1995). Human bone collagen values reflect the isotopic signatures of the plants they consume plus 5‰ due to the fractionation factor between the dietary source consumed and the production of collagen (Krueger and Sullivan 1984). Figure 4 shows the pre-Industrial average $\delta^{13}C$ values for atmospheric CO_2, C_3 and C_4 plants and human bone collagen based on dietary plant source. It is important to note that $\delta^{13}C$ values in collagen predominantly reflect the protein portion of the diet since collagen is synthesized from carbon that comes mainly from protein (Ambrose and Norr 1993).

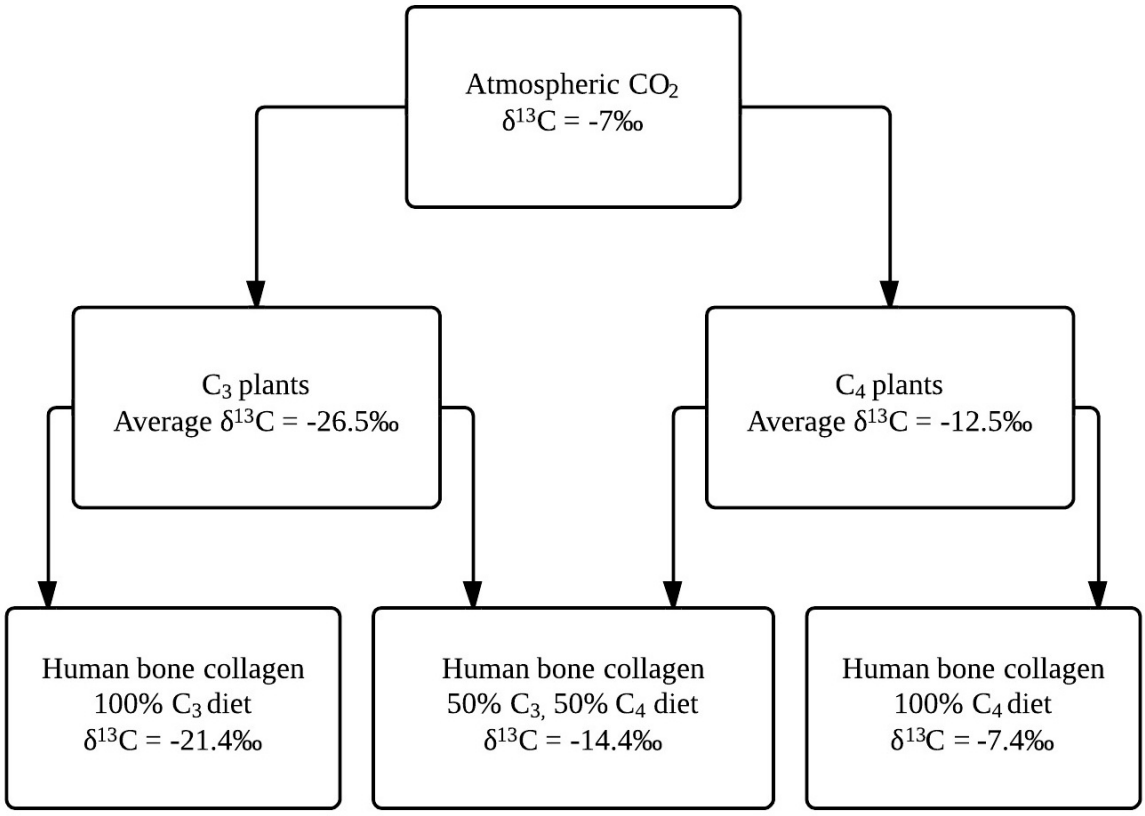

Figure 4. Average $\delta^{13}C$ values of human bone collagen based on carbon sources. Derived from Tykot 2006 and Krueger and Sullivan 1984.

Stable carbon isotope values, when paired with $\delta^{15}N$ values, can provide information on the consumption of marine resources. The consumption of marine resources reflect $\delta^{13}C$ values (in collagen) intermediate to the C_3 and C_4 plant consumption values (Katzenberg 1992, 2000). These values characteristically range from -10 to -15‰ (Katzenberg 1992, 2000; Schoeninger et al. 1983; Ubelaker et al. 1995).

The introduction of maize as a dietary staple in the New World has been the subject of investigation since the inception of carbon isotope analysis in archaeology. Vogel and van der Merwe (1977) were the first to investigate the introduction of maize agriculture in their study population from New York State. This was followed by a number of similar investigations throughout the 80s and 90s (e.g., Bender et al. 1981, Buikstra and Milner 1991, Katzenberg et al. 1995, Larsen et al. 1992, Schurr and Redmond 1991, van der Merwe et al. 1981).

A number of atmospheric, geochemical and biological mechanisms produce distinct isotopic compositions in particular environments (Kohn 1999). In the Americas, Asia and Africa, variations in carbon and nitrogen ratios are often large due to considerable diversity of the isotopic ratios in local environmental resources (van Klinken et al. 2000). In Europe, however, variation in isotopic ratios is much smaller due to much more limited environmental diversity. Van Klinken et al. (2000) discovered carbon enrichment (<2‰) in humans from northwestern to southern Europe historically. They argue these differences are related to climatic variations in temperature and humidity that are reflected in differences between various types of botanical food sources.

Nitrogen isotopes. There are two sources of inorganic nitrogen available to terrestrial plants: atmospheric nitrogen (N_2) and nitrates in soil (van Klinken et al. 2000). There is a minute

fractionation factor of N_2 in nitrogen fixating plants, such as legumes (e.g., peas, beans, clover, alfalfa, etc.) resulting in $\delta^{15}N$ values similar to the atmosphere (0‰) (Schoeninger 1995). However, most plants uptake nitrogen through soil resulting in plant values that are more enriched than atmospheric values. Marine plants exhibit more positive nitrogen values than terrestrial plants since dissolved nitrates in water are isotopically enriched (Schoeninger and Moore 1992).

$\delta^{15}N$ values also reflect trophic level, an organism's position in the food chain. Consumer $\delta^{15}N$ values are enriched over the levels of their dietary sources "due to the specific and roughly constant enrichment in ^{15}N of the consumer's slow turnover body proteins (such as bone collagen) over their diet" (Hedges and Reynard 2007:1240). This means that in an anabolic state, the consumer incorporates dietary protein into bodily tissues (Hatch et al. 2006). As these tissues turnover and protein is utilized or excreted from the body, ^{14}N reacts at a faster rate resulting in higher concentrations of ^{15}N in bodily tissues. The notion of stepwise nitrogen enrichment and ascending trophic levels originated with the work of DeNiro and Epstein (1981), Minagawa and Wada (1984) and Schoeninger and DeNiro (1984). It has been estimated that herbivore $\delta^{15}N$ values are enriched by 3‰ over their dietary source (Schoeninger 1985, Ambrose 1986, Katzenberg 1989). The same is argued for carnivores. However, an increase of up to 5‰ has been argued for human $\delta^{15}N$ values (Hedges and Reynard 2007). Figure 5 plots $\delta^{15}N$ bone collagen values for different European faunal species throughout the Holocene including humans using data from van Klinken et al. (2000). This graph shows the level of enrichment in humans over other animals, due in part to trophic level. Outside of the horse, the other animals are common sources of dietary protein for humans. Deer/elk, horses and cattle are grazing

vegetarians, while sheep/goat and pigs are omnivorous.

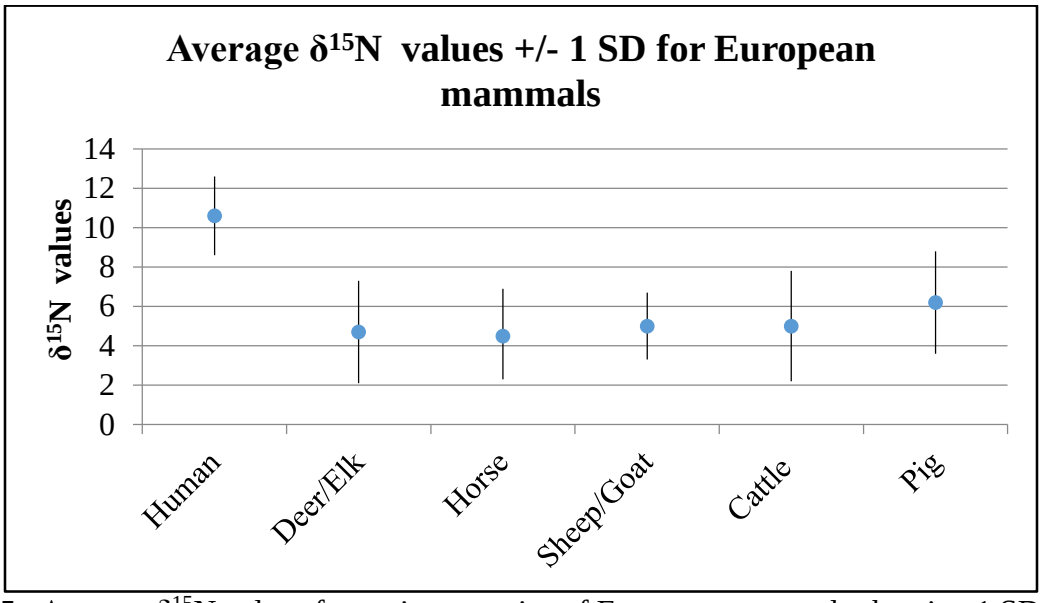

Figure 5. Average $\delta^{15}N$ values for various species of European mammals showing 1 SD (standard deviation) above and below the average (van Klinken et al. 2000).

It has been argued that the increased $\delta^{15}N$ values in humans is likely, in part, related to the "manuring effect" (van Klinken et al. 2000). This occurs when $\delta^{15}N$ values in soil increase due to the introduction or increase of natural fertilizers, such as animal feces. A controlled study by Högberg (1990) showed that the increased nitrogen in soil elevated $\delta^{15}N$ values in plants. This increase continues through the upper trophic levels.

Stable nitrogen isotopes analysis has been used to study consumption patterns beyond trophic level enrichment. Stable nitrogen isotope ratios in bone collagen have been used to interpret marine components in the human diet (e.g., Schoeninger et al. 1983, Walker and DeNiro 1986, Keegan and DeNiro 1988, Sealy and van der Merwe 1988). In these studies and others conducted since, $\delta^{15}N$ values of bone collagen were found to be enriched in the bone collagen of individuals who consumed a significant amount of marine resources. This is because most of the available nitrogen in marine environments is manufactured by bacterial

denitrification and this process creates more ^{15}N than does N_2 fixation in soil (Schoeninger and Moore 1992). The increased available ^{15}N results in more positive $\delta^{15}N$ values in marine organisms over terrestrial plants. This enrichment is passed through the trophic levels resulting in higher $\delta^{15}N$ values for marine vertebrates over their terrestrial counterparts (Schoeninger and DeNiro 1984).

Stable nitrogen isotope values have been used in investigations of changing consumption patterns. Infant weaning age and patterns is a major area of study. Fogel et al. (1989) found that lactating infant $\delta^{15}N$ values are enriched over their mothers' values, consistent with trophic level shifts. These values decrease during and after weaning. Katzenberg et al. (1993), Katzenberg and Pfeiffer (1995), White and Schwarcz (1994) and Dupras et al. (2001) are examples of other studies that describe similar relationships between $\delta^{15}N$ values and infant dietary shifts. The $\delta^{15}N$ values steadily decreases as lactation decreases and consumption of other dietary sources increases until it plateaus when weaning is complete. Bone turnover rates in infants and children is faster than adults and these dietary changes can be tracked.

Enriched nitrogen isotope values in consumer tissues can also be a reflection of climate. A number of studies have argued $\delta^{15}N$ values increase with increased aridity (e.g., Heaton et al. 1986, Sealy et al. 1987, Ambrose 1991, Schwarcz et al. 1999). Heaton et al. (1986) found a linear relationship between $\delta^{15}N$ values and rainfall, with $\delta^{15}N$ values increasing as aridity increases. Ambrose and DeNiro (1987) demonstrated that drought-tolerant herbivorous mammals exhibit enriched nitrogen values in bone collagen due to water stress compared to their water-dependent counterparts. These authors suggest a slower rate of urea excretion as the cause for this enrichment. In a more recent study, Schwarcz et al. (1999) conducted stable nitrogen

analysis on human bone collagen, faunal and plant samples from three desert sites. The authors found enriched $\delta^{15}N$ values for human samples and for a number of plant samples. The authors suggest this may be the result of increased nitrogen in the soil of arid environments, especially at increased depths. The enrichment found in the plants may be passed on to the humans that consume them.

The complexity of interpreting nitrogen isotope values, in relation to diet and other factors, is exemplified in a study by Ubelaker and Owsley (2003). The authors investigated $\delta^{13}C$ and $\delta^{15}N$ values to explore dietary variation in two 17th century American colonial samples from Maryland and Virginia. The samples consisted of adult immigrants from Northwestern Europe and juveniles believed to originate in the area. The authors found high levels of variation in $\delta^{15}N$ values within the samples, with a number of samples exhibiting enriched values. The authors provide a number of possible explanations for these values: marine foods and protein stress related to the voyage (from Europe to America) and early years after arrival (Ubelaker and Owsley 2003). Further investigation into life-ways prior to immigration could help determine the factor most likely impacting the elevated $\delta^{15}N$ values observed in this sample.

Complex factors impact $\delta^{15}N$ values: diet and trophic level, climate and cultural practices such as the use of manure and infant weaning. These factors are dependent upon nitrogen uptake in soil and metabolic processes in consumers. Any one of these factors can lead to nitrogen enrichment in consumer tissues.

Nutritional stress, pathological conditions and $\delta^{15}N$ values

There are a number of factors that can contribute to nitrogen enrichment in human tissue: marine resources, the manuring effect, etc. The following section discusses two sources of

enrichment that was not discussed in previous sections: nutritional stress and pathological conditions. This section begins with a discussion of nitrogen balance in body tissues, followed by a discussion of contemporary and archaeological investigations that investigate nitrogen enrichment caused by nutritional stress and pathological conditions using stable isotope analysis. The relationship between nutritional stress, protein metabolism and nitrogen enrichment are also discussed.

Inter-human variation in $\delta^{15}N$ values is of particular interest to physical anthropologists and contemporary health researchers. A major goal of this type of research is to determine differential animal protein consumption within and between populations. There are two primary perspectives on $\delta^{15}N$ enrichment within human populations: high protein diets result in $\delta^{15}N$ enrichment (Pearson et al. 2003, Sponheimer et al. 2003a,b) and severe protein deprivation leads to nitrogen enrichment (Robbins et al. 2005). As Hedges and Reynard (2007) explained:

> Both arguments are based on the idea that because ^{15}N depleted nitrogen is lost in urea as the main byproduct of protein metabolism, increasing the fraction of nitrogen lost as urea (depleted in ^{15}N relative to protein) results in higher $\delta\,^{15}N$ values of the remaining tissues, while maintaining isotopic mass balance. However, the disagreement arises as to whether increased protein in the diet results in more or less protein metabolism and urea production, thus changing the ^{15}N enrichment. (p 1241)

This research explores the latter mechanism for nitrogen enrichment: protein deprivation.

Nutritional stress can be caused by a number of factors (e.g., metabolic disease, cultural factors, environmental factors) and can be either acute or chronic (Roberts and Manchester 2007). Chronic stress causes metabolic responses that may manifest in bone. Harris lines and rickets are examples of skeletal manifestations of prolonged nutritional stress. In this study, nutritional stress refers to protein deprivation, a severe lack of protein intake in the diet. Protein deprivation may also be acute (as in the case of morning sickness during pregnancy, discussed

below) or chronic (as in the case of anorexia nervosa, discussed below). The relationship between protein deprivation and nitrogen balance are discussed below.

Nitrogen plays an important role in the growth and maintenance of bodily tissues; nitrogen acquired through dietary consumption and the recycling of bodily proteins are the two sources of nitrogen in the body. Nitrogen that is consumed or recycled but not used is excreted from the body, often the form of urea. The difference between nitrogen intake and excretion is known as nitrogen balance. There are three states of nitrogen balance in the body: positive nitrogen balance, nitrogen equilibrium and negative nitrogen balance. Positive nitrogen balance occurs during tissue growth (Katzenberg and Lovell 1999). More nitrogen is consumed than excreted as nitrogen is utilized in new tissue formation. Katzenberg and Lovell (1999) hypothesize that this process results in trophic enrichment comparative to diet due to the preferential excretion of ^{14}N over ^{15}N. Fuller et al. (2004) argue that positive nitrogen balance leads to reduced $\delta^{15}N$ values in body tissues regardless of diet. Nitrogen equilibrium occurs during tissue maintenance when consumption and excretion are equal. According to Katzenberg and Lovell, "bone collagen will largely reflect ingested protein from the diet ($\delta^{15}N$ of diet plus 3‰) averaging over several years" (1999:321). Negative nitrogen balance occurs when there is not enough nitrogen consumption to maintain tissues and the body enters a catabolic state. The resulting $\delta^{15}N$ values are enriched compared to the values of tissues in a state of normal maintenance (Hobson et al. 1993).

There are four types of studies that investigate starvation and/or nutritional stress in mammals through isotopic analysis: nutritional stress related to pregnancy and morning sickness, acute starvation related to anorexia nervosa and/or bulimia, animal studies and

pathological conditions present in bone. These studies investigate the relationship among reduced protein consumption, protein metabolism and stable isotope values. The principal lessons taken from these studies can shed light on isotopic variation in archaeological populations and provide alternative explanations to nitrogen enrichment beyond diet and/or climate.

Fuller et al. (2005) conducted a study on the hair of eight pregnant women experiencing nutritional stress related to morning sickness in the early trimesters. The authors found that while there was no change in $\delta^{13}C$ values during pregnancy, $\delta^{15}N$ values increased during periods of morning sickness and weight loss and decreased later in the pregnancy. The authors argue that this is due to the catabolic state the body enters during nutritional stress, breaking down musculoskeletal tissue for the purpose of retrieving protein in tissue reserves. During this process, ^{14}N is preferentially excreted and the remaining tissue becomes enriched in ^{15}N as protein is recycled rather than consumed resulting in elevated $\delta^{15}N$ values. In a previous study by Fuller et al. (2004), a decrease in $\delta^{15}N$ values was found during gestation in women who did not experience nutritional stress (i.e., morning sickness).

Mekota et al. (2006) conducted the first isotopic study used to track dietary change and changes in isotopic values in anorexia nervosa patients from hospital admittance to recovery. The authors tracked the change in stable carbon and nitrogen isotope values in hair keratin, along with body mass index (BMI) in six anorexia nervosa patients over a 17 week period. They found that the $\delta^{15}N$ values were inversely related to BMI. The $\delta^{15}N$ values were at their highest when BMI was at its lowest. This supports Fuller et al.'s findings (2005). The authors argue this is due to gluconeogenesis, which is the production of glucose from non-carbohydrates, such as

pyruvate, lactate and alanine (Mekota et al. 2006). Glucose is the primary source of energy for tissues and is integral to several vital functions (Orten and Neuhaus 1982). There are three phases of glucose homeostasis during a prolonged period of negative nitrogen balance. The first phase, early starvation, begins approximately sixteen hours after consumption of food and gluconeogenesis is steadily increasing. During this time, muscle tissue is broken down in order to provide amino acids for gluconeogenesis and approximately 2.5% of the body's nitrogen is lost in the form of excreted urea (Orten and Neuhaus 1982). This results in $\delta^{15}N$ enrichment. To prevent an extreme loss of bodily protein, gluconeogenesis begins to decline after approximately two days and ketogenesis becomes increasingly important. This is the intermediate phase of starvation. During ketogenesis, blood glucose is lowered to a minimum and energy for certain functions is provided by ketone bodies (Mekota et al. 2006). During prolonged starvation, the third phase, the production of glucose from amino acids provided by the breakdown of bodily proteins is continues at a minimum (Orten and Neuhaus 1982). Although the process of gluconeogenesis is greatly reduced over a prolonged period of starvations, it does continue at a reduced rate further enriching $\delta^{15}N$ values.

Hatch et al. (2006) conducted stable isotope analysis of hair on females with anorexia and/or bulimia for the purpose of developing a diagnostic algorithm for these two conditions. Their results were similar to that of Mekota et al. (2006). There were distinct differences in $\delta^{15}N$ values for individuals with anorexia, bulimia and the control group. Although their algorithm was problematic, the authors argue a functioning diagnostic algorithm is possible.

Initial dietary investigations utilizing stable nitrogen isotope analysis were based on the assumption that nitrogen enrichment occurs in a step-wise function between dietary product and

consumer tissue. While this is true, the clinical studies above show that there are other possible causes of nitrogen enrichment in body tissues. Animal studies lend further support to these claims. Hobson et al. (1993) determined that both nutritionally stressed captive birds and their fasting wild counterparts show significant nitrogen enrichment in muscle, liver, blood and bone tissues. A controlled study conducted on guinea pigs by Spanheimer and Peterkofsky (1985) demonstrates that collagen production in bone decreases significantly during periods of fasting. The authors argue this the result of decreased collagen synthesis rather than increased catabolism of existing collagen. The decreased production of bone collagen over a long period can contribute to higher incidences of broken bones and contribute to the onset of diseases such as osteopenia and osteoporosis (Spanheimer et al. 1991).

The numerous studies cited in this section demonstrate that dietary consumption and trophic level are not the only causes of $\delta^{15}N$ enrichment in bodily tissues. Prolonged protein depravation causes changes in the body's metabolic production and breakdown of protein in tissues, resulting in the enrichment of $\delta^{15}N$ in these tissues which serve as amino acid pools used in the maintenance of other bodily functions (Orten and Neuhaus 1982). These changes can be measured first in tissues with a fast turnover rate. This relationship is less clear in bone due to slower turnover and the averaging effect in bone collagen. However, severe and prolonged starvation could eventually lead to enriched $\delta^{15}N$ values in human bone. Tissue turnover rates and their relationship with stable isotopes are discussed in greater detail in the following section.

Pathological conditions in human bone tissue can impact stable isotope values in bone collagen due to alterations in bone turnover and healing. Katzenberg and Lovell (1999) investigated variation in stable isotope values of pathological bone comparative to normal bone

within and between individuals. The authors investigated periostitis, osteomyelitis, fracture and atrophy in a modern human cadaver sample. The authors found a small decrease in $\delta^{15}N$ values for repaired fractures but stated the opposite is also possible, similar results for periostitis, no change for bone atrophy related to nerve damage and elevated $\delta^{15}N$ values in osteomyelitic bone were discovered. The authors argue that new tissue deposited during fracture repair reflects short-term diet during the repair process (Katzenberg and Lovell 1999). Traumatic injury increases nutritional stress as the healing process requires large amounts of energy resulting in a catabolic state, where protein reserves in musculoskeletal tissue are broken down and utilized in the body's injury repair processes (Hill 1998). While this sheds light on the relationship between pathological conditions and stable isotope values in human bone, it is important to recognize the limitations of this study and the need for further research in this field of study. The sample size was extremely small (n=7); variation in isotopic values could be attributed to small sample size.

White and Armelagos (1997) investigated the relationship between osteopenia, a pathological condition where bone mass is reduced due to reduced mineralization, and stable carbon and nitrogen isotope values. The authors found that there was no significant difference in carbon isotope ratios between bone in normal and osteopenic individuals. However, they found significantly enriched $\delta^{15}N$ values in individuals with osteopenia relative to individuals without this pathological condition. There are two possible explanations for the relationship between osteopenia and elevated $\delta^{15}N$ values. The authors suggest it is due to urea excretion or problems in renal processing (White and Armelagos 1997). Another possible explanation is that dietary stress, which leads to decreased collagen synthesis and bone mineral formation, may be the primary cause or at least a contributing factor to both enriched $\delta^{15}N$ values and osteopenia. The

latter explanation is particularly relevant to the younger females (<30 years) in the sample since they are less likely to suffer from renal issues prior to menopause.

A recent dissertation investigated the relationship between stable nitrogen isotope analysis, different pathological conditions and tissue turnover in bone collagen (Olsen 2013). Rickets/osteomalacia, degenerative diseases, fracture trauma, osteomyelitis and periostitis were analyzed. No significant differences related to rickets/osteomalacia or degenerative disease and $\delta^{15}N$ values were found. Differences in $\delta^{15}N$ values related fracture repair, periostitis and unaffected bone were found in some samples, but not others. Enriched $\delta^{15}N$ values were found in osteomyelitic lesions compared to unaffected areas of bone by an average of 1.14‰ (Olsen 2013).

These studies demonstrate that pathological bone exhibits $\delta^{15}N$ enrichment similar to the enrichment that occurs in other tissues during periods of nutritional stress. This may be due to similar changes in protein metabolism. These studies provide another cause for nitrogen enrichment in bodily tissues (see Table 2). This section and previous sections illustrate numerous factors that can contribute to enriched nitrogen isotope levels in human tissues. Table 2 summarizes the factors contributing to nitrogen enrichment, the $\delta^{15}N$ value or enrichment factor (+), the tissue enriched and the source of the data.

Table 2. Sources of Nitrogen Enrichment in Human Tissue

Sources of $\delta^{15}N$ enrichment	$\delta^{15}N$ value	Sample and Tissue	Source(s)
Marine-based diet	17 to 20‰	Human bone collagen	Schoeninger 1983
Climate (aridity = <200mm rain/year)	13 to 18‰	Human bone collagen	Schwarcz et al. 1999
Water stress	no data		Ambrose and DeNiro 1987
Lactation	+2 to 3‰	Human bone collagen	Fogel et al. 1989 Dupras et al. 2001
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 conditions in bone (Osteomyelitis)	12.9‰	Human bone collagen	Katzenberg and Lovell 1999
	+1.14‰	Human bone collagen	Olsen 2013
Pathological conditions in bone (Osteopenia)	12.0‰ ± 0.86‰	Human bone collagen	White and Armelagos 1997

Tissue and stable isotope analysis

Stable isotope analysis can be conducted on bone collagen or apatite, tooth enamel and dentin, hair, nails and muscles. Stable isotope analysis of these tissues can provide information about paleoclimate, mobility and dietary information on past human and faunal remains. Bone collagen is more useful when analyzing dietary protein and is less subject to diagenetic processes than bone apatite (Scherer et al. 2007). Approximately 30% of dry bone is organic; of that, a majority consists of collagen (Katzenberg 2008). Roughly 35% of collagen is made up of carbon and 11-16% is made up of nitrogen (van Klinken 1999).

An important factor in dietary estimations using stable isotope analysis is tissue turnover rates. Hair has a fast turnover rate and reflects diet as recent as two weeks when anagen hair is

sampled near the root and can reflect dietary information for several months by sampling sections of hair at increasing distances from the scalp (Mekota et al. 2006, Williams et al. 2011). Bone however, has a much slower turnover rate and reflects average diet over a period of years prior to death (Ambrose 1993). Stenhouse and Baxter (1979) argue 30 years for complete bone turnover, while Libby et al. (1964) argue a period of ten years. Tieszen et al. (1983) propose that tissue turnover rates are directly related to metabolic status in the body. They argue that tissues more active metabolically have higher turnover rates. This partially explains the faster turnover rates in juveniles over adults (Klepinger 1984). Turnover rates vary not only between tissues, but also between bones (Ambrose 1993). This is in part due to the differences in the turnover rates between trabecular and cortical bone (Klepinger 1984).

A study conducted by Hedges et al. (2007) investigated bone turnover rates in the mid-shaft of the femur. The authors found that at birth male and female turnover rates were between 30 and 70% per year, during adolescence it reduced to 12.4-15% for males and 2-10% for females and at cessation of growth the rate further reduced to 1.5-4.5% for males and 3.1-5.1% for females. They estimated that isotopic values derived from human bone collagen reflect average diet for a period far greater than ten years in adults (Hedges et al. 2007). Table 3 shows the average turnover rate per year and average number of years for total bone turnover in males and females at various stages in the life cycle, as determined by Hedges et al. (2007).

Table 3. Turnover Rates and Turnover in Years Adapted from Hedges et al. (2007)

Life cycle stage	Males (age)	Turnover rate (avg %/yr)	Avg turnover (yrs)
Birth	0	50.0%	2.0
Adolescence	16-18	13.7%	7.3
Cessation of growth	23-27	3.0%	33.3
Life cycle stage	**Females (age)**	**Turnover rate (avg %/yr)**	**Avg turnover (yrs)**
Birth	0	50.0%	2.0
Adolescence	14-16	6.0%	16.7
Cessation of growth	18-20	4.1%	24.4

In recent years, there have been several controlled animal studies and clinical studies investigating the relationship between diet, nutritional stress and stable isotope values in body tissues (e.g., Fuller et al. 2005, Hatch et al. 2006, Mekota et al. 2006, Williams et al. 2011). A majority of these studies analyze the isotopic composition of hair to detect dietary changes and nutritional stress in an individual. These studies demonstrated that $\delta^{15}N$ values in hair become enriched during periods of nutritional stress and the authors of these studies emphasize the usefulness of this approach in forensic and archaeological contexts. General trends can be applied to archaeological investigations, however, to make direct quantitative comparisons, the isotopic relationship between hair keratin and bone collagen must be established.

Several controlled animal feeding studies measuring the isotopic composition of both hair keratin and bone collagen have been conducted. These studies found that carbon isotope values were highly correlated between these two tissues, with a collagen enrichment of roughly 5‰ (Ambrose and Norr 1993, Tieszen and Fagre 1993) and a keratin enrichment of +1 to +2‰ meaning that keratin is depleted between 2-3‰ compared to collagen (DeNiro and Epstein 1978, Tieszen and Fagre 1993). The differences in $\delta^{13}C$ between hair keratin and bone collagen have been attributed to the different amino acid composition in these two tissues (O'Connell and

Hedges 1999). Nitrogen isotope studies focused on collagen, keratin and muscle protein found an enrichment of 2-3‰ in $\delta^{15}N$ values in all three tissues over dietary source (DeNiro and Epstein 1981, Sealy et al. 1987, Hare et al. 1991). It has been argued that the similarity in $\delta^{15}N$ values is due to the similarity in nitrogen isotope values in amino acids in different body tissues (Gaebler et al. 1966). It is important to note that these similarities exist when diet is consistent over a long period of time. These patterns do not hold true for major dietary changes over a short period since bone has a much slower turnover rate comparative to hair. In the latter scenario, hair keratin isotope values would reflect recent changes, while bone collagen values would reflect a mixture of recent diet and past diet (O'Connell and Hedges 1999).

O'Connell and Hedges (1999) and O'Connell et al. (2001) conducted studies on archaeological populations and living people to test the consistency of the fractionation factor between diet source and different tissues in humans, specifically hair keratin and bone collagen. In their study conducted on an archaeological population from the UK, O'Connell and Hedges (1999) found that bone collagen was enriched between 0 and 1‰ in $\delta^{13}C$ values and 0 to 2‰ in $\delta^{15}N$ values comparative to hair keratin, however this pattern varied drastically throughout the sample. They argued that the isotopic relationship between hair keratin and bone collagen could not be accurately quantified for their sample (O'Connell and Hedges 1999). They argued more contextual information regarding diet, health and lifestyle prior to death may make this relationship clearer and quantifiable. The study of living individuals produced similar results (O'Connell et al. 2001). Research into isotopic values for specific amino acids and amino acid abundance in various tissues are needed to accurately encapsulate differences in the isotopic composition of various tissues. A major flaw in these two studies that may account, at least in

part, for the inconsistency of the fractionation factor between hair keratin and bone collagen is the growth cycle error. Bulk sampling of hair may include segments of active growing hair and dormant hair, which can lead to temporal error studies tracking diet using stable isotope analysis (Williams et al. 2011). A study accounting for the growth cycle in hair is necessary to determine if there is a consistent and quantifiable relationship in the fractionation factor between diet and different body tissues.

Contemporary clinical studies (Fuller et al. 2005, Hatch et al. 2006, Mekota et al. 2006) demonstrate the relationship between nutritional stress and stable nitrogen values in hair keratin. Although there is a relationship between hair keratin and bone collagen, a consistent fractionation factor has yet to be established. Therefore, direct mathematical comparisons between the $\delta^{15}N$ values for the current sample and hair keratin values in the clinical samples will not be made.

Limitations of isotope studies in archaeology

While isotopic data can provide insight in the reconstruction of past human diets, there are several limitations. When characterizing diet using stable isotope analysis, there are uncertainties related to the characterization of $\delta^{13}C$ and $\delta^{15}N$ values for specific dietary sources at particular spatial and temporal units. These values are subject to alteration due to changes across time and space. Temporal variations may result from the introduction of fossil fuels during the Industrial Revolution (Katzenberg 2008) and spatial variation may result from a number of differences including climate (van Klinken et al. 2000).

As discussed above, there are several non-dietary factors that impact $\delta^{15}N$ values in human tissues (see Table 2). Furthermore, the direct impact of metabolic processes (i.e., positive

nitrogen balance) is poorly understood and often contested. When investigating issues of diet, nutritional stress and/or pathological conditions, alternative lines of evidence may make the cause (or contributing factor) of $\delta^{15}N$ enrichment clearer including of historical evidence, archaeological context, isotopic evidence of fauna and/or plants, etc.

A major pitfall in the study of diet through isotopic analysis of human bone collagen is diagenesis. Diagenetic alteration of collagen can alter stable isotope values making them erroneous (Ambrose 1990). To validate the results of stable isotope analysis and eliminate diagensis as a cause of variation, it is essential to verify the quality of sample collagen. Collagen yield, %N, %C and C:N ratio are measures commonly used to test the quality of collagen in a sample. These measures are discussed in detail in chapter four.

CHAPTER THREE: MATERIALS AND METHODS

Analysis of stable carbon and nitrogen isotopes of bone collagen of 78 individuals from a skeletal sample in Vilnius, Lithuania was performed to ascertain dietary variation. The sample population comes from a mass grave consisting of an MNI of 3269 individuals that were part of Napoleon's Grand Army who perished in their retreat from Russia in 1812 during the Napoleonic Wars (Signoli et al. 2004). Historic documentation and previously published bioarchaeological investigations (Palubeckaitė et al. 2006, Raoult et al. 2006) provide a contextual framework for the interpretation of this isotopic research. This chapter provides a discussion of the samples examined and the methods utilized in their analysis. It begins with a summary of the excavation and preliminary skeletal and archaeological analyses. The subsequent section provides a description of the preparation of samples and methods used in this study.

Sample

A mass grave was exposed in the Fall of 2001 during a construction project in the location of a former World War 2 barracks positioned north of Vilnius (Signoli et al. 2004) (See Fig 6). A number of previous military occupations were initially suspected to be connected with this mass grave, however, preserved clothing and associated artifacts collected during the early excavations of the site indicated the burial contained soldiers believed to be from Napoleon's Grand Army. An examination of buttons from uniform fragments indicated the presence of 40 regiments, signifying predominantly infantry and cavalry (Signoli et al. 2004). Although there may be others, buttons and uniforms from French, Italian, Polish and Bavarian regiments were represented in the grave.

Anthropologists conducted a salvage excavation to clear the site for construction in a timely manner. The grave was situated in a trench approximately 10m wide and 40m long (See Fig 6). Two teams of anthropologists excavated the site using two different approaches outlined in Signoli et al. (2004) (See Fig. 6).

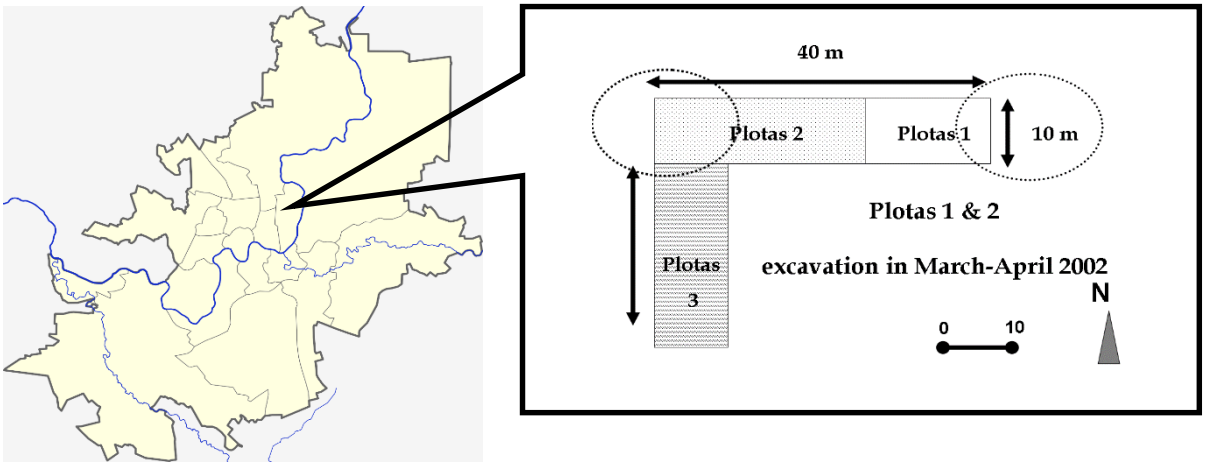

Figure 6. A map of Vilnius, Lithuania with a callout box showing the excavation trenches at the location of the mass grave. Courtesy of R. Jankauskas.

Anthropologists excavated a minimum number of 3269 individuals (based upon the number of left femoral diaphyses). The concentration of bodies within the trench was seven corpses/m^2. The high density of corpses within the burial trench indicated a single burial event and the positioning of the bodies led to the conclusion that the individuals were buried shortly after death, prior to rigor mortis (Raoult et al. 2006). Figures 7 and 8 show the density of skeletons within the mass grave at Vilnius

Figure 7. Photograph of excavation (image courtesy of R. Jankauskas).


Figure 8. Close-up of commingled skeletons in this mass grave (image courtesy of R. Jankauskas).

Osteological and paleopathological analyses of this skeletal population were conducted by a team of French and Lithuanian anthropologists. Pelvic and skull morphology were assessed

to determine sex, and standard methods (i.e., epiphyseal fusion, cranial suture closure, etc.) were employed to determine age at death, all following Buikstra and Ubelaker (1994). The total number of females excavated was 29 (18 probable females, 11 definite females), 1883 males (22 probable males, 1861 definite males) and 1317 individuals of indeterminate sex (Signoli et al. 2004). A majority of the individuals excavated were in their twenties. Table 4 lists the age at death and sex estimates for the 445 individuals for which these variables were able to be determined (Signoli et al. 2004).

Table 4. Age at Death Estimates for Males and Females in Burial

Age Range (in years)	Males	Females
15-20	46	-
18-20	-	4
~20	-	2
>20	-	2
20-25	211	5
25-30	115	1
30-35	39	1
35-40	10	-
40-45	5	-
~50	3	-
50-60	1	-
Age Determination Total	430	15

Historical evidence suggests the presence of louse-transmitted pathological conditions in Napoleon's Grand Army during the Russian Campaign (Weiss 1988). Raoult et al. (2006) analyzed the DNA of ancient lice discovered in the grave and dental pulp of 72 unerupted teeth from 35 individuals to confirm the presence of relapsing fever (*Borrelia recurrentis*), trench fever (*Bartonella quintana*) and epidemic typhus (*Rickettsia prowazekii*). The authors detected the presence of *B.quintana* in body lice and in the dental pulp of seven soldiers. The presence of

R. prowazekii was discovered in the dental pulp of three soldiers but not in the lice. The authors concluded that individuals in the Napoleonic Army suffered from trench fever and typhus. Table 5 lists the pathological conditions diagnosed in this burial population including the louse-bourne pathogens discovered in the dental pulp of these soldiers.

Table 5. Osteological Pathological Conditions Diagnosed in Sample

Sample	Sex	Age Range (in yrs)	Pathological Condition
NAP-K7	PM	35-40	enthesopathy pit in left clavicle
NAP-K19	M	>40	louse-bourne pathogen in tooth 33
NAP-K23	M	20-25	periostitis in right tibia
NAP-K26	M	25-30	louse-bourne pathogen in tooth 35
NAP-K29	M	25-35	healed stress fracture of 3rd right metatarsal
NAP-K36	M	25-35	perimortem spiral fracture in right humerus
NAP-K38	M	35-40	enthesopathy pit in right clavicle louse-bourne pathogen in tooth 22
NAP-K40	M	25-30	healed fracture of left clavicle
NAP-K75	M	30	healed fractures of left clavicle and left tibia
NAP-K127	M	20-25	spondylolysis of L5
NAP-K128	M	20-25	louse-bourne pathogen in tooth 28
NAP-K158	M	30-35	louse-bourne pathogen in tooth 17 and tooth 43
NAP-K176	M	35-45	spondylolysis of L5

The study by Raoult et al. (2006) indicates that Napoleonic soldiers suffered from the febrile illnesses of typhus and trench fever. During periods of febrile illness, the body enters a state of negative nitrogen balance where amino acids are utilized from the nitrogen pool created by the catabolizing of muscle protein (Beisel 1975). Therefore, the body expends large amounts of muscle protein to increase the nitrogen pool. The process of muscle protein catabolism and the febrile response allowing this to take place "requires the excessive expenditure of body energy…with continued or increased excretory losses through urine, stool, and sweat, these

changes lead inevitably to negative body balances of many substances" (Beisel 1975:11). Diarrhea can increase nitrogen losses from body tissues.

Palubeckaitė et al. (2006) conducted a dental health analysis of this burial sample to determine dental health status and dietary trends of these soldiers. Dental health was evaluated for 293 adult males using the presence of antemortem tooth loss, dental attrition, caries, abscesses and linear enamel hypoplasias as criteria. Enamel hypoplasias are indicative of childhood nutritional stress and/or pathological conditions, while the other criteria indicate adult health and dietary trends. Low rates of dental attrition and antemortem tooth loss and prevalence of calculus and abscesses indicated good dental health in Napoleon's young soldiers (Palubeckaitė et al. 2006). A majority of individuals in this sample had one to two hypoplasias of mild to moderate degree, occurring between the third and fourth years of life (Palubeckaitė et al. 2006). Although the overall rate of carious lesions was low, the location of the lesions and "a high number of carious teeth in some individuals suggest increased vulnerability to disease" (Palubeckaitė et al. 2006:361). The location of the lesions also points to non-abrasive, sticky food that when it remains in contact with teeth, leads to tooth decay (Hillson 1986). Rounded tooth notches and stained tartar rims indicate routine tobacco use in some of the individuals in this sample (Palubeckaitė et al. 2006).

Seventy-eight of the 3269 individuals from the mass grave were selected for this study. The sample size is limited by circumstance; only a limited number of individuals were removed from the grave for further investigation during this salvage excavation (Signoli et al. 2004). A majority of the individuals removed were selected due to perceived high-levels of preservation and visible pathological conditions in the bone. This may be a source of selection bias for this

sample. Seventy-two individuals were male, one was a probable male, two had unknown sex and three were female. Age at death ranged from 16 to 50 years old (See Fig 9). The age of each individual was reported with age ranges. In order to make comparisons between age groups, ranges were converted to median age for each individual. Seven to 20 grams of bone were collected from the mid-shaft of the femoral diaphysis of these individuals.

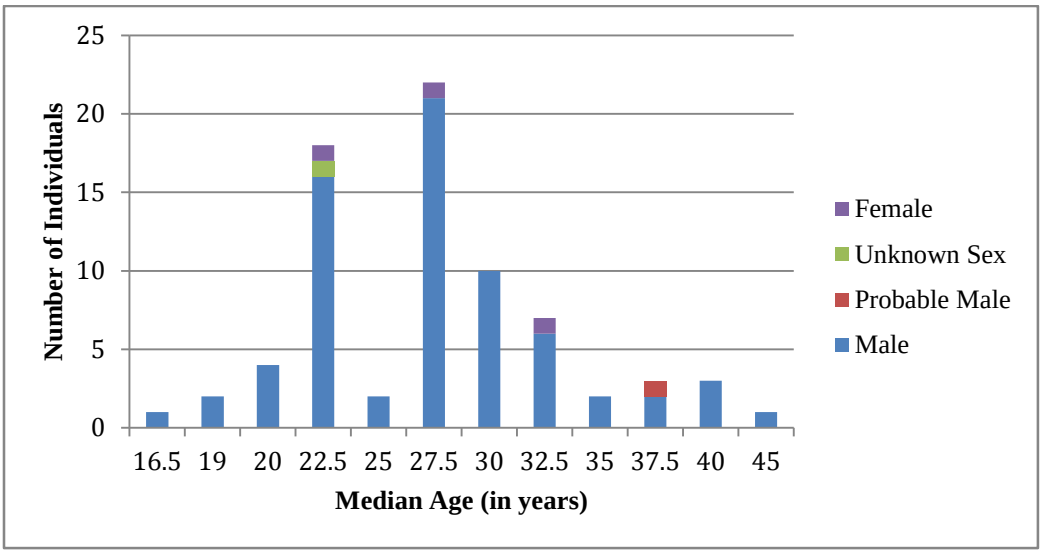

Figure 9. Graph showing the distribution of age and sex for this sample.

Methods

Up to 20 grams of sample was removed from each left femoral diaphysis using an 8100 8V Max Rotary Dremel® tool with a 1¼-inch fiberglass reinforced cutoff wheel. From this, four to six grams were extracted and crushed into small pieces using a mortar and pestle. Samples were weighed using a Fisher Scientific balance and placed in labeled centrifuge tubes. Each sample was then placed in a 50 mL beaker with distilled water and ultrasonicated for 10 minutes. After ultrasonication, the samples were removed and scrubbed with a toothbrush before being placed back into beakers with fresh distilled water. Cycles in the ultrasonicator were repeated until the distilled water remained clear. Bone samples were removed from the water, dabbed

with paper towels and placed in labeled centrifuge tubes in an oven to dry overnight at 60°C. After drying, the samples were weighed and recorded prior to undergoing collagen extraction.

Collagen Sample Preparation. Bone collagen preparation for isotopic analysis was completed in UCF's Laboratory for Bioarchaeological Sciences following laboratory protocol based on Longin (1971), and modified by Western University's Laboratory for Stable Isotope Science. Although archeological samples rarely contain lipids, lipid extraction was performed due to the relatively recent age of the sample (ca. 200 years), and the presumed high level of preservation. Approximately 15 mL of 2:1 chloroform:methanol after Jim et al. (2004) was added to each sample in 50 mL plastic centrifuge tubes. These samples were stored in the fume hood for ~20 minutes, and then spun down in the centrifuge for 10 minutes at 2400 rpm. Afterward, the chloroform:methanol waste was removed. The chloroform:methanol process was repeated once more and samples were then left uncapped in the fume hood overnight.

After any possible lipids were extracted from the samples, the inorganic portion of the bone was dissolved using 0.5 molar (M) hydrochloric acid (HCl). During demineralization, acid was changed on a regular basis and pH was tested to ensure it did not drop below 1.0. Due to the high level of preservation and the size of the pieces, the samples took between four and six weeks to demineralize. Once the samples were demineralized, they were rinsed with distilled water and spun down in the centrifuge for three cycles of 10 minutes at 2400 RPM to remove remaining acid from the samples.

Soil contaminating acids (humic and fulvic) were removed using sodium hydroxide (NaOH). Due to the impact that long treatments of NaOH may have on isotopic analysis (DeNiro and Epstein 1981, Katzenberg 1989), the lab protocol called for short (~20 minutes)

rinses and 10 minute spins of NaOH in the sample tubes. Once any contaminating acids were removed, samples were rinsed six times and pH tested for a value of ~7.0 to ensure removal of NaOH. After the appropriate pH was obtained, samples were rinsed once with 0.25 M HCl, and then with deionized water and placed in the oven at 90°C for approximately 16-48 hours in order to extract water-soluble collagen from the remaining sample.

The collagen-containing liquid was placed back in the oven at 90°C in a capless glass dram vial for 48-96 hours to break down collagen bonds. After a period of 48-96 hours, the vial was removed, weighed and collagen yields were calculated. All samples were placed into 3.5x5 mm tin capsules and weights ranged from 0.4 to 0.7 mg.

Analysis. All samples were sent to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University to undergo mass spectrometry. Stable isotopes were measured in the samples using gas isotope-mass spectroscopy using a Delta V Advantage Mass Spectrometer with a Carlo Ebra NC2100 Elemental Analyzer. The Colorado Plateau Stable Isotope Laboratory (CPSIL) reported the $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values, %C and %N values, and the C:N ratios. The C:N ratios were then converted to atomic C:N ratios.

CHAPTER FOUR: RESULTS

This chapter presents the results of the stable isotope analysis and evaluates preservation for this sample. Level of preservation is determined through atomic C:N ratios, collagen yields and carbon and nitrogen concentrations (%C and %N). Plant protein consumption is evaluated using a modified equation from White and Schwarcz (1989). Differences in isotopic values between groups based on age and sex are also reported in this section. Statistical analyses between groups were not conducted due to differences in samples sizes.

Stable isotopes were measured in the samples using gas isotope-mass spectroscopy using a Delta V Advantage Mass Spectrometer with a Carlo Ebra NC2100 Elemental Analyzer by CPSIL. The precision of this instrument as reported by CPSIL is < ±0.1 for carbon and ±0.2 for nitrogen. The laboratory also ran eight samples twice. The absolute value of the differences between the two values was averaged for the eight samples to determine accuracy for stable carbon and nitrogen isotope measures. The accuracy of each measure is presented below in Table 6.

Table 6. Calculation of Accuracy for each Measure

Sample	Differences in $\delta^{13}C$	Differences in $\delta^{15}N$
NAP-K1	-0.28	0.00
NAP-K25	-0.10	0.02
NAP-K44	-0.06	-0.03
NAP-K65	-0.04	0.04
NAP-K91	-0.07	-0.10
NAP-K126	0.03	0.01
NAP-K148	-0.15	-0.16
NAP-K168	-0.02	0.04
Accuracy	**0.09**	**0.05**

Sample Preservation

Bone becomes unstable post-deposition during soft tissue decomposition; during this time it is subject to diagenetic processes. Contact with microbial agents leads to the deterioration of organic and mineral portions of bone (Stodder 2008). Three ways to measure the organic preservation of bone is through calculated percentages of collagen yield, carbon and nitrogen, and atomic C:N ratio (Ambrose 1990). Table 7 presents a summary of the preservation and isotopic data for each of the 78 individuals sampled.

Table 7. Summary of Preservation and Isotopic Data for Napoleonic Samples.

Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	Collagen Yield (%)	Atomic C:N ratio
NAP-K1	-16.96	9.37	45.40	17.30	7.07	3.06
NAP-K3	-18.36	11.28	35.37	13.78	18.45	2.99
NAP-K6	-19.19	12.26	42.79	16.34	3.53	3.05
NAP-K7	-18.47	10.93	45.02	17.20	17.64	3.05
NAP-K9	-17.43	11.56	44.93	17.13	18.04	3.06
NAP-K17	-18.91	10.60	42.70	16.53	3.95	3.01
NAP-K19	-17.77	9.22	43.74	16.96	17.20	3.01
NAP-K23	-18.65	9.90	46.93	17.79	18.90	3.08
NAP-K25	-18.14	10.44	45.13	17.36	16.39	3.03
NAP-K26	-19.22	8.68	45.24	17.21	18.81	3.07
NAP-K29	-18.60	10.36	43.75	16.95	16.02	3.01
NAP-K36	-18.04	13.46	43.65	16.86	12.53	3.02
NAP-K37	-18.86	12.35	39.80	15.33	20.50	3.03
NAP-K38	-18.79	11.85	43.98	16.94	14.87	3.03
NAP-K40	-16.74	11.47	44.43	16.97	15.09	3.05
NAP-K41	-18.48	8.48	44.90	17.37	16.54	3.02
NAP-K42	-18.45	8.28	44.38	17.11	15.02	3.03
NAP-K44	-18.18	9.08	43.82	16.97	7.26	3.01
NAP-K45	-17.61	10.69	37.63	14.91	20.04	2.95
NAP-K46	-17.59	11.85	44.26	17.38	17.10	2.97
NAP-K50	-17.94	12.25	44.66	17.26	15.56	3.02
NAP-K52	-18.74	7.59	44.82	17.62	14.94	2.97
NAP-K54	-16.92	10.27	44.27	17.41	17.53	2.97
NAP-K61	-18.08	10.78	45.48	17.66	13.75	3.01
NAP-K62	-17.83	11.05	45.36	17.75	13.69	2.98
NAP-K63	-18.08	8.03	46.02	17.88	13.39	3.00

Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	Collagen Yield (%)	Atomic C:N ratio
NAP-K64	-15.02	10.78	45.69	17.70	12.80	3.01
NAP-K65	-12.05	9.27	47.44	18.31	14.96	3.02
NAP-K66	-18.10	10.31	37.44	14.65	19.48	2.98
NAP-K68	-14.41	10.91	44.61	17.38	14.26	2.99
NAP-K69	-17.10	9.03	44.60	17.22	14.62	3.02
NAP-K73	-17.80	12.60	45.38	17.68	12.66	2.99
NAP-K75	-16.68	7.84	43.87	17.32	16.57	2.95
NAP-K78	-17.66	11.08	45.45	17.74	17.03	2.99
NAP-K79	-15.83	9.50	43.91	17.18	14.65	2.98
NAP-K82	-15.46	10.62	44.65	17.34	13.20	3.00
NAP-K87	-17.39	11.42	44.52	17.47	12.82	2.97
NAP-K91	-17.51	8.43	46.93	18.35	18.00	2.98
NAP-K96	-17.08	10.70	45.27	17.59	19.01	3.00
NAP-K105	-12.28	8.66	45.13	17.56	17.95	3.00
NAP-K106	-17.67	11.57	40.50	15.90	5.28	2.97
NAP-K107	-17.37	13.46	37.70	14.65	6.64	3.00
NAP-K111	-17.32	11.56	23.90	9.47	10.74	2.94
NAP-K112	-16.75	8.12	44.31	17.31	9.33	2.99
NAP-K113	-18.00	9.34	45.48	17.63	16.52	3.01
NAP-K116	-17.28	7.14	38.00	15.00	14.34	2.95
NAP-K121	-16.48	11.54	44.16	17.17	13.35	3.00
NAP-K126	-17.72	11.11	43.04	16.97	6.66	2.96
NAP-K127	-17.29	9.29	24.64	9.91	10.91	2.90
NAP-K128	-17.75	11.94	42.73	16.85	13.36	2.96
NAP-K131	-17.84	10.78	43.35	17.00	17.69	2.97
NAP-K133	-17.58	8.28	43.59	17.22	13.48	2.95
NAP-K134	-17.36	10.59	44.90	17.80	8.72	2.94
NAP-K142	-16.35	10.91	42.80	16.46	16.92	3.03
NAP-K143	-16.82	8.06	23.37	9.41	14.31	2.90
NAP-K148	-14.32	8.70	42.84	15.51	16.95	3.22
NAP-K150	-17.50	11.36	43.44	17.39	13.28	2.91
NAP-K151	-17.03	11.93	44.10	17.61	15.07	2.92
NAP-K153	-16.77	11.11	43.55	17.34	11.57	2.93
NAP-K154	-17.44	10.95	43.35	17.05	11.99	2.97
NAP-K158	-16.89	10.24	44.64	17.64	14.64	2.95
NAP-K159	-17.20	11.13	43.56	17.39	11.71	2.92
NAP-K164	-16.20	12.01	44.52	17.54	4.68	2.96
NAP-K165	-17.45	10.99	42.15	16.62	15.59	2.96
NAP-K167	-17.51	10.46	41.48	16.63	17.55	2.91
NAP-K168	-16.71	9.62	42.56	17.04	8.48	2.91

Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	Collagen Yield (%)	Atomic C:N ratio
NAP-K169	-17.15	10.80	44.12	17.38	4.80	2.96
NAP-K170	-17.36	11.33	44.43	17.61	3.92	2.94
NAP-K171	-15.95	10.06	44.32	17.68	13.65	2.92
NAP-K174	-13.18	10.65	43.10	17.28	14.84	2.91
NAP-K175	-17.88	12.00	47.45	18.77	10.30	2.95
NAP-K176	-16.82	10.58	24.71	9.94	17.62	2.90
NAP-K177	-18.16	8.82	44.66	17.67	12.69	2.95
NAP-K182	-11.76	11.03	43.53	17.45	8.60	2.91
NAP-K183	-17.03	10.45	43.52	17.26	13.01	2.94

Collagen yield is the proportion of extracted collagen to the initial clean, dry bone. It is calculated using Equation 4.1:

$$\text{collagen yield (\%)} = \frac{\text{collagen weight (g)}}{\text{sample dry weight (g)}} \times 100 \qquad (4.1)$$

The average collagen yield in fresh bone samples is 20 to 25% collagen by weight (Schoeninger et al. 1989). The minimum collagen yield for archaeological samples deemed acceptable for isotopic analysis varies. White and Schwarcz (1989) and White et al. (1993) suggest a value above 1%, DeNiro and Weiner (1988) suggest a value above 2% and Schoeninger and DeNiro (1982) and Tuross et al. (1988) suggest a more conservative value above 5-6%. The threshold value used for this research is 2%, following DeNiro and Weiner's study (1988), which demonstrated that the sample quality declined significantly below 2%. All samples in this study exhibit collagen yields above 2% (Figure 10).

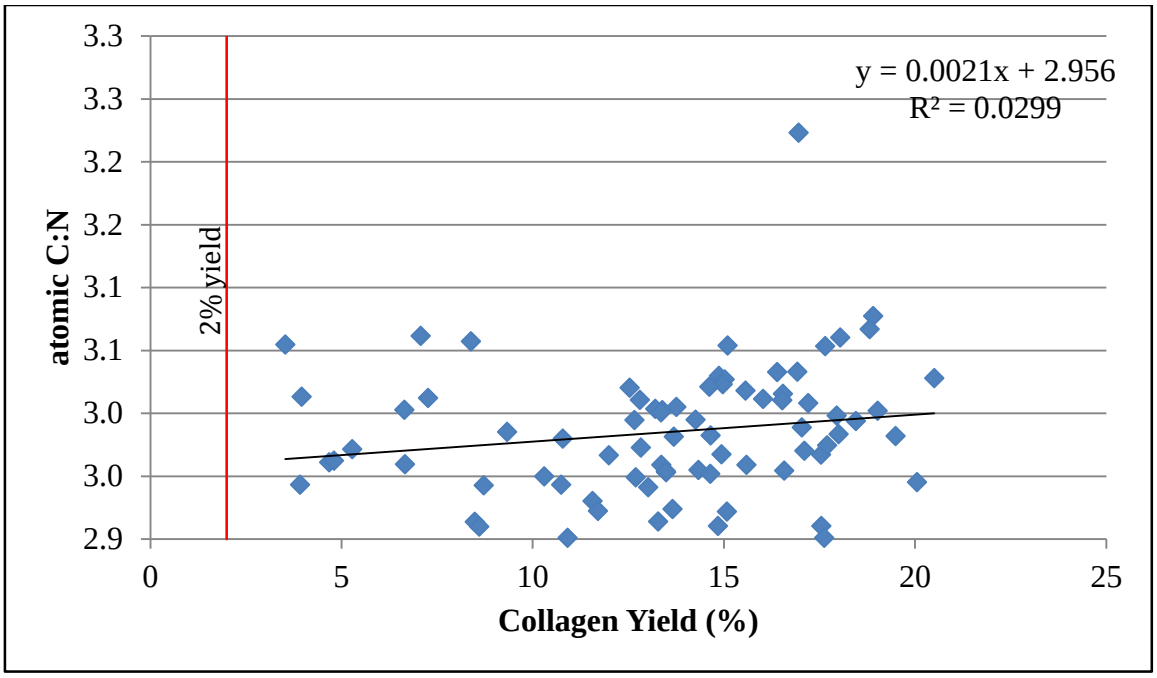

Figure 10. Graph showing collagen yield vs. atomic C:N ratio. The cut off limit for preservation measured by 2% collagen yield is shown on left (red). A trend line and equation also appear on the graph.

Ambrose (1990) argues that carbon and nitrogen concentrations in bone collagen serve as additional measures of bone preservation. These indicators denote the concentration of carbon (%C) and nitrogen (%N) within a collagen sample. They are calculated from the quantity of CO_2 and N_2 in the collagen, which is measured using an elemental analyzer on a mass spectrometer. Ambrose (1990) found that modern animals have %C of 15% to 47% and %N of 5% to 17% in bone collagen. These ranges are used as a standard for acceptable ranges of carbon and nitrogen concentrations in archaeological human bone samples. Two samples from this study exhibited %C outside the accepted range (NAP-K 65=47.44 and NAP-K 175=47.45) (See Fig 11). However these values are only slightly above the accepted range and are still acceptable to include in the analyses since the other measures of preservation (C:N ratio and collagen yield) indicate these samples are well-preserved. Figure 11 shows that there is no relationship between

%C and $\delta^{13}C$ for this sample. Forty-nine of the 78 samples exhibited %N concentrations above 17% (see Fig 12). Only three of the 49 samples exhibit values above 18 and none exhibit values above 19. The other measures of collagen quality (C:N ratio and % collagen) are well-within the accepted range, therefore, the isotopic analysis for all these samples will be accepted. Figure 12 shows that there is no relationship between %N and $\delta^{15}N$ for this sample. There is a strong linear relationship (R^2=0.97834) between carbon and nitrogen concentrations in this sample (Figure 13). This is expected. Table 8 presents the average and standard deviation of %C, %N, collagen yield and atomic C:N ration for this sample.

Table 8. Average and Standard Deviations of Preservation Measures

Measures of Preservation			
Average %C	Average %N	Collagen yield (%)	Atomic C:N ratio
42.7±4.9	16.7±1.8	13.5±4.2	3.0±0.1

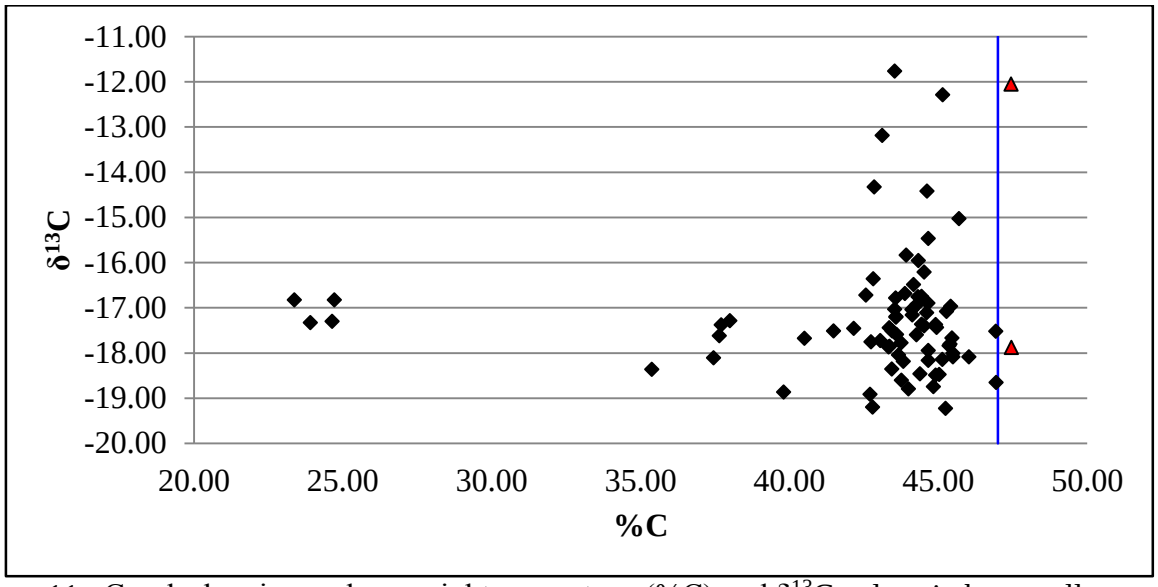

Figure 11. Graph showing carbon weight percentage (%C) and $\delta^{13}C$ values in bone collagen samples. The line (blue) represents the maximum accepted %C, the diamonds (black) represent values within the accepted range and the triangles (red) represent values above the range.

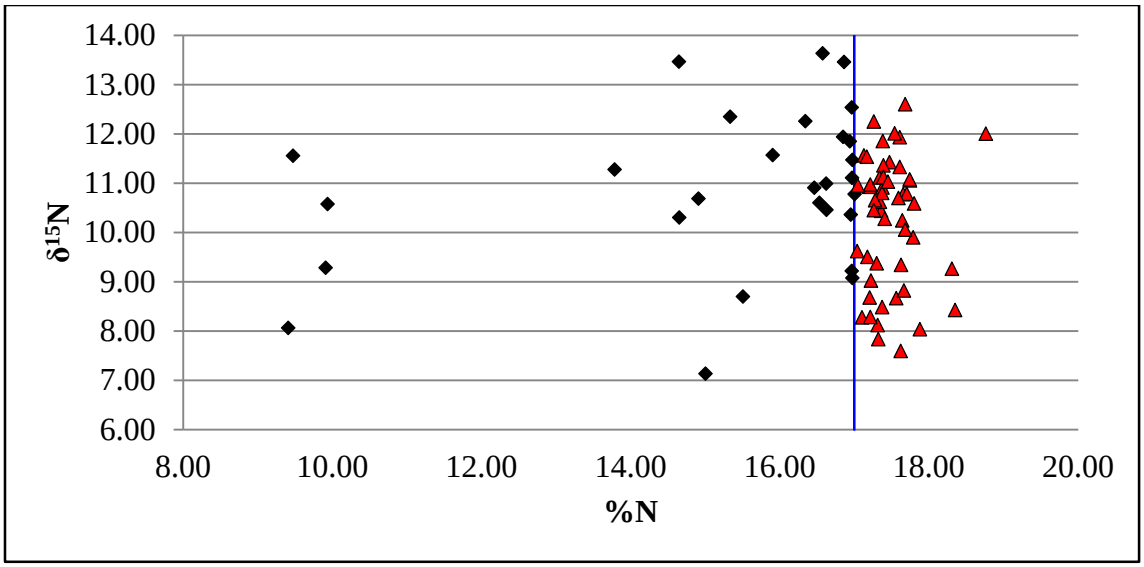

Figure 12. Graph showing nitrogen weight percentage (%N) and $\delta^{15}N$ values in bone collagen samples. The line (blue) represents the maximum accepted %N, the diamonds (black) represent values within the accepted range and the triangles (red) represent values above the range.

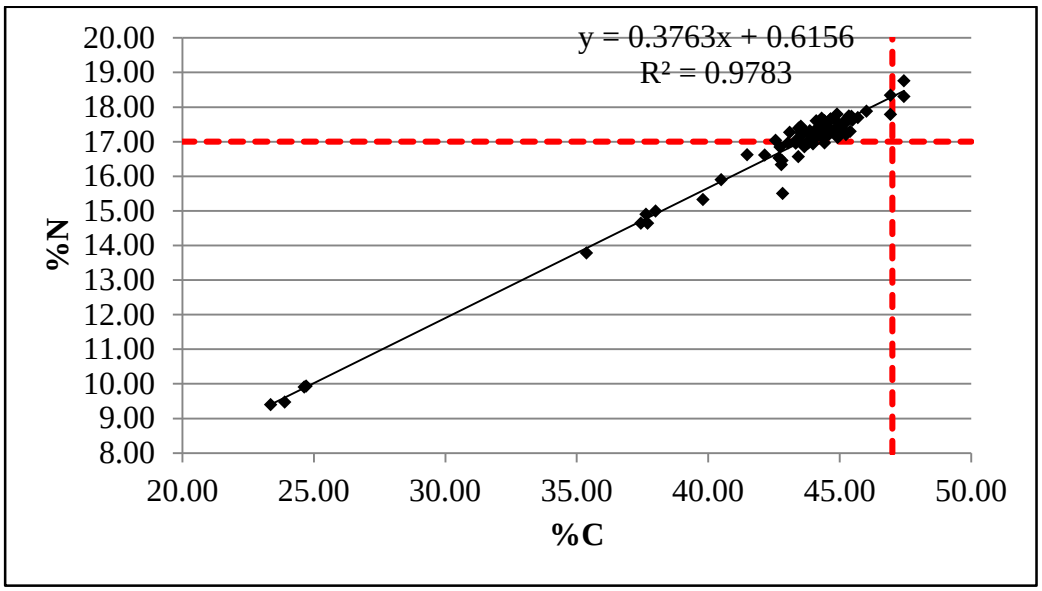

Figure 13. Graph showing the linear relationship (through a trend line) between carbon weight percentage (%C) and nitrogen weight percentage (%N) in bone collagen samples. The dashed lines (red) indicate the maximum accepted %C and %N.

Weight percent C:N ratios are calculated using %C and %N. Atomic C:N ratio is calculated using Equation 4.2:

$$\text{atomic C: N} = \frac{14}{12} \times (\text{weight \% C: N}) \qquad (4.2)$$

Modern mass spectrometers calculate weight ratios of C:N that are lighter than the ratios proposed by DeNiro (1985) by a factor of 14/12 making it necessary to calculate the atomic C:N ratio (Katzenberg 2008). The average atomic C:N ratio in modern bone collagen is 3.2. DeNiro (1985) suggests an atomic C:N ratio for a well-preserved archaeological sample will fall between 2.9 and 3.6. This range was proposed to account for "analytical error and some slight alteration over long periods of time" (Katzenberg 2008:418). All 78 samples fall within the accepted range (Figure 10).

Van Klinken (1999) argues that collagen yield and %C together is the best indicator of collagen degradation. Figure 14 displays the relationship between collagen yield and %C for the current sample. The two triangles (red) represent the two samples with %C values above the accepted range. There is no linear relationship between these two indicators (R^2=0.00002). Both %C and collagen yield show wide ranges of variation. However, with all but two samples below the accepted %C and all samples above the accepted collagen yield, this graph indicates an acceptable level of preservation for this sample.

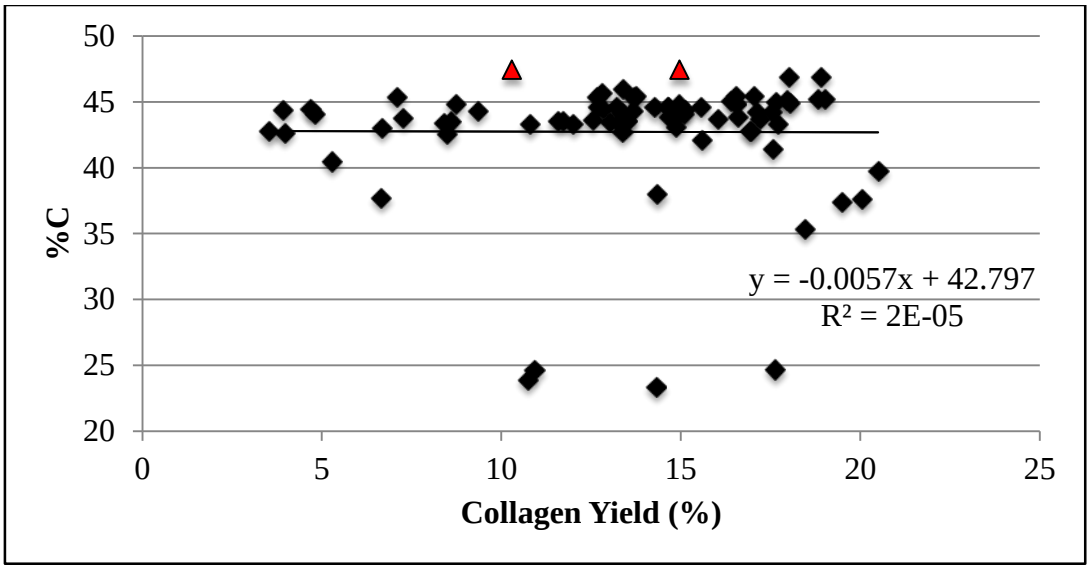

Figure 14. Graph showing the relationship between collagen yield (%) and carbon percent weight (%C) in collagen bone samples. The triangles (red) represent the two samples above the accepted %C range. There is also a trend line and equation.

Ambrose (1990) argues that carbon and nitrogen concentrations in collagen decrease as the collagen yield decreases in a non-linear fashion. However, figures 14 and 15 show that the lowest percentages of carbon and nitrogen do not correspond with the lowest collagen yields for this sample.

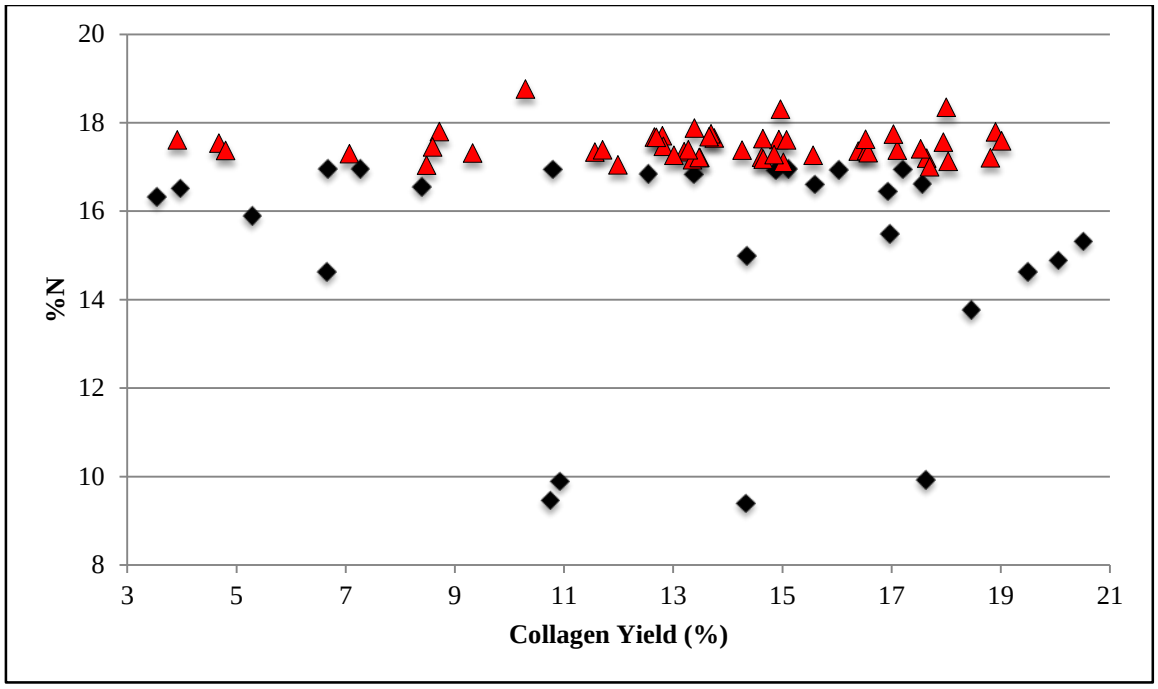

Figure 15. Graph showing collagen yield (%) and nitrogen percent weight (%N) in collagen bone samples. The triangles (red) represent samples above the accepted %N range.

Interpretation of diet

Stable carbon and nitrogen isotope analysis was conducted on samples from 78 Napoleonic soldiers from Vilnius to assess diet and identify possible cases of chronic starvation. Carbon and nitrogen isotope ratios are reported in the standard delta (δ) notation, in per mil (‰) in relation to international referencing standards discussed in Chapter Two.

The $\delta^{13}C$ values plotted against $\delta^{15}N$ values are presented in Figure 16. The $\delta^{13}C$ ratios range from -19.22‰ to -11.76‰ with a mean of -17.18‰ ± 1.48‰ (1σ). The $\delta^{15}N$ values range from 7.14‰ to 13.63‰ with a mean of 10.49‰ ± 1.44‰ (1σ). There is no linear relationship between $\delta^{13}C$ and $\delta^{15}N$ for this sample (R^2=0.0156).

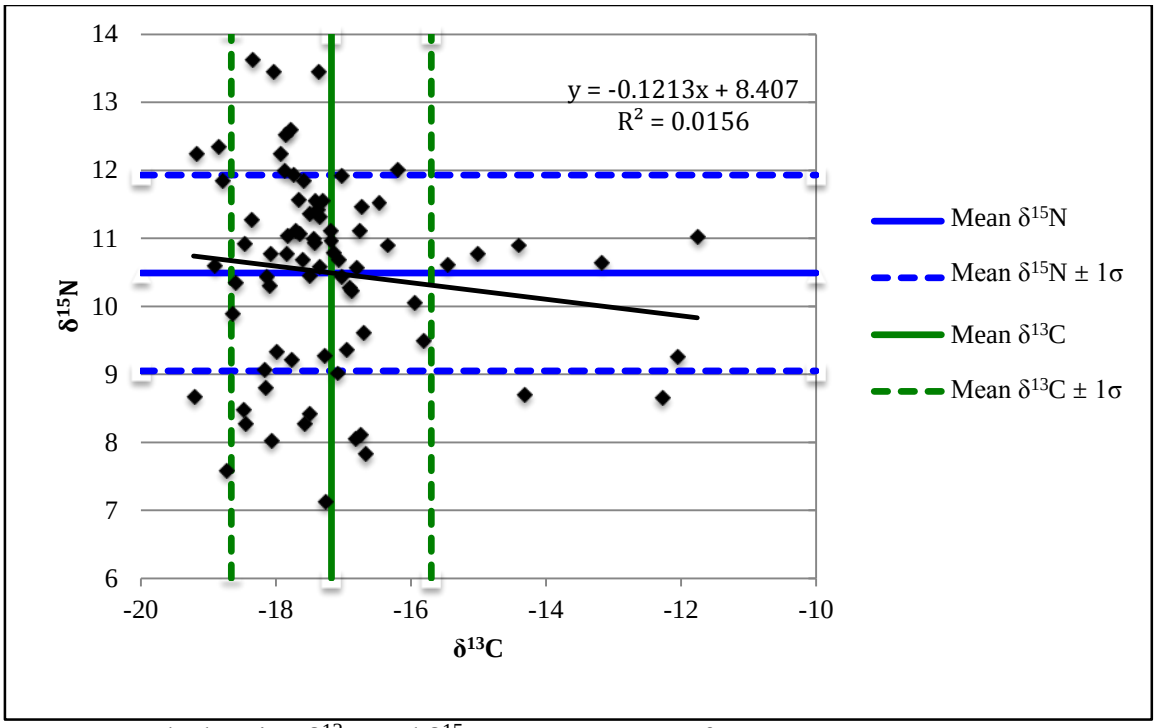

Figure 16. Graph showing $\delta^{13}C$ and $\delta^{15}N$ with the range of variation in this sample.

Figures 17 and 18 show the relationship between median age and stable carbon and nitrogen isotope values. Both $\delta^{13}C$ and $\delta^{15}N$ values appear to be fairly evenly distributed, however, all $\delta^{15}N$ values above +13‰ occur in individuals 30 years of age and below, as shown in Figure 17.

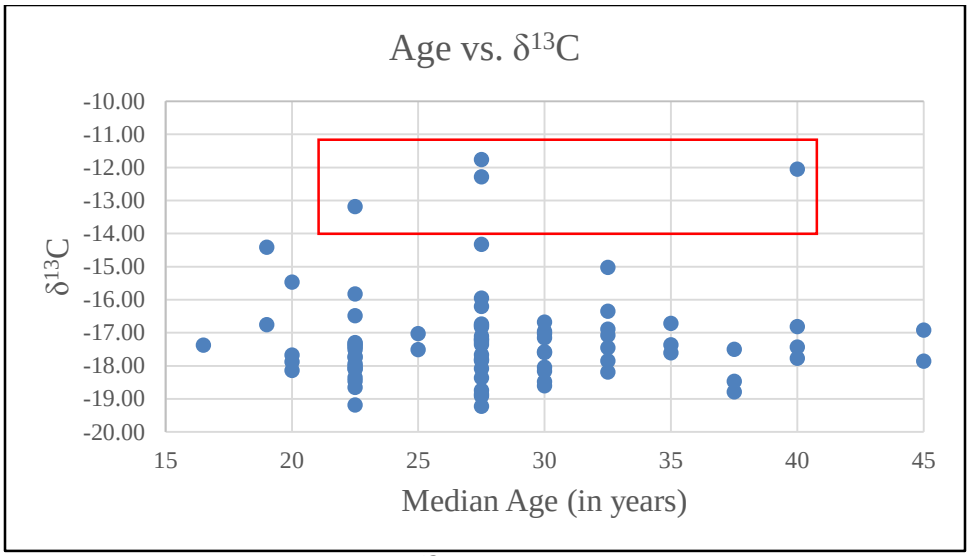

Figure 17. Graph plotting median age vs. $\delta^{13}C$. The rectangle (red) highlights the individuals with $\delta^{13}C$ values above -14‰.

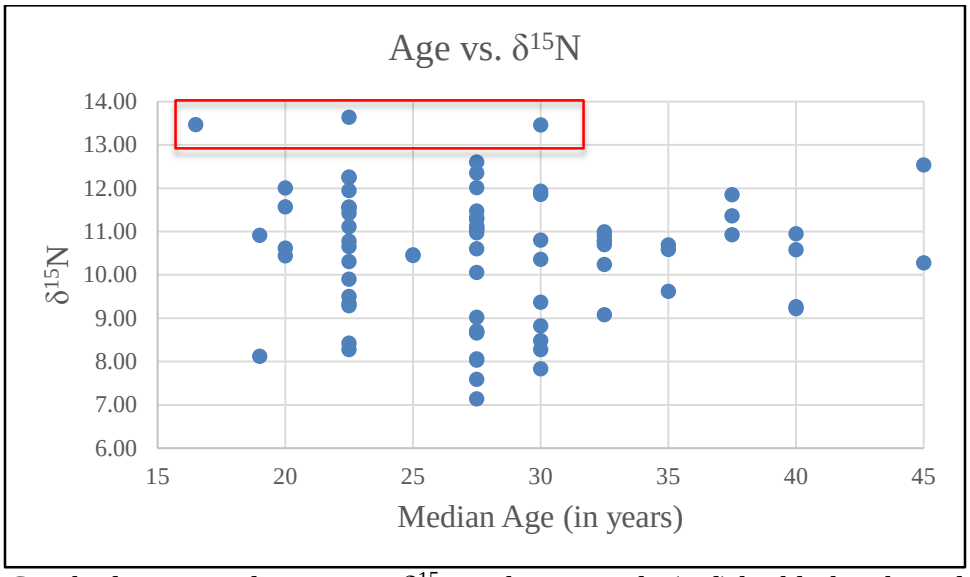

Figure 18. Graph plotting median age vs. $\delta^{15}N$. The rectangle (red) highlights the individuals with $\delta^{15}N$ values above 13‰.

Figures 19 and 20 show box and whisker plots of the range of $\delta^{13}C$ and $\delta^{15}N$ values for known males (n=72) and known females (n=3). The probable male and unknowns were not included. The median $\delta^{13}C$ value for males is -17.41‰, the minimum is -19.22‰ and the maximum is -11.76‰. The median $\delta^{13}C$ value for females is -17.72‰, the minimum is -18.91‰

and the maximum is -15.02‰. The median $\delta^{15}N$ value for males is 10.74‰ with a minimum of 7.14‰ and maximum of 13.63‰. The median $\delta^{15}N$ value for females is 10.78‰ with a minimum of 10.60‰ and a maximum of 11.11‰. In both cases, males show a greater range of values than females. This may be due to differences in sample size.

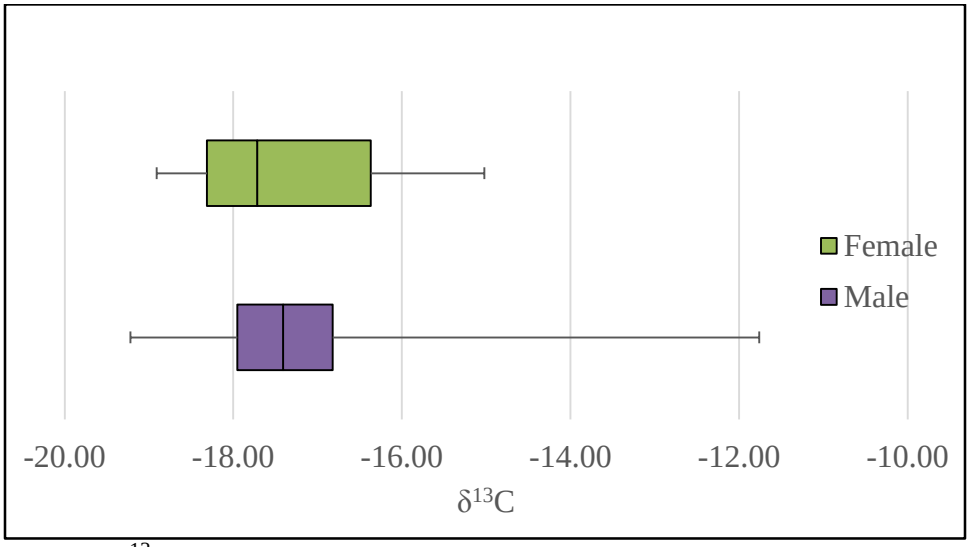

Figure 19. Box plot of $\delta^{13}C$ values for males and females.

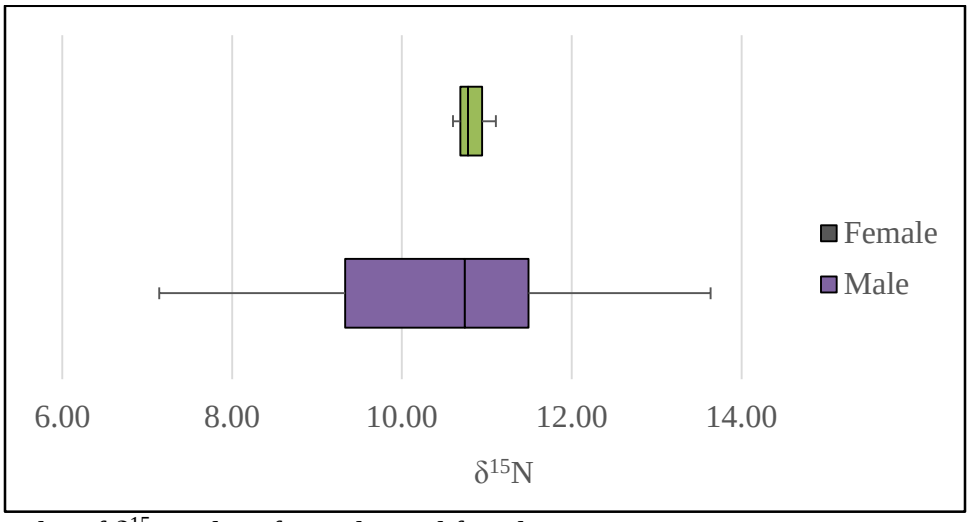

Figure 20. Box plot of $\delta^{15}N$ values for males and females.

White and Schwarcz (1989) developed an equation to calculate the percentage of C_4 plants consumed by an individual using $\delta^{13}C$ values, as displayed in Equation 4.3:

$$\text{Percentage } C_4 = \frac{(\delta_c - \delta_3 + \Delta_{dc})}{(\delta_4 - \delta_3)} \times 100 \qquad (4.3)$$

where δ_c= sample $\delta^{13}C$ value, Δ_{dc}=-5, δ_3=-26 and δ_4=-9. White and Schwarcz (1989) based the δ_3 value on van der Merwe and Vogel's (1978) average $\delta^{13}C$ value of -26.5‰ for C_3 plants. The δ_4 value is based on Schwarcz et al.'s (1985) C_4 value of maize and the Δ_{dc} value is based on Van der Merwe and Vogel's (1978) value for isotopic fractionation between bone collagen value and dietary product. This study modifies White and Schwarcz's (1989) equation to better fit the current sample, utilizing a δ_4 value of -12.5‰, which is the average $\delta^{13}C$ value for C_4 plants in a number of populations (van der Merwe 1982). This value is used because maize was not a staple C_4 plant consumed in Europe during the 1800s. This study uses a δ_3 value of -26.5‰ and a Δ_{dc} value of -5.1 in order to more accurately calculate the percentage of C_4 plants consumed by Napoleon's Grand Army. Equation 4.4 is the alternative equation utilized for the current sample:

$$\text{Percentage } C_4 = \frac{(\delta_c - (-26.5) + (-5.1))}{(-12.5 - (-26.5))} \times 100 \qquad (4.4)$$

Table 9 shows the $\delta^{13}C$ values and the estimated percentage of C_4 plants consumed for each sample in ascending order. The percentage of C_4 plants (PC_4) consumed by the individuals in this sample range from 15.57% to 68.86%, with a mean of 30.17% ± 10.59 (1σ).

Table 9. Data for $\delta^{13}C$ and Percentage of C_4 Plants in the Diet for each Sample sorted from Lowest to Highest C_4 Consumption.

Sample	$\delta^{13}C$	PC_4	Sex	Median Age (in years)
NAP-K26	-19.22	15.57	M	27.5
NAP-K6	-19.19	15.81	M	22.5
NAP-K17	-18.91	17.80	F	27.5
NAP-K37	-18.86	18.18	M	27.5
NAP-K38	-18.79	18.64	M	37.5
NAP-K52	-18.74	19.01	M	27.5
NAP-K23	-18.65	19.66	M	22.5
NAP-K29	-18.60	20.01	M	30
NAP-K41	-18.48	20.85	M	30
NAP-K7	-18.47	20.93	PM	37.5
NAP-K42	-18.45	21.04	U	22.5
NAP-K3	-18.36	21.73	M	27.5
NAP-K35	-18.35	21.80	M	22.5
NAP-K44	-18.18	22.99	M	32.5
NAP-K177	-18.16	23.16	M	30
NAP-K25	-18.14	23.31	M	20
NAP-K61	-18.08	23.70	M	22.5
NAP-K63	-18.08	23.73	M	27.5
NAP-K36	-18.04	24.00	M	30
NAP-K113	-18.00	24.28	M	22.5
NAP-K50	-17.94	24.74	M	22.5
NAP-K175	-17.88	25.14	M	20
NAP-K132	-17.86	25.28	M	45
NAP-K131	-17.84	25.41	M	32.5
NAP-K62	-17.83	25.47	M	27.5
NAP-K73	-17.80	25.73	M	27.5
NAP-K19	-17.77	25.93	M	40
NAP-K128	-17.75	26.08	M	22.5
NAP-K126	-17.72	26.31	F	22.5
NAP-K106	-17.67	26.63	M	20
NAP-K78	-17.66	26.72	M	27.5
NAP-K45	-17.61	27.08	U	35
NAP-K133	-17.58	27.28	M	30
NAP-K91	-17.51	27.79	M	22.5

Sample	$\delta^{13}C$	PC_4	Sex	Median Age (in years)
NAP-K167	-17.51	27.79	M	25
NAP-K150	-17.50	27.83	M	37.5
NAP-K165	-17.45	28.21	M	32.5
NAP-K154	-17.44	28.32	M	40
NAP-K9	-17.43	28.37	M	22.5
NAP-K87	-17.39	28.62	M	22.5
NAP-K107	-17.37	28.77	M	16.5
NAP-K134	-17.36	28.84	M	35
NAP-K170	-17.36	28.88	M	27.5
NAP-K111	-17.32	29.12	M	22.5
NAP-K127	-17.29	29.35	M	22.5
NAP-K116	-17.28	29.45	M	27.5
NAP-K159	-17.20	29.97	M	27.5
NAP-K130	-17.19	30.08	M	27.5
NAP-K169	-17.15	30.33	M	30
NAP-K96	-17.08	30.89	M	32.5
NAP-K151	-17.03	31.22	M	30
NAP-K183	-17.03	31.23	M	25
NAP-K1	-16.96	31.72	M	30
NAP-K54	-16.92	32.01	M	45
NAP-K158	-16.89	32.19	M	32.5
NAP-K143	-16.82	32.70	M	27.5
NAP-K176	-16.82	32.74	M	40
NAP-K153	-16.77	33.04	M	27.5
NAP-K112	-16.75	33.21	M	19
NAP-K40	-16.74	33.30	M	27.5
NAP-K168	-16.71	33.49	M	35
NAP-K75	-16.68	33.74	M	30
NAP-K121	-16.48	35.14	M	22.5
NAP-K142	-16.35	36.05	M	32.5
NAP-K164	-16.20	37.13	M	27.5
NAP-K171	-15.95	38.91	M	27.5
NAP-K82	-15.46	42.41	M	20
NAP-K64	-15.02	45.55	F	32.5
NAP-K68	-14.41	49.92	M	19
NAP-K148	-14.32	50.55	M	27.5

Sample	$\delta^{13}C$	PC_4	Sex	Median Age (in years)
NAP-K174	-13.18	58.70	M	22.5
NAP-K105	-12.28	65.11	M	27.5
NAP-K65	-12.05	66.76	M	40
NAP-K182	-11.76	68.86	M	27.5

Figure 21 displays the $\delta^{13}C$ and $\delta^{15}N$ values with percentages of C_4 pathway plants in the diet of the individuals in this sample. Twenty-two individuals consumed <25% C_4 plants, 51 consumed between 25% and 50% C_4 plants, 5 consumed between 50% and 75% C_4 plants and no individuals consumed >75% C_4 plants.

There is no visible relationship between the percentage of C_4 plants consumed and median age of the individuals in this sample (see Figure 22). However, a majority (>50%) of the individuals in every age group consumed a diet of less than 40% C_4 plants.

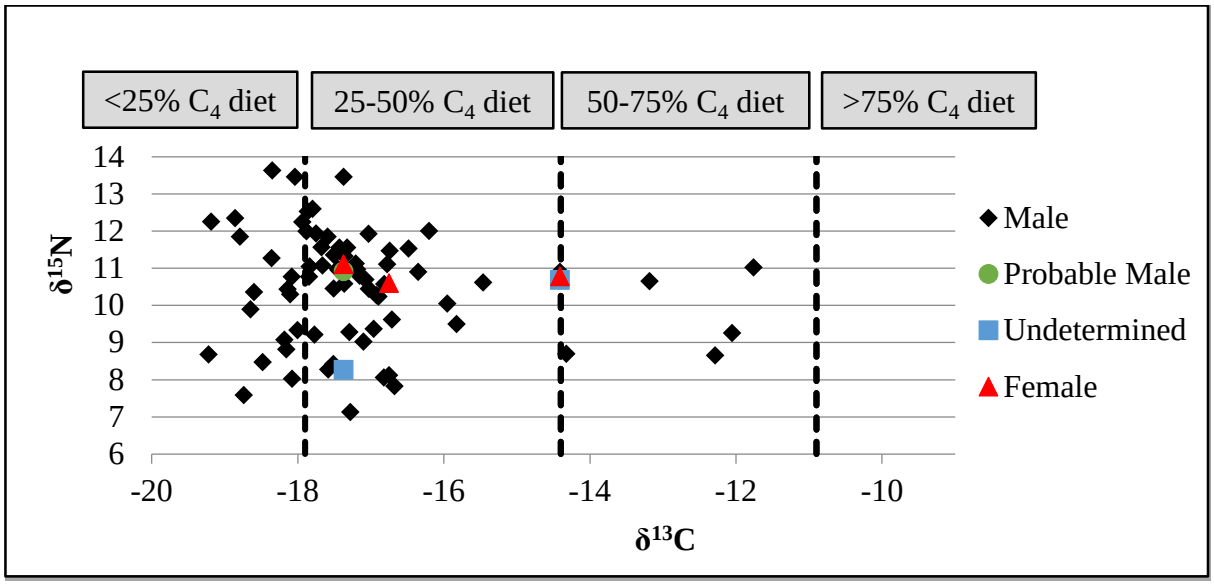

Figure 21. Graph showing the percentage of C_3 and C_4 plants consumed.

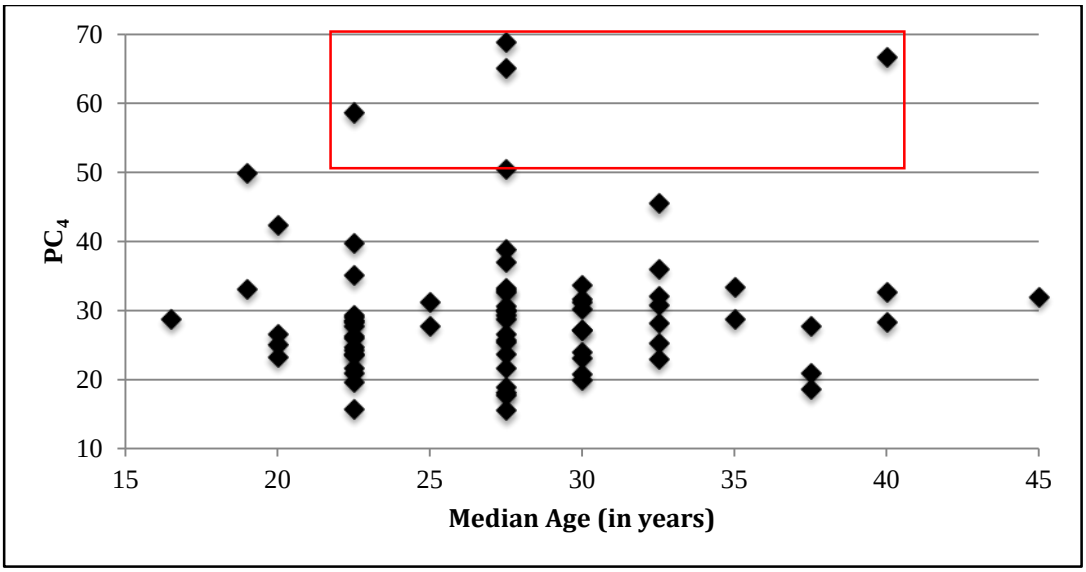

Figure 22. Graph showing median age vs. percentage of C_4 plants consumed in the diet. The rectangle highlights the individuals with C_4 plant consumption above 50%.

A food web was unable to be reconstructed for this sample. This is because stable isotope data for flora and fauna were unattainable for this region and time-period. Furthermore, a food web would be erroneous due to the heterogeneity of the geographic origin of the individuals in this sample. Historic accounts, contextual evidence and comparisons of stable isotope values with other European populations were used to reconstruct diet for this sample. It is discussed in the following chapter.

CHAPTER FIVE: DISCUSSION

The following sections provide a detailed analysis of the results in the context of historical accounts and literary and archaeological evidence. Dietary trends are evaluated and interpreted. Comparisons with other historical populations are also made in this chapter.

General trends: career soldiers, conscripts and non-soldiers

Annual conscription in France began in 1799; it affected men over the age of 18, with single young men in their early 20s most often selected (Forrest 2002). Previous studies indicate that soldiers were taller and healthier than their contemporary non-military counterparts (Costa 1993, Sandberg and Steckel 1980). Recruitment and conscription were aimed at young, healthy individuals. Due to constant conflict in the early 19th century, many conscripted soldiers became career soldiers and served in multiple campaigns throughout the Napoleonic Wars. The process of military conscription fluctuated depending on level of conflict (Forrest 2002). It has been argued that military service in the 19th century "turned relatively healthy young men into physical wrecks, unless they perished in combat first" (Sledzik and Sandberg 2002:185). Individuals in this sample with a median age of 20 years and below may be young conscripts and their stable isotope values likely reflect diet prior to military service. Individuals from 22.5-27.5 years of age may have served in previous campaigns and their diet likely reflects a combination of diet prior to and during military service. Individuals 30 years and above may be career soldiers and their stable isotope values would reflect average diet throughout their career. Five individuals exhibit $\delta^{13}C$ values above -14.40‰, making C_4 plant consumption for these individuals above 50%. Table 10 reports the sex, age and stable isotope values for these

individuals. All three individuals with $\delta^{15}N$ values above 13.00‰ have a median age of 30 years and younger. These differences are discussed further in the following sections.

Table 10. Summary of Data for Individuals with C_4 Plant Consumption above 50%.

Sample	Sex	Median Age (in years)	$\delta^{13}C$ (in ‰)	PC_4	$\delta^{15}N$ (in ‰)
NAP-K148	M	27.5	-14.32	50.55	8.7
NAP-K174	M	22.5	-13.18	58.7	10.65
NAP-K105	M	27.5	-12.28	65.11	8.66
NAP-K65	M	40	-12.05	66.76	9.27
NAP-K182	M	27.5	-11.76	68.86	11.03

Archaeological evidence in the form of material remains indicates the predominance of infantry and cavalry soldiers in the mass grave discovered in Vilnius. Due to problems of fragmentation and commingling, it is unclear exactly which individuals were infantry and which were cavalry. Therefore, consumption differences between these groups were unable to be determined.

There is a lack of dietary discrimination based on sex. Mean $\delta^{13}C$ and $\delta^{15}N$ values were similar for males and females. Males showed a much larger range of variation for stable isotope values. However, these differences may be a reflection of error created by differences in sample size (n=3 for females, n=72) rather than similarity in diet.

Historical and archaeological evidence demonstrate that the mass grave from Vilnius contains the skeletal remains of soldiers from Napoleon's Grand Army (Signoli et al. 2004). However, reports indicate that convoys of non-soldiers followed the French Army during their retreat from Moscow (Nicolson 1985). Local individuals who died around the time of occupation by the Napoleonic Army could also be present in the grave. This is supported by the presence of females in the grave. Although the analyses presented make the assumption that all

of the male individuals were soldiers, however, non-soldiers may also be represented in the grave. Variation in $\delta^{13}C$ and $\delta^{15}N$ values could be partially attributed to differences between soldiers and non-soldiers.

Nitrogen enrichment: A comparison with other samples

Determining what $\delta^{15}N$ values constitute nitrogen isotope enrichment is often unclear in the literature. Authors rarely state how enrichment is determined for their sample, although it often appears to be population-based since $\delta^{15}N$ values reflect diet. For example, nitrogen enrichment would be different for a population with marine resources as a dietary staple compared to a population with a predominantly terrestrial diet. Determining a value based on nitrogen equilibrium and expected dietary resources for a sample would be ideal for determining a "normal value". Since the current sample is heterogenic in regional origin (and, therefore diet), neither a population-based approach or expected dietary resources approach are appropriate for this sample. Instead, this sample was compared with an arbitrarily determined "normal" European population with a terrestrial diet and a soldier sample to evaluate nitrogen enrichment and dietary patterns for the current sample.

An average $\delta^{15}N$ value from a Medieval site in the UK was used as a "normal" European population for comparison with the current sample. The average adult $\delta^{15}N$ value from the site of Wharram Percy was used because isotopic values from this sample reflect what is typical for most of western and northern Europe (i.e., C_3 plants, a lack of marine resources and mixed animal and plant protein). The isotopic values in the Wharram Percy sample reflect a diet with "no measurable input of marine foods or C_4 pathway foods in average diet" (Richards et al. 2002:207). Furthermore, evidence points to a diet of mixed plant and animal protein. The

average $\delta^{15}N$ value for adults in the Wharram Percy sample is approximate 9‰ ±1. Two standard deviations from the mean value will indicate values enriched or depleted in $\delta^{15}N$. Figure 23 shows the spread of $\delta^{15}N$ values for the Napoleonic sample with lines indicating the thresholds for enrichment and depletion. None of the individuals in this sample are depleted in $\delta^{15}N$, however, 29 of the 78 (37.18%) individuals are exhibit enriched $\delta^{15}N$ values compared to the Wharram Percy sample. No statistical comparison was made between the Wharram Percy and Napoleonic samples because individual $\delta^{15}N$ values were not reported for the Wharram Percy sample.

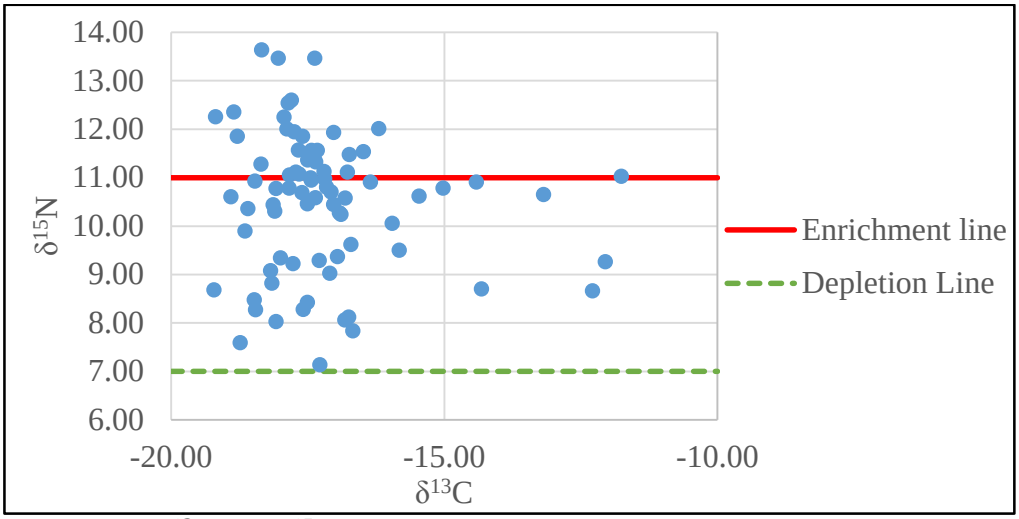

Figure 23. Graph of $\delta^{13}C$ and $\delta^{15}N$ values for the Napoleonic sample with lines indicating predicted nitrogen enrichment and depletion. Lines are based on Wharram Percy sample (Richards et al. 2002)

There is a lack of linear relationship between $\delta^{13}C$ and $\delta^{15}N$ values for the current sample (R^2=0.0156). A lack of linear relationship between these two variables is not surprising since these soldiers come from all of Europe with varying social backgrounds. A similar "social and regional heterogeneity…reflected in the extremely poor linear relationships between C and $\delta^{15}N$ ratios" was found in a sample of English soldiers from a 15th century mass grave in the village of

Towton, England (Müldner and Richards 2005:45). The individuals from this grave are suggested to be the casualties from the battle of Towton in 1461 during the Wars of the Roses. Eleven of the 38 samples excavated underwent stable isotope analysis.

Although this sample comes from a different time-period, there are a number of similarities between these two samples that make them appropriate for comparison. Both sets of samples come from mass graves in Europe with drafted soldiers that come from diverse social and regional backgrounds. Historical evidence on the Napoleonic sample and healed lesions associated with weapon trauma in the Wharram Percy sample suggest that both samples contain professional soldiers (Forrest 2002, Müldner and Richards 2005).

Figure 24 shows the isotopic values of the Napoleonic soldiers in this sample plotted against the isotopic values of the Towton soldiers published in Müldner and Richards (2005). The Townton soldiers' $\delta^{15}N$ values fall on the higher extreme of the Napoleonic sample with far less variation, although this could be due to the large differences in sample size. The Towton soldiers exhibit enriched $\delta^{15}N$ values comparative to their 19th century counterparts. The authors argue that the $\delta^{15}N$ values in the Wharram Percy sample are atypical for a Holocene sample from the UK and consumption of terrestrial protein alone do not account for the unusually high values (Müldner and Richards 2005). Similarly, $\delta^{13}C$ values cluster at the more negative end of the Napoleonic sample indicating consumption of little to no C_4 plants. This corresponds to expectations for a historical sample from Western Europe (Müldner and Richards 2005).

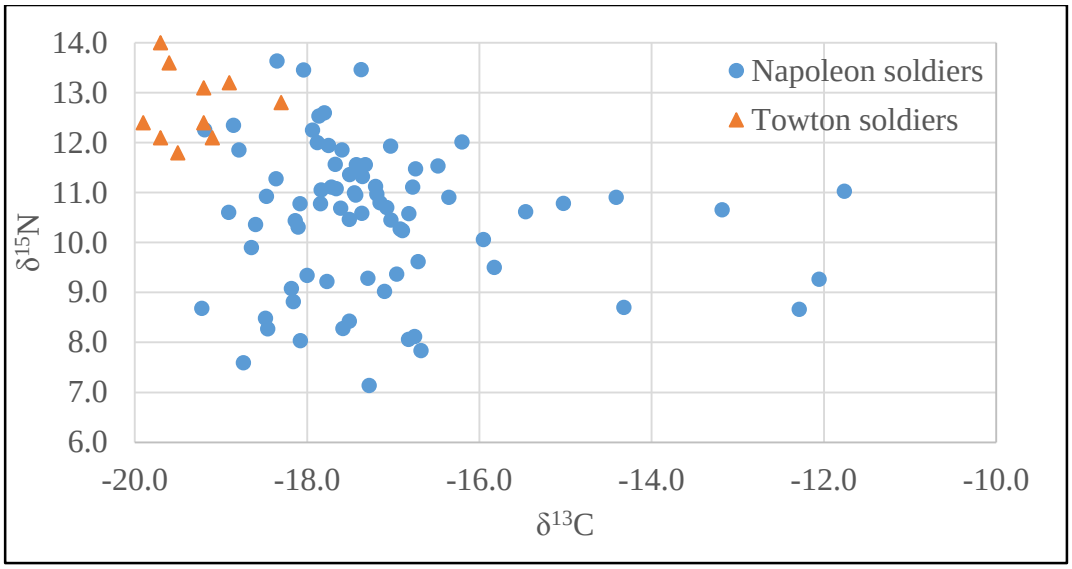

Figure 24. Graph comparing Napoleonic soldier isotope values with Towton soldier isotopic values. Data on Towton soldiers comes from Müldner and Richards (2005)

Possible causes of nitrogen enrichment

Nitrogen isotope enrichment in bone collagen of adult humans can be explained by a number of factors: prolonged nutritional stress, marine resources as dietary staple or pathological conditions affecting bone.

Prolonged nutritional stress. The enriched $\delta^{15}N$ values in this sample may indicate nutritional stress associated with years of military service in harsh environmental conditions, a lack of protein in the diet prior to military conscription or both. Several studies show that during periods of nutritional stress, $\delta^{15}N$ values become enriched in bodily tissues (Spanheimer and Peterkofsky 1985, Hobson et al. 1993, Fuller et al. 2005, Hatch et al. 2006, Mekota et al. 2006. How long it takes for a tissue to become enriched depends on the turnover rate of that tissue. Although bone, particularly the femur, has a very slow turnover rate (>10 years) (Hedges et al. 2007), it is possible that the individuals in this sample exhibit enriched $\delta^{15}N$ values due to the severity of starvation over a prolonged period of time. The prolonged period(s) of starvation possibly stem

from career-long military service in numerous campaigns throughout the Napoleonic Wars and/or from nutritional stresses prior to military conscription. Age is an important factor in determining which of these scenarios may be the cause of nitrogen enrichment in the individuals in this sample. As discussed in the previous sections, nitrogen enrichment in the younger soldiers (≤ 20 years of age) may reflect nutritional stress prior to military service. The high prevalence of LEH (78.5%) in individuals in this sample indicates periods of stress in childhood, which may or may not be the result of nutritional stress, specifically (Palubeckaitė et al. 2006). Periods of nutritional stress could have continued following dental formation and eruption into adolescence and early adulthood.

Marine-based diet. Historic and archaeological evidence points to a marine-based diet as a possible source of enrichment for the individuals in the sample. Nicolson (1985) and uniform fragments from the mass grave indicate geographic origin of these individuals from all over Europe. Communities throughout coastal Europe relied on marine resources as a major dietary source. Stable nitrogen isotope values are approximately 4‰ higher in marine resources over terrestrial resources (Ambrose 1993). Figure 25 shows the $\delta^{15}N$ values for the current sample, with a line (black) representing the average $\delta^{15}N$ value for the Wharram Percy sample and a dashed line (red) representing a 4‰ enrichment level. Three individuals exhibit values above 13‰, which could be the result of a marine based diet. However, $\delta^{13}C$ values are more negative than the typical range for marine resources of -10 to -15‰.

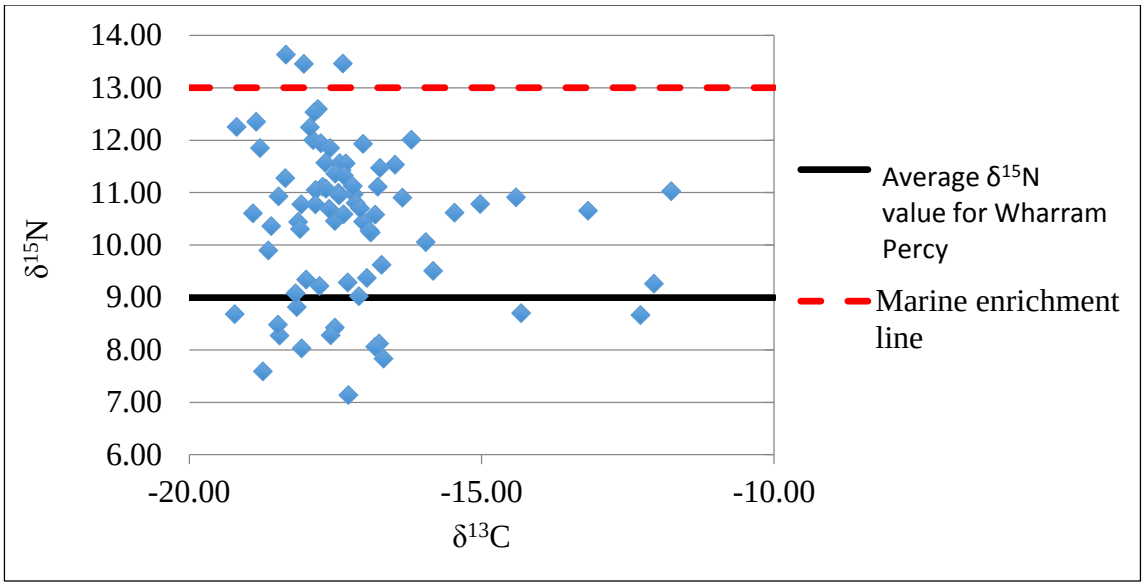

Figure 25. Graph of $\delta^{13}C$ and $\delta^{15}N$ values for the Napoleonic sample with lines indicating average diet and marine enrichment.

Pathological conditions. In the current sample, stable isotope analysis was conducted on healthy appearing portions of bone. Bone with obvious pathology was not included, however, protein imbalance caused by pathological conditions not visible on the surface of the bone cannot be eliminated. Table 5 in Chapter 3 shows that there were individuals in this sample with pathological conditions.

Katzenberg and Lovell (1999) argued that periostitis does not impact $\delta^{15}N$ values unless the periosteal area affected is sampled. The samples in this study upheld Katzenberg and Lovell's argument for $\delta^{15}N$ values in bone with periostitis. Figure 26 shows the $\delta^{15}N$ values for this sample, differentiating individuals with pathological conditions from normal individuals. Normal individuals are those with no diagnosed skeletal pathological conditions. It is important to note that the healed and perimortem fractures in this study occurred in different bones that what was sample for stable isotope analysis. Therefore, these individuals are highlighted in the

Figure 26, but the results were not compared with Katzenberg and Lovell's sample because they did sample affected and unaffected portions of fractured bone (1999).

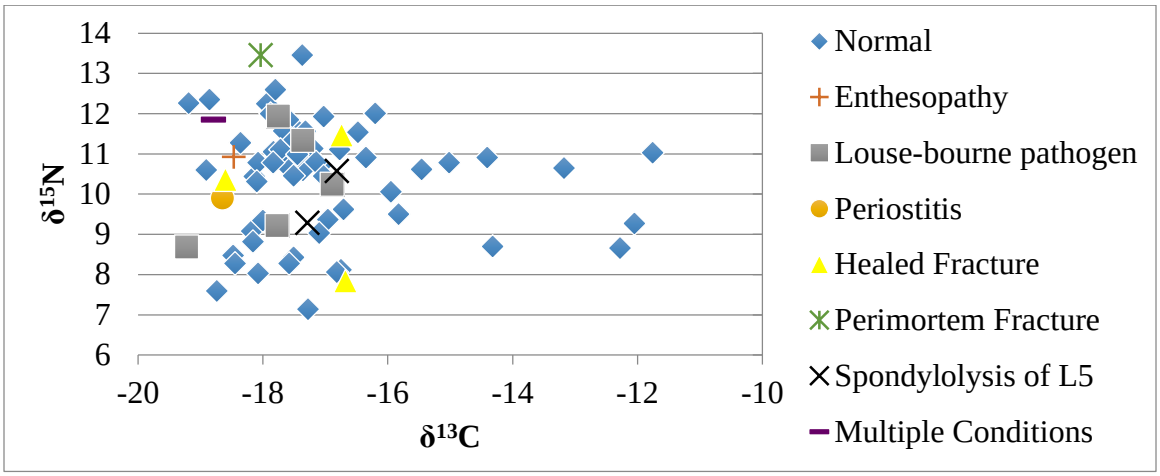

Figure 26. Graph of stable carbon and nitrogen isotope values for Napoleon's Grand Army highlighting individuals with pathological conditions.

C_3 and C_4 plant consumption

Collagen $\delta^{I3}C$ values for humans in a temperate environment with little to no marine or C_4 resources are typically between -19 and -22‰ (van Klinken 1999). Environmental conditions in a majority of Europe, where this sample originates, mirror these characteristics. Although collagen $\delta^{I3}C$ values were very negative indicating a diet of primarily C_3 plants for a majority of the sample, most values fall above -19‰. Approximately 94% of individuals in this sample received more than half of their dietary plant protein from C_3 plants. These results align with previous studies on other historic European populations (e.g., Privat et al. 2002, Richards et al. 2002, Müldner and Richards 2005). C_3 pathway plants dominate edible vegetation in Europe. Wheat and barley consumed in various forms are staple C_3 plants in European diet (Müldner and Richards 2005). Bread, soup and porridge were staple dietary sources in Napoleon's Army (Forrest 2002). The reliance on porridge, a starchy and sticky food, could account for the

prevalence of carious lesions found in Palubeckaitė et al.'s study (2006). The abundance of these domesticated species throughout Europe accounts for the dominance of C_3 plants in the Napoleonic soldiers in this sample. C_4 plants were supplementary to the diet of these individuals since values indicate that no individuals consumed 100% of dietary plant protein from C_3 plants. Millets are the dominant C_4 plant consumed in Europe prehistorically and historically, especially *Panicum miliaceum* (broomcorn) and *Setaria italica* (foxtail) (Tafuri et al.2009). It was often consumed in the form of porridge or unleavened bread. Poor people often consumed millet, particularly during the Middle Ages. It was also cultivated in southern Europe as an alternative to certain C_3 grains (Andrews 2000). Table 9 highlights the five individuals with dietary plant protein consumption of C_4 plants above 50%. All five individuals are males, however, there is no relationship between the $\delta^{13}C$ values of these individuals and age or $\delta^{15}N$ values. There are a number of plausible explanations for the large amounts of C_4 plants consumed by these five individuals. These individuals may be poor conscripts (that consumed large amounts of millet) who were either drafted or volunteered for the Grand Army as was common during this period. The higher $\delta^{13}C$ values may reflect diet prior to military service. Another possibility is that these individuals were from or were stationed for a significant amount of time in southern Europe, where millet consumption was more common. Another possibility is that these individuals were not soldiers. Historical and archaeological evidence suggest that the mass grave represented Napoleon's Grand Army, however, non-soldiers are known to have travelled with armies providing goods and/or services. As previously noted, a convoy followed the Napoleonic Army during the retreat from Moscow (Nicolson 1985); it is possible that some of these individuals

transpired in the mass grave. The $\delta^{13}C$ values of these individuals would reflect diet over a period long before the French retreat from Moscow.

CHAPTER SIX: CONCLUSIONS AND FUTURE RESEARCH

This research allows for an insight in to the consequences of long-term conquest. Upon failure to capture the Russian capital of Moscow, the soldiers in Napoleon's Grand Army retreated through the harsh Russian winter in an attempt to escape back to France. However, thousands of soldiers never made it back to France. Instead, these soldiers perished due to cold, hunger and disease (Signoli et al. 2004). According to historical accounts, a number of these soldiers were buried in mass grave in Vilnius, Lithuania.

Stable nitrogen isotope analysis shows that more than a quarter of this sample were enriched in ^{15}N. It is less clear as to why these individuals exhibited these values. Based upon historical evidence, contextual evidence and research of the literature on this subject, nitrogen enrichment is most likely the result of prolonged nutritional stress, although this has not been demonstrated with reasonable scientific certainty. More research into the relationship between bone turnover, protein metabolism and nitrogen enrichment may shed light on this currently murky subject. Furthermore, analysis of specific amino acid abundances in bone collagen may also contribute to the clarity of the relationship between diet, fractionation and nitrogen isotope values.

From stable carbon isotope analysis, it is clear that a majority of these soldiers consumed primarily C_3 plants for plant protein in the years prior to their death. Five soldiers consumed more C_4 plants, however, this is not surprising as it is well-known that Napoleon recruited professional soldiers from all over Europe to join his Grand Army. The heterogeneity of this sample also explains the high degree of variation in $\delta^{13}C$ values for this sample.

This research makes a number of contributions to the field of anthropology. Investigations into the diet and health of historic military populations are rare. This investigation contributes to the body of knowledge on diet and health in this unique sample. It also illuminates the complexities in stable isotope research, particularly related to elevated ^{15}N values. It makes clear the importance of future research into the mechanisms of protein formation related to nutritional stress and nitrogen balance.

Other stable isotope analyses could provide further dietary information for this sample. Analysis of carbon in bone apatite could elucidate whether the three individuals with ^{15}N values above 13.0‰ were consuming marine foods. Because marine resources consumed in diet are typically animals, there should only be a small difference between carbon apatite and collagen values (Lee-Thorp et al. 1989). Stable oxygen isotope analysis could also contribute to the understanding of the variation in $\delta^{13}C$ and $\delta^{15}N$ values for this sample. Stable oxygen isotope analysis provides information on mobility related to water source. This type of analysis on tooth enamel can be used to determine the geographic origin of individuals, which may shed light on C_4 consumption in some individuals and the possibility of marine consumption.

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