AN ISOTOPIC EVALUATION OF HUMAN HAIR FROM BELLEVILLE, ONTARIO

YOU ARE NOT WHAT YOU EAT DURING STRESS: AN ISOTOPIC EVALUATION OF HUMAN HAIR FROM BELLEVILLE, ONTARIO

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Abstract

Carbon and nitrogen isotope values in sequential segments of human hair keratin provide an archive of temporal fluctuations in isotopic composition close to the time of an individual's death. By combining stable isotope analysis with a microscopic examination of hair, this thesis explores health status prior to the death of early settlers from St. Thomas' Anglican Church cemetery in Belleville, Ontario (1821-1874). The purpose of this thesis is to determine if there is a consistent difference in carbon and nitrogen isotopic signatures along sequentially segmented hair in individuals who have observable pathological conditions versus individuals who display no osteological evidence of pathology. Elevated nitrogen values can be associated with physiological stressors such as chronic illness, infection, or injury that affect an individual's metabolic state. Elevated nitrogen values represent a recycling of nitrogen derived from the breakdown of existing proteins in the body and subsequent tissue repair. Results from 10 individuals indicate that $\delta^{15}N$ values increase greater than 1‰ if an individual was suffering from a pathological condition (e.g., periostitis) or decrease by 1‰ if an individual was possibly pregnant, while $\delta^{13}C$ values remained relatively constant. The variability in nitrogen values over 1‰, coinciding with less change in $\delta^{13}C$ values, may be indicative of physiological stress. These results suggest that $\delta^{15}N$ values are not only useful for studying diet, but may also be used as indicators of physiological stress.

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DECLARATION OF ACADEMIC ACHIEVEMENT

This is a declaration that the content of this thesis has been completed in entirety by Lori D'Ortenzio, recognizing the critical evaluation of Dr. Tracy Prowse, Dr. Megan Brickley, and Dr. Henry Schwarz. All samples were collected and prepared for stable isotope analysis by Lori D'Ortenzio and the samples were run in the mass spectrometer by Martin Knyf, the lab manager for the Research Group for Stable Isotopologues (MRSI). The trichogram was conducted by the author and all other work presented in this thesis was performed by the author.

Chapter 1

Introduction

1.1 Bioarchaeological Analysis of Hair Keratin

An important component of bioarchaeological research is the ability to elucidate the lives of individuals who lived in the past. By combining stable isotope analysis with microscopic examination of hair, this thesis explores diet and metabolic status prior to death among early settlers from Upper Canada who were buried in the 19th century St. Thomas' Anglican Church cemetery in Belleville, Ontario (1821-1874). Metabolic status refers to biochemical processes that occur within living organisms that consist of anabolism (the build-up of tissue) and catabolism (the breakdown of tissue) that are required for the maintenance of life (LaPorte et al., 2011). Bones and teeth are typically used for isotopic analysis; however hair keratin can be utilized as an alternative tissue in the study of past diet, but on a shorter time scale than other tissues (Katzenberg et al., 2000; O'Connell and Hedges, 1999; Schwarcz and White, 2003). Hair grows incrementally and records dietary information at approximately one cm/month, therefore sequential stable isotope analysis of hair keratin has the potential to provide insights into the dietary preferences and metabolic status of individuals prior to death (Paus and Costarelis, 1999).

1.2 Research Objectives

The main objective of this research is to analyze $\delta^{13}C$ and $\delta^{15}N$ values of hair specimens from the St. Thomas' Anglican Church cemetery sample to explore patterns in diet and to identify possible indicators of physiological stress. The goal is to determine if there is a consistent difference in carbon and nitrogen isotopic signatures along sequentially segmented hair in individuals who have observable pathological conditions versus individuals who display no osteological evidence of pathology. Individuals with known physiological conditions or compromised health status were selected to reconstruct their metabolic status prior to death. Dietary patterns are also explored to facilitate a greater understanding of individual life histories and consumption practices from19th century Belleville, Ontario

This study examines how human $\delta^{15}N$ values are affected by nutritional or physiological stress and was motivated in part, by Katzenberg and Lovell's (1999) study that analyzed stable isotope ratios in pathological bone from individuals with known medical histories, and in which they found elevated $\delta^{15}N$ values that were possibly the result of tissue catabolism (breakdown).

Stable isotopes in general and nitrogen isotopes in particular can be used to monitor fluctuations in nitrogen balance caused by conditions such as nutritional or physiological stress (Katzenberg and Lovell, 1999; Tomé and Bos, 2000). Elevation or depletion of $\delta^{15}N$ values in body tissues is due to the fact that under periods of substantial physiological or nutritional stress, organisms can transition into a catabolic (tissue breakdown) or anabolic (building tissue) state. Whether stress is accidental (i.e., from

broken bones or burns), or from illness, the body reacts to stress much as it does to the stress of starvation, causing the body's metabolic rate to alter profoundly (Cahill, 1988). As the body responds to injury or illness (within 36-48 hours), there is an increase in nitrogen excretion that results in fluctuations in nitrogen values that take approximately six to twelve days to be detected in the human body (Cahill, 1988; Jones et al., 1981; Oddoye and Margen, 1979). This study analyzes the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ values along the hair fiber in order to obtain an isotopic archive to assess the metabolic state of individuals just prior to death. This thesis had two complimentary objectives:

1.2.1 Objective 1:

The first goal is to investigate a method to detect physiological or nutritional stress prior to isotopic analysis through the microscopic identification of the growth phases of hair. The trichogram (microscopic) analysis determines how many hairs from an individual have transitioned from the active growth (anagen) phase to the resting or inactive phase (catagen/telogen). Identification of the percentage of hair actively growing versus that in the resting phase is an indicator of physiological stress, because during times of stress hair growth patterns can be altered to reflect the metabolic reactions of an individual (Kligman, 1961; Tomé and Bos, 2000; Williams et al., 2011). Telogen effluvium or acute hair shedding is usually accompanied by increased levels of glucocorticoids, which may be a response to a number of physiological stresses including infection, injury, illness, or pregnancy (Kligman, 1961; Vermes and Beishuizen, 2001). In a normal healthy individual, it is assumed that approximately 80% of scalp hairs are

actively growing at any given time and 20% of hairs are inactive or resting (O'Connell and Hedges, 1999; Williams et al., 2011). In healthy individuals, the timing of growth phases tends to remain constant because it is genetically determined, however intrinsic, hormonal, and physiological changes can influence the duration of each phase permitting fluctuations in the proportion of resting and growing hair to be an indicator of stress (Botchkarev, 2003; Kligman, 1961; Mekota et al., 2006; O'Connell and Hedges, 1999; Williams et al., 2011).

1.2.2 Objective 2:

The second goal is to compare isotopic data of hair samples obtained from individuals with pathological conditions to those without. The stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in hair samples provide information on short-term dietary changes and may indicate the timing of a stressor before the death of an individual. During stress the human body converts to a negative nitrogen balance associated with elevated $\delta^{15}N$ values and this 'enrichment in loss' has been reported in pregnant women with morning sickness, individuals diagnosed with eating disorders such as anorexia nervosa, and individuals who have experienced trauma or infection (e.g., Beisel, 1977; Fuller et al., 2004; Huelsemann et al., 2009; Lovejoy and Heiple, 1981; Mekota et al., 2006). The increased $\delta^{15}N$ values are produced under periods of physiological or nutritional stress because an organism catabolizes amino acids to meet the demands of protein synthesis; therefore an organism literally 'lives on its own meat' (Waterlow, 1968). Conversely, conditions such

as pregnancy produce an increased demand for energy and protein (i.e., an anabolic state) to enable a fetus to grow resulting in depletion of $\delta^{15}N$ values.

1.3 Overview of Methods

1.3.1 Trichogram Analysis

The trichogram is a diagnostic technique based on the microscopic examination of hair fibers, which can be used to determine the presence of metabolic stress. This method examines the appearance of hairs, looking at morphological features of the proximal hair root under a microscope. The aim is to determine the rate of hair loss progression by establishing how many of the hairs are in each of the three stages of the hair growth cycle anagen (growth), catagen (transition to resting) and telogen (resting). Roughly 20% of the hairs should be in the resting phase; any more is an indicator of potential stress (Fuller et al., 2004; Huelsemann et al., 2009; Kligman, 1961; Mekota et al., 2006; Petzke, 2010; Williams et al., 2011).

1.3.2 Stable Isotopes

Stable isotope analysis is a method that utilizes carbon and nitrogen isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in hair keratin and other body tissues and reflects the corresponding isotopic ratios in the diet consumed (Pollard and Heron, 2008). An isotope is an atom of the same element with the same number of protons but different numbers of neutrons. In a chemical reaction isotopes that have a higher mass (heavier isotope) react more slowly than the lighter isotope creating an isotopic ratio for the element of interest.

This is referred to as fractionation and can be used to explain systematic differences in the isotopic composition of human diet and metabolism (Katzenberg and Saunders, 2008; Petzke, 2010). The stable isotopes of carbon and nitrogen are used for this study because they can be employed in combination to differentiate dietary shifts from shifts caused by physiological/metabolic changes in the human body (Meier-Augenstein, 2010).

The combination of methods employed in this thesis allows for the understanding of positional-temporal relationship of hair in different growth phases, which is essential for accurate interpretation of the isotopic data. The isotope values will be also be interpreted within the context of historical evidence for diet and nutritional/health stressors from this time period. This will be done in conjunction with demographic information provided in burial records. The integration of these various lines of evidence permits a study of variability in dietary patterns and metabolic status in the time preceding death. The identification of pathological conditions can be important in the determination of cultural or behavioural practices when combined with contextual data. When applied to archaeological samples and combined with historical data that incorporates information such as living conditions or access to food sources, an understanding of how an injury or a specific condition may have impacted an individual can be assessed.

Stable isotope analysis of hair offers a non-invasive method to reveal information about dietary habits, some of which may lead to impaired nitrogen balance (Petzke et al., 2010). The comparison of hair in different growth phases refines the timing of physiological stress in an individual's life that can be reflected in the isotopic data. Hair

can yield important information on dietary intake and physiological stress in past and modern human populations, but on a different scale of analysis than the more commonly used bone, and provides another analytical tool for the interpretation of archaeological evidence.

1.4 Thesis Organization

Chapter two presents a description of the Belleville region and its history to provide a framework within which to interpret the isotopic results. Chapter three is an overview of the basic components of hair biology including a description of hair structure and growth cycles. Chapter four is a summary of stable isotopes literature, followed by a discussion of the specific use of hair keratin for stable isotopes in chapter five. A detailed description of the trichogram and isotopic methodology is provided in chapter six, with presentation of the results in chapter seven. An explanation of the results is discussed in chapter eight including the limitations inherent in the study of a small historic sample, followed by conclusions in chapter nine.

Chapter 2

The History of Belleville, Ontario

2.1 Introduction

Regional and historical contexts are essential in the analysis of archaeological remains as they provide the framework for the interpretation of results. Due to the importance of context, a detailed examination of the Belleville region and history is necessary before the results of the stable isotope analysis can be discussed. This chapter provides a historical background of Upper Canada and Belleville, an overview of the St. Thomas' Anglican Church Project, and an examination of the information available on cause of death from burial records. The final section provides a description of the health status and diet of individuals residing in Belleville during 1821-1874, and concludes with a brief discussion on the isotopic analysis of food sources and skeletal material from Belleville.

2.2 Immigration to Upper Canada in the 19th Century

During the early 19th century, a wave of British emigrants arrived in Upper Canada and settled initially in the backwoods and clearings along the shores of Lake Ontario. In 1789, approximately 50 Loyalist families arrived in the region along the Moira River near Belleville and founded Thurlow Village. This area became a major farm market center by the early 19th century (Jimenez, 1991). Early settlers were encouraged to move into the

Belleville region because it was considered a place where job and business opportunities were plentiful (Mika and Mika, 1986).

From 1826-1851 the population of Upper Canada increased by 470 percent from 166,379 to 952,004 (Lewis and Urquhart, 1999). Canada received over 50,000 landed immigrants in 1832 and by the 1850s a multifaceted society had developed (Moodie, 1853). Deteriorating economic conditions in Britain in the 1830s hastened emigration to Canadian colonies and immigration to Upper and Lower Canada reached a peak during the mid-19th century, as many Irish tried to escape the potato famine of the 1840s (Jimenez, 1994; Saunders, 1999).

According to Catherine Parr Traill (1836), immigrants who travelled to Canada in the early 1800s encountered a widely forested region in which success was equated with clearing the land and developing agriculture (Ennals, 1975; Parr Traill, 1836). Early settlers were granted large tracts of land and encouraged to clear small areas in order to incorporate subsistent farming.

2.3 Regional and Historical Background of Belleville

Belleville is located in Hastings County, Ontario on the north shore of Lake Ontario's Bay of Quinte (Figure 2.1). Early in the 18th century a small village was located on the eventual site of Belleville, Ontario. This initial village consisted of the earliest settlers of European origin in the region and was situated on a bluff of land overlooking the east bank of the Moira River. The strategic location provided easy access to many waterways: The Bay of Quinte, Lake Ontario, and the Trent and Moira River systems

(Herring et al., 1991). Because of Belleville's location near the waterways, a steamship service operated between Kingston, Prescott, and Belleville, accelerating trade that contributed to the eventual prosperity of the town. Foreign trade played an important role in the development of newly settled villages like Belleville. Imported goods provided Canadians access to a wide variety of foodstuffs and manufactured articles that could not otherwise be produced in Canada (Lewis and Urquhart, 1999).

Figure 2.1 Map of Southern Ontario illustrating the location of Belleville, Ontario (source: Albert College, http://www.albertcollege.ca/).

In 1816 the town of Belleville was first established and settled by pioneers who occupied less than 200 acres of land (Figure 2.2). These early settlers were United Empire Loyalists who maintained allegiance to the British crown and had fled the former colonies of the United States. Further waves of settlement occurred after the War of 1812 and into

the midcentury. The vast majority of these early settlers were British subjects from England and Scotland and a large number of Irish immigrants settled in Belleville in the early to mid-19th century (Boyce, 1967). According to the 1851 census for Belleville, of the 2,201 individuals who were not native to Ontario, 290 persons were emigrants from the United States and nine were from various Canadian provinces (Canada Census 1851-2). A small number of other immigrants to Belleville included those from Prussia, Holland, Germany, and the East Indies (Jimenez, 1991).

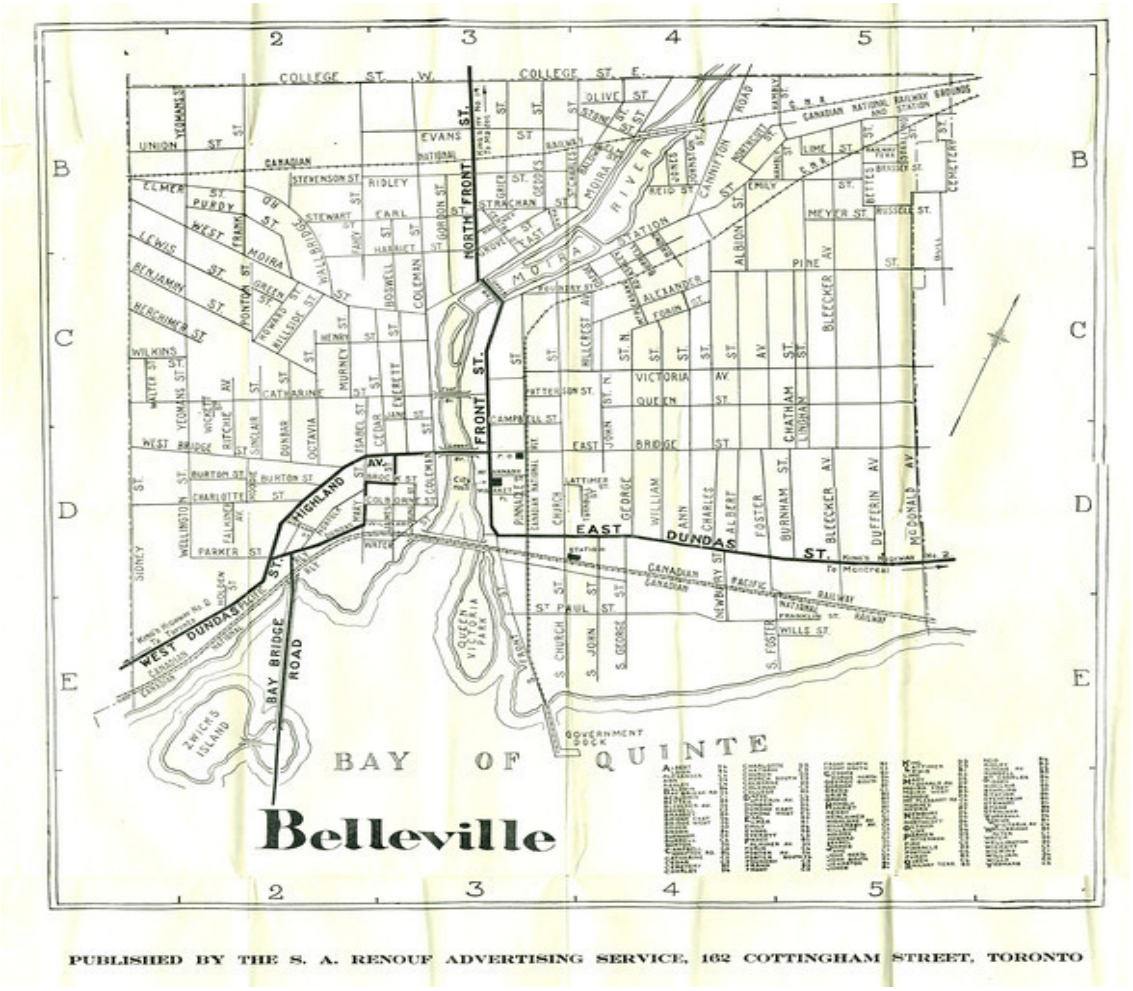

Figure 2.2 Map of Historic Belleville (Lantz, 2012 http://paullantz.smugmug.com/History/Map-of-Belleville-Ontario).

At the time of the founding of Belleville in 1816 the population consisted of only 100 people, but grew to approximately 700 by the end of the 1820s (Boyce, 1967). Belleville inhabitants constructed a port that became one of Upper Canada's busiest ports and local merchants exported barrels of flour, wheat, potash, and timber. By the 1840s, Belleville had become a major center for lumber manufacturing and iron foundries were built to supply machinery for the various manufacturing industries located in the town (Jimenez, 1991). In 1856, the Grand Trunk Railway began to provide service between Montreal and Toronto, passing through Belleville (Mika and Mika, 1986). The arrival of the railway stimulated local business and the locomotive industry employed many of Belleville's inhabitants. Aside from industry related to the railway, Belleville was also an important sawmilling centre throughout the 19th century, primarily because of its location on the Moira River. As Belleville's businesses grew in number and variety, the population expanded rapidly to 4,569 by 1851 and by 1874 Belleville had grown from a small village to a dynamic town consisting of 7,500 people (Boyce, 1967; Herring et al., 1991). There were two women who lived in Belleville around the time the cemetery was in use who wrote books about their lives (Moodie, 1852; Parr Traill, 1836). Their books contain valuable information about food, cooking, health, and illness in Belleville during the 19th century that was useful for this research.

St. Thomas' Anglican Church was founded in 1818 and the cemetery was the first public burial ground in Belleville. The cemetery contained individuals from the local population, most of whom were of British descent, although there were some people from other European countries (Herring et al., 1991). Methodists, Presbyterians, and

individuals of other denominations were initially buried in St. Thomas' cemetery until several other cemeteries opened. In 1874 St. Thomas' cemetery ceased to be used largely due to health reasons. The town council passed a bylaw prohibiting further burials within the town boundaries; therefore a new municipal cemetery was established where further burials were located.

2.4 The St. Thomas' Anglican Church Project

The St. Thomas' Anglican Church Project developed out of the decision by church administrators to build a new parish hall on the site of the abandoned 19th century burial ground. The area slated for construction was thought to contain 80 graves because all but 15 gravestones had been destroyed or removed due to fires in 1876 and 1975 (Herring et al., 1991). In 1989, Dr. Heather McKillop of Northeastern Archaeological was contracted to remove and relocate the graves in preparation for the new parish hall. The parish agreed to allow study of the human remains for a period of one year and this was overseen by Dr. Shelley Saunders of McMaster University.

The cemetery was located in an ideal region for the preservation of human tissue, particularly hair. Resting on a raised sandy knoll approximately one kilometer from the shoreline of the Bay of Quinte, St. Thomas' cemetery occupied one of the highest elevations of land in Belleville (Herring et al., 1991). The sandy soil consisted of coarse texture soils with a low moisture content that frequently promotes desiccation (Fiedler and Graw, 2003; Santarsiero et al., 2000). Sandy soil can inhibit decomposition and result in the natural preservation of a body for thousands of years (Micozzi, 1991). This is due

to the large pore content of the coarse textured soils that allow gases and moisture to move rapidly through the soil matrix. Sandy or coarse textured soils with low moisture contents also promote desiccation because hydrolytic enzymes associated with the cycling of carbon and nutrients are retarded (Skujins and McLaren, 1967). Generally, the environmental conditions of St. Thomas' cemetery aided in preserving human bone, hair, and teeth as well as coffin handles, nails, and hinges. Clothing, shoes, and other personal effects such as dentures, glasses, and buttons were also recovered from the graves (Herring et al., 1991).

In total, 579 graves were excavated from the portion of the cemetery that was in use from 1821 to 1874. There was no cemetery plan indicating who was buried in each plot. Some name plates on the coffins were preserved so there were 80 individuals who were positively identified, including their age-at-death, sex, and family affiliation (Herring et al., 1991; Katzenberg et al., 2000). Parish records indicated that there were 1,564 individuals interred in the cemetery and the recovered samples made up 37% of the total recorded interments (Herring et al., 1991; Katzenberg et al., 2000 Saunders et al., 1995).

Research on the Belleville collection generated a number of research projects on the skeletal biology of this historic sample. Katzenberg et al. (2000) studied diet by combining bone chemistry with the isotope values of food (discussed in more detail in section 2.7.1 below). Dr. Shelley Saunders and colleagues (1995) estimated age-at-death for individuals in the Belleville sample and compared the documentary records with the age estimates derived from the skeletal remains. Saunders and colleagues' (1995) study

focused on children's health and growth and development patterns for subadults. Combining the burial registers with the skeletal analysis, their study indicated that children were exposed to serious acute infections, such as cholera, smallpox, measles, meningitis, scarlet fever, typhoid fever, and whooping cough (Saunders et al., 1995). Rogers' (1991) study used 17 pelvic and 17 cranial features to estimate adult skeletal sex and age-at-death in the Belleville sample. Rogers (1991) determined that the Suchey system was more accurate than the auricular surface method for estimating skeletal age-at-death, while pelvic and cranial morphology produced sex ratios indistinguishable from the parish documentation (Rogers, 1991). An analysis of pathological changes was completed by Jimenez (1991), in which she examined the collection for the presence of specific and non-specific infections in the Belleville skeletal remains, as well as indicators of trauma. This study found that traumatic injuries were common in the Belleville sample and non-specific infections (i.e., periostitis) were also found (Jimenez, 1991). Infant mortality patterns were examined by Herring and colleagues (1991). Analysis of data from burial records and from the large skeletal sample (n=576) indicated that males and females were equally likely to die during infancy and that environmental factors played a role in Belleville's mortuary profile (Herring et al., 1991). Herring and colleagues' (1991) study found that the parish records revealed elevated risks of infant death in the summer months, probably due to diarrhea owing to the unsanitary conditions and the presence of acute infectious diseases in Belleville. A later study by Saunders and colleagues (1997) examined the presence of dental caries and antemortem tooth loss in the Belleville sample. They found that the occurrence of caries and antemortem tooth loss

was higher in females than males and suggested that the caries and antemortem tooth loss in the Belleville sample was similar to a pre-1850 British group, but higher than American samples (Saunders et al., 1997). All of the skeletal remains were reburied in 1990 at St. Thomas' after the project was completed (Herring et al., 1991).

2.5 Parish Records for St. Thomas Anglican Church Cemetery

In 1991, Dr. Ann Herring (McMaster University) supervised the transcription of the St. Thomas' parish registers for 1821 to 1874 from microfilm copies held at the archives of the Anglican Church of Canada in Toronto, Ontario. Separate databases were created for burials, baptisms, and marriages. The personal information contained in each burial record was very complete for the 53-year period studied; however cause of death was not recorded for over 90% of the burials (Herring et al., 1991).

The age-at-death registers were also fairly complete with all but 32 burials (2%) containing enough information to allow for the classification of the skeletal material into adult (15 years or older) and subadult (under 15) age categories (Herring et al., 1991). The exact ages-at-death were recorded for 1,434 burials (92%) and sex was assigned for all but six individuals in the burial register (Herring et al., 1991; Saunders et al., 1995a).

The transcription of the St. Thomas' Anglican Church parish registers helped to create a data set that synthesized information from archaeological, documentary, and skeletal sources (Herring et al., 1991). This enabled a comprehensive analysis of the St. Thomas' cemetery skeletal collection to reveal aspects of pioneer life during the formation of the town of Belleville.

2.6 Health Status in 19th Century Upper Canada

While Upper Canada was considered a healthy country to settle, diseases such as ague and rheumatism were reportedly more common in 19th century Canada than in Britain (Parr Traill, 1836). Ague was the disease most feared by new settlers, especially for those who inhabited forested lands (Parr Traill, 1836). According to Saunders (1991) ague was believed to be a form of malaria carried by mosquitos in the warmer summer months. However, Parr Traill (1836) thought that poor diet was a contributing factor to the development of ague because those individuals who could afford to eat well seldom suffered from it. Ague was characterized by a cycle of high fevers that occurred seasonally, particularly in the autumn and after a wet summer. It was often a chronic condition for people who had recently settled in frontier lands (Valencius, 2002). To date, we do not actually know the etiology of this disease, as ague was believed by people in the 19th century to have been arisen from toxic miasmas.

Medical doctors started to open practices in Canada around 1855 (Parr Traill, 1836). However, most settlers could not afford to pay the fees charged by the physicians and many people bartered for medical care by supplying property or farm animals to the attending physician (Jimenez, 1994). It was customary for people to take care of themselves or rely on neighbours for assistance. Only in extreme cases, such as accidents resulting in life-threatening fractures or near drowning, was a medical practitioner summoned (Blackbourn, 2005; Herrington, 1915). According to Herrington (1915) the physician was rarely called upon and then only when amputations were necessary. Many deaths occurred as a result of infection before antiseptic treatment came into general use

and no anaesthetic was used during surgery. It is estimated that two out of every three surgical patients died (Jimenez, 1994).

It is clear that early Canadian settlers were not spared from infectious diseases. In 1832, an estimated 20,000 lives were lost in Upper and Lower Canada from a cholera epidemic (Roland, 1983; Sevigny, 2004). In an attempt to contain the disease, the Canada Board of Health created quarantine stations for the inhabitants of Belleville and other towns in Ontario. Quarantine measures were enforced by the military to prevent the spread of the disease through Upper and Lower Canada (Sevigny, 2004). In 1847, the next wave of infectious disease, typhus, killed 6,000 of the estimated 100,000 Irish settlers fleeing the potato famine in their home country (Duffin, 1999). When typhus reached Belleville in 1847, hospital administrators turned patients away fearing contagion. As a result, a building was erected on a property in town that served as a second hospital for those with contagious illnesses (Jimenez, 1991). Again, quarantines were instituted; unfortunately this may have actually fuelled the spread of typhus since people in quarantine were more likely to contract the disease.

Women's health in 19th century Upper Canada was exceedingly poor. According to Moodie (1853:40) Canadian women looked "sickly and sallow after a few years and the loss of their teeth was a great drawback to their personal charms". The women of the frontier weathered quickly and "hardly a woman could be found that had not already lost her teeth by the age of twenty" (Gullett, 1971:19). In the 19th century, the Canadian medical community believed that women were susceptible to ill health, simply because they were women (Mitchinson, 1984). Corroborating this was the number of females (28

out of 104) in the Belleville skeletal sample that exhibited healed traumatic injuries and antemortem tooth loss (Jimenez, 1991). This could have contributed to the image of the pioneer female as frail and in failing health.

During the 19th century maternal deaths in childbirth were frequently the result of puerperal fever (Watson, 1939). From the parish records, Jimenez (1991) noted several cases of deaths by puerperal fever within the St. Thomas' skeletal sample (n=14). The fever usually originates from a streptococcal infection and was common before the age of antiseptic use (Miller and Keane, 1987). The hazards of giving birth during the preanesthetic and preantiseptic days could be severe and sometimes fatal, a situation exacerbated by the absence of trained medical assistance during childbirth (Roland, 1983).

2.6.1 Causes of Death in Belleville

There were 157 (out of 1,564) causes of death listed in the St. Thomas' registers for Belleville; however some of those listed were not very detailed. For example, one cause of death was recorded as 'found dead in bed,' or deaths caused by complications from childbirth were not stated as the cause in church records (Saunders et al., 1994). This combined with the fact that some infectious diseases are not observable on the skeletal remains makes it impossible to infer cause of death. It is known that during this time period Belleville inhabitants suffered from infectious diseases such as cholera, typhus fever, scarlet fever and similar acute epidemic disease (Jimenez, 1991). Cholera,

whooping cough, measles tended to cause death in children and accounted for a large proportion of infant mortality (Siegel, 1984).

According to parish records, it is clear that the causes of death for individuals buried within St. Thomas' cemetery were diverse. Although a one-to-one correspondence of cause of death to specific individuals is impossible because of a lack of personal identification for individuals, Jimenez (1991) was able to compile a general list of causes of death from the parish registers (Table 2.1). As can be seen the causes of death can be classified under three main categories: (1) accidents, (2) diseases, and (3) medical conditions. It is interesting to note that accidents appear to account for the largest number of deaths within the Belleville sample followed by Asiatic cholera, Emigrants disease, and childbirth. Scarlett fever was a common fatal disease for infants in the 19th century (Roland, 1983), and 8 of 157 individuals listed with causes of death died of scarlett fever, all of them under four years of age (Jimenez, 1991).

Table 2.1: Causes of Death for Adults from Parish Records, St. Thomas' (adapted from Jimenez, 1991:123)

Cause of Death	**Number**	**Notes**
Accidents:		
Drowning	35	
Falls	7	
Gunshot wounds	5	
Lumbering accidents	2	In 2 cases drowning was a factor
Industrial accidents	5	
Exposure	3	
Lightning	2	
Kicked by a horse	2	
Vehicle accidents	2	

Cause of Death	**Number**	**Notes**
Railroad accidents	2	
Alcohol	2	One was a minor
Knife wound	1	
Bayonet wound	1	
Poison	1	
Burns	1	
Choked on food	1	
A blow	1	
Runaway horse	1	
Falling Tree	1	
Sunstroke	1	
Mining accident	1	
Frozen	1	
'Accidents'	1	
Diseases		
Asiatic cholera	14	
Emigrants disease	12	Unspecified disease contracted by emigrants from the British Isles in 1847
Scarlett fever	8	
Consumption	5	
Small pox	3	
Typhoid fever	1	
Fever	1	
Ague	1	
Whooping cough	1	
Inflammatory croup	1	
Medical Conditions		
Childbirth	14	Maternal death after childbirth
Apoplexy	9	
Congestion of brain	1	
Epilepsy	1	
Paralysis	1	
Heart disease	1	
Prouperal mania (?)	1	
Unknown	16	

2.6.2 Infectious Diseases

By the 1830s the population of Belleville had increased from 100 to 1,200 (Mika and Mika, 1986) and early sanitary conditions were less than adequate. Mika and Mika (1986) describe the sanitary conditions in Belleville during this time period and it can be inferred that Belleville was an ideal environment for the spread of infectious disease:

> Garbage lay scattered for days in the streets and pedestrians making their way past the rubbish not only were apt to encounter a nauseating stench but more often than not, a pack of pigs scrounging through the refuse (Mika and Mika, 1986:17).

The parish records indicate that infectious diseases were present in Belleville during the time period the St. Thomas' cemetery was in use. Tuberculosis is a specific infection that was discovered on the skeletal material from Belleville and during the 19th century it played a key role in the mortality rates of many North American populations (Jimenez, 1991; Roland, 1983). As many as 25% of all deaths in cities in Europe and North America can be attributed to tuberculosis and it was not until the later part of the 19th century that the germ theory of disease transmission was recognized (Daniel, 1981). Consequently, individuals suffering from tuberculosis were not isolated in sanitariums until the early part of the twentieth century (Dupos and Dubos, 1987; Jimenez, 1991). It was not until the introduction of streptomycin in 1948 that a cure for tuberculosis was found; therefore the risk of transmission to others was not greatly reduced by isolating individuals afflicted with tuberculosis. According to Steinbock (1976) only 5-7% of tuberculosis cases are expressed on the skeleton; however this rate will vary with population incidence of the disease and may be affected by epidemic waves. Evidence of tuberculosis was expected to be present in skeletal material from the 19th century Upper

Canada; however because of the low incidence of tuberculosis lesions affecting bone, the skeletal sample may not accurately reflect the true incidence of tuberculosis in the Belleville sample (Jimenez, 1991). Within the Belleville sample, over the 54 year period that the cemetery was in use (1820-1874), a total of 12 out of 251 adult individuals (males and females combined) exhibited skeletal evidence of tuberculosis (Jimenez, 1991).

The effects of smallpox on Belleville are not entirely clear from the historical records. St. Thomas' Anglican Church parish records stated that there were three cases of smallpox listed as causes of death in the Belleville sample (Jimenez, 1991; Saunders et al., 1995). Smallpox is a viral infection that was re-introduced into Canada along with typhus and cholera during the middle of the 19th century (Jimenez, 1991). Similar to tuberculosis, the amount of skeletal involvement in smallpox is minimal and ranges from 2% to 5% (Ortner and Putschar, 1985). Smallpox lesions are not observable in adults who have survived the disease and it presents as osteomyelitic infections in children, which are not always distinguishable from other infections on the skeleton (Ortner and Putschar, 1985). Non-specific infections such as osteomyelitis and periostitis may occur as the result of trauma, soft tissue infection, or as a complication of degenerative arthritis (Steinbock, 1976). These types of pathological changes to bone may be induced either accidentally or intentionally (Jimenez, 1991). Because smallpox is not usually observable in the adult skeleton it is difficult to confirm if the individuals listed with the disease actually contracted smallpox. The small number of subadults within the Belleville sample who exhibited mild periostitis (n=7), exhibited no osteological evidence of osteomyelitis that is associated with smallpox (Jimenez, 1991).

According to the parish records there were 12 cases of Emigrant's disease found in the Belleville sample. Emigrant's disease was used to describe the cause of death for individuals who had arrived in Belleville by ship from the British Isles (Boyce, 1967; Jimenez, 1991). These individuals died two days to three months after their arrival and parish records suggest that they died from a form of disease that they contracted on the voyage to Belleville (Boyce, 1967; Jimenez, 1991). Emigrant's disease is also known as 'ship fever' and this was a form of typhus fever which killed approximately 20,000 British immigrants on their voyage to Quebec (Boyce, 1967; Jimenez, 1991; MacDougall, 1983).

Asiatic cholera and cholera are synonymous and both are recorded as causes of death for some individuals in the Belleville sample. During the summer of 1832, a cholera epidemic swept through areas of Upper Canada, probably brought by immigrants arriving by ship from the United Kingdom (Boyce, 1967). There was a chronic lack of fresh fruit and vegetables aboard the ships and water was often contaminated. Conditions during these voyages were unsanitary due to overcrowding and rats were also a problem and likely carried disease (Guillet, 1963). Relatively few deaths within the Belleville sample can be directly attributed to cholera (14 out of 157) however, considering the effects of the disease can not be observed on the skeletal remains, it is important to consider the potential devastating consequences cholera may have had within the Belleville sample. Of particular concern was infant mortality from cholera (Herring et al., 1991). The Moira River that supplied water to the town was polluted from several sources, including manufacturing, and concern for the death of infants escalated (Jimenez, 1991). In 1869,

alarms were raised over Belleville's access to fresh drinking water and by 1873 the town administrator's implemented sanitary regulations to avoid further contamination of the drinking water (Boyce, 1967; Jimenez, 1991).

2.6.3 Traumatic Injuries

Traumatic injuries within the Belleville sample included healed and unhealed antemortem fractures as well as several cases of dislocation, spondylolysis, and one case of iatrogenic (caused by medical intervention) trauma (Jimenez, 1991). Twenty-eight of 104 females and 69 out of 146 males demonstrated evidence of either healed or unhealed fractures (Jimenez, 1991). Healed fractures were common among the Belleville skeletal sample and may represent occupational stresses associated with the rigors of pioneer life. Accident-induced trauma can be the result of occupational hazards and are a response to pressures imposed during certain activities (Jimenez, 1991; Kennedy, 1989). Alternatively, traumatic injuries such as the humeral fracture found on individual B339 may be the direct result of violent acts or accidents that occurred prior to or at the time of death. Several causes of death were recorded for individuals within the Belleville skeletal sample as a result of railroad construction, lumbering, mining, and industrial accidents (n=8) (Jimenez, 1991). Various other types of accidents such as falls, blows, or gunshot wounds resulting in serious injury or loss of life were frequent in Belleville. When the accidental deaths from the Belleville sample are combined into one category, these accidents total 79 out of 170, or 47% of all reported causes of death.

2.7 Food in Belleville during the 19th Century

Kenyon and Kenyon (1992) describe the diet of 19th century Ireland and Scotland as 'moist' because the major components were based on milk and butter, producing various porridges and pottages of cereal or meat. In England, 'moist' foods had been replaced by tea, sugar cane, and baker's bread by the late 18th century (Kenyon and Kenyon, 1992). Working people disliked liquid foods and the English distaste for liquid foods was transferred to Upper Canada whose diet had a 'dry' yet greasy consistency (Kenyon and Kenyon, 1992). Food such as soups and porridge were not popular among British immigrants. The methods of preparing food in Canada varied from preparation methods typically used in Britain **(**Boyce, 1967; Guillet, 1963). Guillet (1963) states that all Canadian cooking was done in the frying pan, however settlers were reported to take advantage of the many new food sources found in North America, particularly maple sugar, maize, pumpkins, and wild fruits (Boyce, 1967; Saunders et al., 1997).

Upper Canada had a wide variety of meat available to settlers including pork, venison, beef, mutton, chicken, goose, turkey, hare, and duck (Boyce, 1967; Kenyon and Kenyon, 1992; Parr Traill 1836). Pigs were raised in the backwoods and did not need extensive pastures or extra care required to raise beef cattle or sheep, therefore fried pork was considered Canada's national dish by many settlers (Kenyon and Kenyon, 1992). When cattle were introduced into Bellville around 1845, settlers began producing and consuming dairy products such as cheese, milk, and butter, which comprised an important part of their diet (Boyce, 1967). Situated in the midst of a dairy-producing region, Belleville became renowned for producing world-famous cheddar cheese (Boyce, 1967).

Although Belleville was located on the Moira River where abundant fresh water fish were available, the settlers of Upper Canada did not consume fish as part of their regular diet (Boyce, 1967; Herrington, 1915). Backwoods people in Upper Canada were able to procure venison that was brought into towns by Native hunters in exchange for salt-pork, flour, or vegetables (Parr Traill, 1936).

Wheat was an important cash crop in Upper Canada and a major agricultural export item (Kenyon and Kenyon, 1992; Parr Traill, 1936). Wheat flour was used to make yeast-leavened loaves of bread, griddle cakes, puddings, cakes, and pies (Parr Traill, 1936). Bread was an important component in 19th century Canadian diet and a wide variety of flours were available in Belleville's stores including wheat flour, cracked wheat, corn, oatmeal, bran, and oats (Boyce, 1967). Domesticated animals were also fed wheat, barley, oats, potatoes, and corn (Parr Traill, 1936; Saunders et al., 1997). A portion of the grain produced in Upper Canada was used to distill hard liquor, which was plentiful and inexpensive (Herrington, 1915). It was common practice for farmers to set aside a small amount of their inferior quality grain to be distilled into spirits. A distillery built by Henry Corby just north of Belleville quickly developed a reputation as a superb whiskey production center (Danyleyko, 2010).

Parr Traill (1836) states that although potatoes were the staple vegetable used by 19th century settlers, a wide variety of fruits and vegetables were grown including green beans, melons, cucumbers, corn, beets, squash, broccoli, apples, and raspberries. Apple orchards were common and apples were used to make cider, puddings, pies, and tarts.

Apples were dried and used year round in the form of jellies, syrups, and butters (Guillet, 1963; Parr Traill, 1836).

Sugar made up a large proportion of the diet of 19th century settlers. Maple sugar supplied most of the sugar component of their diet in the first half of the 19th century and written records show that sugar cane was imported into Canada from the West Indies (Saunders et al., 1997). As prices dropped, sugar cane use became more prevalent in the latter half of the 19th century. The heavy reliance on maple sugar consumption has been estimated by Saunders and colleagues (1997) to be 93 pounds per capita from 1850 to 1880. The consumption of foods prepared with refined flour, which is softer and stickier than unrefined flour, and the increased sugar intake is implicated in the rise in the prevalence of caries found in the teeth of the Belleville sample by Saunders and colleagues (1997).

2.7.1 Stable Isotopes Analysis of Diet in the Belleville Sample

Accounts from early settlers indicate that Belleville residents ate a variety of foods, including various native and domestic animals and plants (Katzenberg et al., 2000). Belleville diets were dominated by C_3 foods as was demonstrated by Katzenberg and colleagues' (2000) study. C_3 sources typically consist of temperate grasses (e.g., wheat), trees, shrubs, nuts, fruits, and vegetables. Katzenberg et al.'s (2000) study investigated the diet of 19th century Belleville by combining historical sources with the stable isotopes of carbon and nitrogen for 439 individuals. Foods were prepared following recipes in historical documents, using traditional cookware. Stable isotopes of carbon and nitrogen

were analyzed in preserved bone collagen, hair, and the prepared food. The raw ingredients from baked goods and stews were also analyzed isotopically (Katzenberg et al., 2000). Overall, the range of variation in the values from bone collagen and hair supported the historical sources on the composition of the 19th century diet (i.e., largely based on C_3 plants) and Katzenberg and colleagues (2000) concluded that while there was abundant food, the diet of Belleville inhabitants was rather monotonous.

Katzenberg and colleagues (2000) also analyzed $\delta^{15}N$ and $\delta^{13}C$ in 24 hair and bone samples from the Belleville sample to compare diet between the sexes and among all age classes; seven individuals analyzed for $\delta^{15}N$ in both hair and bone were children under the age of five (Katzenberg et al., 2000). The authors concluded that there was no evidence that males and females were eating different foods and the only evidence for age differences (higher $\delta^{15}N$ values in infants) was derived from the trophic level shift during breastfeeding (Katzenberg et al., 2000). In contrast to Katzenberg and colleagues (2000) study, this research analyzed nine adults and one subadult (~7 years) to examine how human $\delta^{15}N$ values are affected by nutritional or physiological stress.

2.8 Summary

This chapter discusses the historical evidence on the origins of the town of Belleville and its development during the use of the St. Thomas' Anglican Church cemetery (1821-1874) to provide historical context for the isotopic analysis of hair. The St. Thomas' Anglican Church Project and the research conducted by various authors has assessed the level of infectious disease, traumatic injury, and causes of death in the

Belleville sample to permit a more comprehensive picture of life and death in Belleville. The historical evidence suggests that individuals from Belleville were at high risk for infectious disease, and faced many hazards such as accidental deaths. Katzenberg and colleagues (2000) study was also discussed in detail due to its importance in formulating a specific picture of the diet consumed by Belleville inhabitants which consisted of bread (whole wheat and corn bread), meat (beef, mutton, and pork), vegetables (potatoes, turnips, and carrots), and sugar cane. The foundation for this study is that the combination of various sources of information including stable isotope analysis of hair keratin, skeletal pathology, and historical records provides insights into diet, and health in historic Belleville.

Chapter 3

The Biology of Hair

3.1 Introduction

Hair is important in stable isotope studies because unlike bone, hair is not remodeled after formation; therefore its isotopic composition reflects diet, and possibly physiological stress, at the time of tissue formation. Various studies demonstrate that, like bone, the carbon and nitrogen isotopic composition of hair reflects an individual's diet during life (e.g., Macko et al., 1999b; Nakamura et al., 1982; O'Connell and Hedges, 1999; Schwarcz and White, 2004). Hair grows between 0.35 to 0.44 mm per day and based on the growth rate of 0.35mm/day, one centimeter of hair corresponds to approximately one month of diet (Saitoh et al., 1967; Williams et al., 2011). Because hair grows incrementally, and is an inert tissue once formed, its isotopic composition provides a short term record of diet for the weeks/months/years (depending on the length of hair) preceding death. Although inferences about physiological stress based on isotopic evidence are necessarily general, human hair can provide an important supplement to, or confirmation of skeletal, historic, and artifactual evidence.

This chapter provides an overview of the basic components of hair with a focus on hair structure and growth. A discussion of the factors that affect the growth cycle of hair illustrates how intrinsic, hormonal, and physiological changes can influence the duration of each phase. The final section of this chapter discusses the preservation of hair in archaeological contexts and a brief review of early anthropological studies of hair.

3.2 Structure of Hair

Human hair growth starts during the third month of fetal life. Hair fibres are created through a cyclic pattern of cell proliferation and differentiation that continues throughout life and is genetically determined (Valkovic, 1988). Each hair fiber consists of a strong exterior layer or cuticle, a cortex, and an inner layer called the medulla. The medulla is only present in large thick hairs and the cortex provides strength, texture, and colour of hair. The cuticle is thin and colourless and serves as protection for the cortex (Figure 3.1).

Hair is composed of the protein keratin which is a component of nail and horn (Meier-Augenstein, 2010). Keratins are proteins, long chains of amino acids that form a cytoskeleton (miniature skeleton within a cell) (Baden, 1990; Valkovic, 1988). Keratin filaments that run within a cell form the inside of the outer membrane to weave a basket around the nucleus of the cell (Baden, 1990). There are two types of keratin proteins called 'soft' keratins or 'hard' keratins. Soft keratins are found in the skin and break down relatively easily, whereas hard keratins found in hair are very resistant to degradation. Hard keratins do not dissolve in water and are highly resistant to proteolytic enzymes (Baden, 1990). Unlike bone, the surface of the hair or cuticle is made up of flat overlapping scales that protect the hair from diagenesis in a burial context. The fibrous protein structure of hair makes it resistant to degradation; therefore it can preserve isotopic information over thousands of years (Benfer et al., 1978; Lubec et al., 1987).

Below the surface of the skin is the hair root, which is enclosed within a hair follicle. At the base of the hair follicle is the dermal papilla (Figure 3.1). The dermal

papilla is fed by the bloodstream that carries nourishment to produce new hair and is important to hair growth because it contains the receptors for male hormones and androgens that regulate hair growth (Valkovic, 1988). Cells associated with the development of hair fibers are among the fastest dividing cells in the human body (Valkovic, 1988; Williams et al., 2011). Rapidly dividing cells in the hair matrix produce a variety of differentiated cells types every 24-72 hours (Van Scott et al., 1963). This high rapidity of differentiation and high-metabolic activity explains why hair is sensitive to physiological and dietary shifts (Williams et al., 2011).

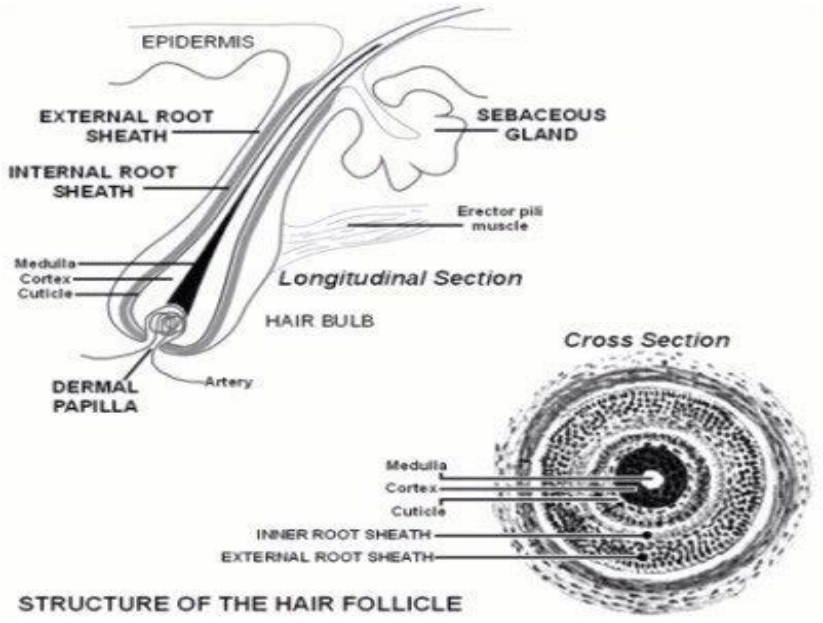

Figure 3.1 Diagram of a hair follicle (www. follicle.com).

As mentioned earlier, the primary component of a hair fibre is keratin. Keratin occurs in two forms in the hair, Hair Fibrous Proteins (HFP) and Hair Matrix Proteins (HMP)

(Bonnichsen, et al, 2001). On average, hair is composed of 50.65% carbon, 20.85% oxygen, 17.14% nitrogen, 6.36% hydrogen and 5.0% sulphur (Baden, 1990; Valkovic, 1988). Hair also contains trace amounts of magnesium, arsenic, iron, chromium, and other metals and minerals (Baden, 1990).

The cuticle of the hair fiber is made up of polymerized protein in a homogenous high-sulfur protein matrix (Baden, 1990). The matrix consists primarily of the amino acid cysteine. While hair is growing, the matrix cells at the dermal papilla of the follicle have a high rate of metabolic activity, which causes them to absorb elements introduced from the diet (Valkovic, 1988).

3.3 Hair Growth Phases

Hair follicles grow in repeated cycles that consist of three phases; i) anagen, the growth phase, ii) catagen, the transitional phase, when hair has stopped growing, and iii) telogen, the resting phase. Saitoh and coworkers (1970) were the first to demonstrate that hair follicles undergo this repetitive sequence of growth. Approximately 80% of all hairs are in the growing phase at any one time (Valkovic, 1988; Williams et al., 2011). The anagen phase can vary from two to seven years and the average hair grows 10 to 12 cm per year (Stenn and Paus, 1999; Valkovic, 1988).

Research on the pattern of hair growth compared summer and winter hair cycles and it was apparent that summer hair growth was not longer than winter hair growth, regardless of the age of the subject or body region (Saitoh et al, 1970). The authors concluded that the difference in growth patterns between summer and winter months is

that telogen hairs (resting) stay embedded in the hair follicle longer during the summer months (Saitoh et al, 1970). This contributes to the perception that summer hair is longer or grows more rapidly than winter hair. The overall result is that summer hair is approximately the same length as winter hair (Saitoh et al., 1970). Stenn and Paus (1999) suggested that the growth phase signal is under the control of a unique genetic biological clock, which is probably based on different molecular controls other than circadian rhythms. To date, researchers have not unravelled the molecular controls for hair follicle cycling or the mechanisms that stimulate the hair follicle remodelling process (Stenn and Paus, 1999).

Anagen

Anagen is the active growth phase of hair follicles where the root of the hair is dividing rapidly, adding to the hair shaft. Scalp hair stays in this active phase of growth for 2–7 years, and the amount of time the hair follicle stays in the anagen phase is genetically determined. At the end of the anagen phase an unknown signal causes the follicle to go into the catagen phase (Parakkal, 1990).

Catagen

The catagen phase is the short transition stage that occurs at the end of the anagen phase. It signals the end of the active growth of a hair and is considered the start of the resting phase. During the catagen phase the hair follicle shrinks to about 1/6 of the normal length and the dermal papilla breaks away to rest further below the surface of the skin. This phase lasts for about two weeks to two months while the hair converts to a club hair (Baden, 1990; Parakkal, 1990). A club hair is formed when the part of the hair follicle in

contact with the lower portion of the hair becomes attached to the hair shaft. This process cuts the hair off from its blood supply and from the cells that produce new hair (Baden, 1990; Parakkal, 1990). When a club hair is completely formed, the hair follicle enters the telogen phase.

Telogen

The telogen phase is the final resting phase of the hair follicle. When the body is subjected to extreme stress, as much as 70% of the hair can prematurely enter a phase of rest, called the telogen phase (Kligman, 1961). Telogen hair begins to fall out, causing a noticeable loss of hair. This condition is called telogen effluvium. The club hair is the final product of a hair follicle in the telogen stage, and is a dead, fully keratinized hair. Fifty to 100 club hairs are shed daily from a normal scalp (Parakkal, 1990). During the telogen phase the hair does not grow but remains attached to the follicle while the dermal papilla stays in a resting phase (Figure 3.2). Approximately 10-20% of all hairs are in this phase at one time (Baden, 1990; Valkovic, 1988).

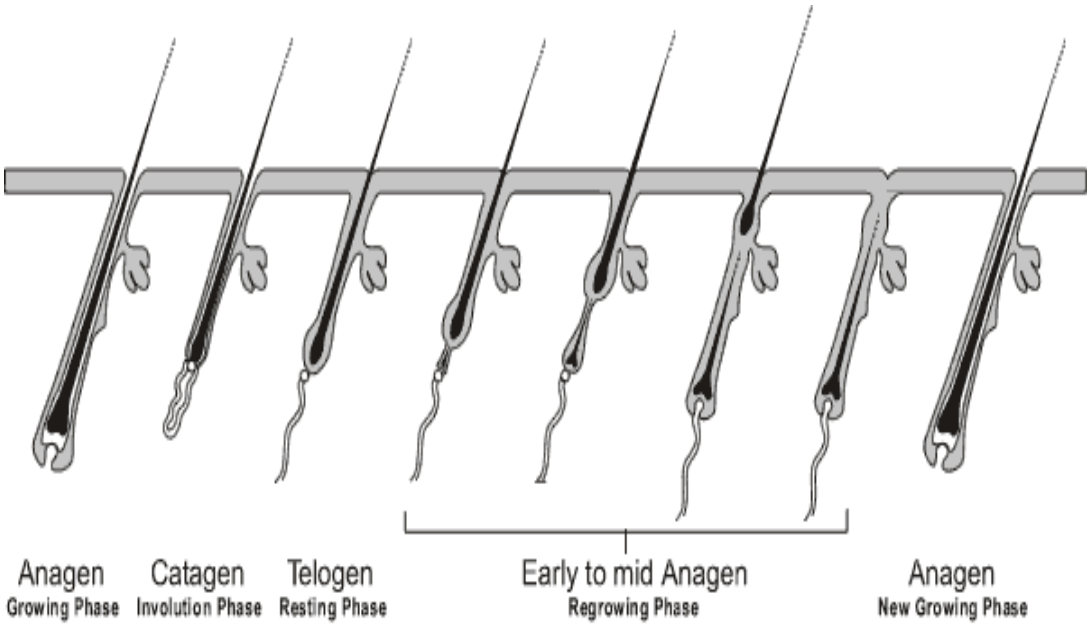

Figure 3.2 Growth phases of the hair cycle (AcuSkin Wellness Center LLC, http://acuskin.com/laser_hair_removal_weight_loss).

Van Scott and coworkers (1963) biopsied the scalp hair of 11 healthy individuals and examined thin sections of the hair roots by microscope to investigate the proportional relationships between the volume of the hair matrix and the size of the papilla (Van Scott et al., 1963). They reported that approximately 80% of hairs were in anagen (growing) and 20% in catagen/telogen (resting) (Van Scott et al., 1963). This is the standard that current researchers use to estimate the percentage of hairs in the different growth phases regardless of the individual's physiological condition (e.g., Hatch, 2006; Jones et al., 1981; Macko et al., 1999b, Nakamura et al., 1982; O'Connell and Hedges, 1999).

3.4 Physiological Factors That Affect Hair Growth Cycle

Data from clinical studies provide information about hair growth cycling among individuals with a compromised health status (e.g., Botchkarev, 2003; Kantor et al., 2003; Kligman, 1961). Botchkarev (2003) demonstrated that systemic stress response can significantly influence hair growth, for example, audio stressors applied to mice caused actively growing hair follicles to transition into the resting phase.

In healthy individuals, the timing of growth phases in hair tends to remain constant, however intrinsic, hormonal, and physiological changes can influence the duration of each phase. Intrinsic factors such as epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1) are important for maintaining follicle development and initiating transition to the catagen/telogen (resting) phase (Ebling, 1990). Specific hair follicle structures, cytokines, receptors, genes, and hormone related-proteins are critical for normal follicle development and cycling (Sato et al., 1999). The connective tissue and dermal papilla also serve as an immunoprotective barrier for the hair follicle and Class 1 histocompatibility complex (Class 1 MHC) expression increases during the catagen/telogen phase and activate surrounding macrophages, which serves to prompt the immune system (Paus and Costarelis, 1999). Deficiency of the follicle can affect immunity and has been associated with various immune related diseases such as alopecia areata, which is the development of patchy hair loss on the scalp, and is thought to be an autoimmune response to hair follicles in the anagen (growing) phase (Paus and Costarelis, 1999; Williams et al., 2011).

Different hormones can also alter the growth cycle of hair. An early study by Ebling (1990) showed that decreasing hormonal concentrations affect hair growth phases as demonstrated by spayed rats that had significantly longer hair in every region of the body than intact rats. Hormones artificially implanted into rats, such as oestradiol and thiouracil decreased the length of the hair by altering the number of hair fibers in the different growth phases, whereas the hormone thyroxine significantly increased hair length (Ebling, 1990). Similar evidence for the effect of oestrogen and thyroxine on the follicle in anagen has been observed in sheep. When thyroxine is administered to sheep, there is an increase in the rate of wool growth, as measured by weight per unit area, mainly through an increase in the rate of fiber elongation that occurs in the anagen (growing) phase (Ebling, 1990).

Hormonal effects on hair growth cycles are not limited to animal studies. Freinkel and Freinkel (1972) studied the hair roots of nine patients with hypothyroidism in which catagen/telogen (resting) hairs were expressed as a percentage of the total number of hairs counted. The researchers found that patients with untreated hypothyroidism (i.e., a lack of thyroid hormone) displayed hair loss, and in all cases, a deficiency of thyroid hormone was associated with an increase in the percentage of telogen hairs (Freinkel and Freinkel, 1972). Because hair growth depends on the proper functioning of the thyroid gland, abnormal levels of thyroid hormone produced by this gland can result in changes to the hair growth cycle (Freinkel and Freinkel, 1972). When there is too much thyroid hormone, hair can become fine or thin and when there is too little hormone, there can be hair loss.

Conversely, high levels of estrogen such as those found during pregnancy have been shown to decrease the percentage of hair in telogen (resting) phase and extend the anagen growth phase (Ebling, 1990; Lynfield, 1960). Lynfield (1960) examined the hair roots of eight women during and after a normal pregnancy, recorded the number of hairs found at various times during and after pregnancy, and found that seven of them showed a significantly higher percentage of anagen hairs during pregnancy than postpartum (Lynfield, 1960). However, three to twelve months postpartum the conversion to catagen/telogen was accelerated resulting in increased hair loss due to estrogen withdrawal around three to four months after delivery (Lynfield, 1960). During the postpartum period, the proportion of hairs in catagen/telogen (resting) can be as high as 60%, whereas in late pregnancy it may be less than 5% (Williams et al., 2011).

The term 'telogen effluvium' was first coined by Kligman (1961) and refers to the loss of club hair (telogen). Excessive shedding of normal club hairs from healthy telogen follicles is brought about by a variety of physiological stresses such as febrile illness, postpartum stress, and systemic disease (Kligman, 1961). For example, hair loss after febrile illness usually begins one to two months after the febrile episode. Kligman (1961) observed four cases of febrile illness in children that were suffering from pertussis, lobar pneumonia, and influenza and noted that hair loss developed suddenly and continued copiously for two to three weeks, ranging between 34% and 53% telogen counts (Kligman, 1961).

Psychological stress can also produce extended periods of telogen effluvium, mainly resulting from the effects of neurotransmitters (Botchkarev, 2003; Kligman,

1961). Kligman (1961) studied a physically healthy prisoner implicated in a murder and noted on two occasions (after trial and after sentencing) his telogen counts were 41% and 54%. His average daily loss during the height of shedding was 1,100 hairs and there were no histological changes except for an increased number of telogen follicles, suggesting that stress was the primary causative factor in the prisoner's hair loss (Kligman, 1961).

Kligman (1961) also conducted telogen counts on hospitalized patients with a variety of chronic illnesses such as terminal carcinoma, ulcerative colitis, leukemia, tuberculosis, who had a range of telogen counts between 26% and 64%. It was clear in Kligman's (1961) study that abnormal telogen counts were correlated with morbidity; the less sick patients had normal telogen counts. One hypothesis for the increased telogen counts during illness is that the conversion of growing hairs to resting hairs constitutes a useful defense against illness by conserving protein (Harrison and Sinclair, 2002). It has also been shown by Stenn and Paus (1999) that the anagen (growing) phase is suppressed by glucocorticoids, which have immunosuppressive properties. Telogen effluvium lasting less than six months is usually accompanied by increased levels of glucocorticoids that are a response to a number of physiological stresses such as febrile illness, infection, chronic illness, or injury (Stenn and Paus, 1999; Williams et al., 2011). These studies demonstrate that there is a consistent relationship between high telogen counts (i.e., above 20%) and the likelihood of clinical illness (including severe psychological stress).

More recently, Hatch and coworkers (2006) found a means of diagnosing anorexia nervosa and bulimia nervosa using nitrogen and carbon isotope ratios in hair. When the body is in an anabolic (or normal) state, it is building and repairing tissue and

incorporates dietary proteins into growing hair (Hatch et al., 2006). Body tissues are enriched in ^{15}N with respect to diet due to the preferential excretion of the ^{14}N in urea, commonly known as the trophic level shift (Hatch et al., 2006; Mekota et al., 2006). As an individual loses weight, entering a catabolic state, the body consumes its own energy and protein stores, the remaining enriched proteins are recycled and some of the amino acids are incorporated into new hair growth, providing a record of the change in diet (Fuller et al., 2005; Hatch et al., 2006; Mekota et al., 2006). Changes in diet related to anorexia and low protein intake can produce fluctuations between growing hair (anagen) and resting hair (telogen), subsequently increasing the number of hair fibers in the telogen phase (Hatch et al., 2006). Both telogen counts and increased $\delta^{15}N$ values can be used effectively as indicators of physiological stress associated with illness, as well as changes in diet.

3.5 Early Studies of Hair in Biological Anthropology

Hair has been of interest to biological anthropologists since the 1920s as a means of exploring aspects of human variation. Yet in most archaeological contexts, with the exception of relatively unique preservation conditions at sites like Belleville, hair is preserved as fragments only, or not at all. Therefore, the problem with hair has been one of preservation and specimen size. Bone has been used more often than hair in stable isotope analysis to infer diet and published work on stable isotope analysis of hair to date has used either modern hair (e.g., Nakamura et al., 1982; Webb et al., 1980) or mummy hair (e.g., Macko et al., 1999a,b; White, 1993; Williams et al., 2011). These studies were

undertaken because there were large quantities of hair specimens available for study. Studies have been conducted on ancient hair (discussed below), despite small sample sizes, but few have attempted sequential isotopic analysis of hair (for notable exceptions see Fuller et al., 2004, 2005; Mekota et al., 2006; Williams and Katzenberg, 2012; Williams et al., 2011).

3.5.1 Studies of Variation in Hair Morphology Related to Age

In the 1920s, Hrdlicka (1922) studied 'Old Americans' to compare skin, hair and eye colour to detect anomalies of pigmentation, and regional distribution. He also looked at the greying of hair and hair loss, finding that men turn grey faster and lose their hair at an earlier age than women, although he found considerable individual variation (Hrdlicka, 1922). Wynkoop (1929) collected information on cuticle scales, medullas, and shaft diameters to determine a correlation with the age of an individual. He found no correlation between age and morphological type of hair, the size of the cuticle scales, or the hair shaft diameter (Wynkoop, 1929). Trotter (1943) looked at the life cycles of human hair in order to better understand irregularities of hair growth and noted that the cycles of individual hair follicles are of relatively constant duration.

3.5.2 Racial Typology and Hair

Early researchers were also interested in hair morphology in association with the demarcation of race and focused on morphological features such as colour, diameter, and texture to determine a correlation between race and the cross-sectional shape of hair

(Trotter, 1943). They were using a typological model that focused on the notion that there were 'pure' unmixed races in the past (O'Neil, 2012).

Egyptian mummy hair specimens were examined by Pruner-Bey (1877) for colour and structure to identify variation between Egyptian and Peruvian hair specimens. Woodbury and Woodbury (1932) and Trotter (1943) examined ancient Peruvian material using metric techniques and found that ancient hair colour and texture generally fell in the range of modern variation. In the 1930's Kneberg (1935) sought to improve cross sectional techniques for determining hair colour, form, and texture, and reported that a hair index was not a reliable racial criterion.

Steggerda and Seibert (1941) also examined hair morphology in relation to 'racial' groups. While six groups were studied, intraracial variation in hair shaft dimensions was found to exceed the interracial differences observed (Steggerda and Seibert, 1941). In the early 1970s, Strouhal (1971) examined hair from pre-dynastic Egyptian skulls and described the hair samples in terms of both texture and colour to explore racial variation. Similarly, Hrdy (1978) analysed pigmentation and hair morphology, including hair shaft diameter and scale count in hair specimens from Sudanese Nubia using electrophoresis and fluorescence microscopy. He concluded, based on the curling variable of hair, that males from the Sudanese Nubian sample showed more African elements (i.e., more curly) than females in the curling variable (Hrdy, 1978). Both researchers identified Negroid or African elements as hair that had a higher curling variable and stated that the cross-sections of the hairs were flattened which resulted in curly hair and they believed they could differentiate hair specimens from Melanesians,

European, African, and Mongoloid groups based on the combination of high ratio of hair curvature and hair shaft diameter (Hrdy, 1978; Strouhal, 1971).

3.6 Preservation of Hair in the Archaeological Record

There are two factors involved in the preservation of hair in archaeological environments; the survival of hair fibres in different post-depositional environments, and the chemical stability of hair over long periods of time. Keratin is very stable and resistant to degradation (Brothwell and Spearman, 1963; O'Connell et al., 1999; Sandford and Kissling, 1994). Moreover, hair is resistant to microbial attack due to its protective fibrous protein structure; however despite the chemical robusticity hair can undergo structural decay (Lubec et al., 1987). Damp climates and alkaline soils inhibit the survival of hair, whereas acidic waterlogged conditions (i.e., bogs) are better, as are dry or freezing conditions (Lubec et al., 1987). Mummies often have extremely well preserved hair, including the shaft and follicles that are preserved as a result of naturally-occurring environmental conditions, such as extreme arid or cold conditions.

Changes that occur in archaeological hair include loss of pigment and weight. Brothwell and Spearman (1963) noted that archaeological hair appears ginger in colour, due to chemical changes in the pigment after death. It also appears to shrink with time due to moisture loss; therefore ancient hair becomes brittle and must be treated with care (Benfer et al., 1978; O'Connell and Hedges, 1999). Brothwell and Spearman (1963) were the first authors to study the preservation of hair specimens from North Africa using a variety of techniques, including microscopic examination, fluorescence microscopy, and

reflectance spectrophotometry and found that the state of preservation of the hair samples closely corresponded to environmental factors of the burial context.

Benfer and coworkers (1978) studied hair samples from burials on the arid Central Coast of Peru, using SEM, atomic absorption and spectrophotometric analysis, with modern hair samples used as a control. The well preserved burials were from the sites of Paracas, Asia, and Chilca, ranging in date from nearly 9,000 BP to 600 BP. The percentage of nitrogen and the estimated percentage of protein were compared across the samples and both were lost over time, as with bone, but at a slower rate (Benfer et al., 1978). Between the modern sample and the 9,000 year old sample, only 2% of nitrogen was lost and 11.5% of the protein (Benfer et al., 1978).

The structural stability of hair over time was demonstrated through the analysis of hair samples from an Egyptian Coptic cemetery dating from the 8th to the 10th century AD, where organic materials were found to be well preserved (Lubec et al., 1987). X-ray diffraction analysis revealed that there was no difference between ancient and comparative modern hair samples, and the only change was dehydration of the archaeological hair samples (Lubec et al., 1987).

The studies by Lubec and coworkers (1987) and Benfer and coworkers (1978) suggest that hair is very stable over time both structurally and chemically. It is important to note that little research on the preservation of hair in archaeological contexts has been completed since the 1980s. Current research explores how hair is degraded by microorganisms in forensic investigations for hair samples that have been either buried or left exposed on the ground surface (e.g., Wilson et al., 2010). Wilson and coworkers

(2010) concluded that hair fiber has increasing importance in forensic cases, since it is now possible to retrieve detailed biomolecular information on recent life history using individual fibres (e.g., on diet, drug use, and DNA).

3.7 Summary

Once formed hair tissue is metabolically inactive; therefore it can be used to provide a temporal archive of dietary and physiological changes at the time of formation. Although the timing of growth phases is relatively constant in healthy individuals many factors can influence the duration of each phase. Hair loss can be caused by excessive physical or emotional stress, like that associated with injury or chronic illness. A review of clinical studies indicates that the timing of the growth cycle can be affected by compromised health status or fluctuating hormones, such as those that occur during pregnancy. This provides a basis for understanding individual metabolic reactions to stressful events and illustrates how physiological stress can change hair growth cycles. Although early biological anthropological studies focused on the physical characteristics of hair largely for racial typological analysis, more recent research on hair has shown that even when hair is exposed to UV light, humidity, insects, and bacterial attack, hair has remained intact for thousands of years. Hair preservation studies are useful to understand the chemical reactions and changes in hair morphology that occur during deposition in archaeological contexts. Sites that most commonly contain hair fibers are arid or cold burials, like those found in Egypt or Peru.

Chapter 4

Stable Isotopes

4.1 Introduction

Stable isotope analysis of human bone and hair provides an objective means of generating dietary profiles for past populations (White, 2000). Not only can stable isotope analysis of hair keratin reveal sources of dietary protein, it can also provide information on the timing of stress events (e.g., fever or infection) prior to the death of an individual. This chapter provides an introduction to stable isotopes analysis and presents an overview of studies that have been conducted using the isotopes of carbon and nitrogen to document past diet. Nitrogen balance of the human body will be discussed in relation to how this affects $\delta^{15}N$ values and how elevated $\delta^{15}N$ values can potentially reflect nutritional or physiological stress. No methodology is without its drawbacks and this chapter will end with a discussion on the limitations of stable isotope analysis and the constraints on the type of dietary information that it can provide.

4.2 What is a Stable Isotope?

All organisms are comprised of common elements such as hydrogen (H), carbon (C), nitrogen (N), and oxygen (O). Many of these elements exist as different isotopes, which share the same chemical properties but vary in their atomic masses (Katzenberg et al., 2000; White, 2000). An isotope is an atom of the same element with an equal number of protons and electrons, but a different number of neutrons. The number of protons and

neutrons determines the atomic weight of an element, therefore adding an extra neutron changes the atomic weight by one (Price and Burton, 2011). Carbon, which normally has six protons and six neutrons, and an atomic weight of approximately 12, has six protons and seven neutrons in its stable form of ^{13}C. These two isotopes are referred to as 'stable' isotopes because, unlike radioactive isotopes (e.g., carbon 14), they do not decay over time. This allows the relationship between stable isotopes in certain organic tissues to remain constant (stable) even after a plant or animal dies. Alternatively, ^{14}C in a skeleton decays to ^{14}N over time, which allows for radiocarbon dating, but the amount of ^{12}C and ^{13}C in a skeleton remain constant through time, which has important implications for dietary studies (Meier-Augenstein, 2010; Price and Burton, 2011). The charge of a ^{13}C atom remains the same as the charge of the ^{12}C atom and as a result, the two atoms behave the same way in chemical reactions with the exception of a fractionation effect due to weight (Pollard and Heron, 2008). In chemical reactions the heavier isotopes react at a slower rate, which results in mass-dependent differences in organic tissues (Hall, 1967; Keegan, 1989). It is these mass-dependent differences in organic tissues that are used to distinguish dietary relationships. For example, when plants convert CO_2 into glucose, the ratio of ^{12}C to ^{13}C in the plant tissue is different from the ratio found in atmospheric CO_2 (Katzenberg et al., 2000). This is because plants contain less ^{13}C than the atmospheric CO_2 on which they rely for photosynthesis. They are therefore 'depleted' in ^{13}C relative to the atmosphere and this depletion is caused by enzymatic and physical processes that discriminate against ^{13}C in favor of ^{12}C (Katzenberg, 2008; Marshall et al., 2007). The C_3 pathway begins with the diffusion of CO_2 from the atmosphere into the air-

filled spaces within the leaf. This diffusion occurs through the air occupying stomatal pores where the plant metabolizes the CO_2 (Marshall et al., 2007). Such metabolism causes a chemical reaction that has a fractionation of around 4‰ due to the slower reaction of the heavier ^{13}C containing CO_2 molecules (Katzenberg, 1992; Marshall et al., 2007). Isotopic fractionation is the enrichment of one isotope relative to another in a chemical reaction and as a result there is a change in the relative concentration of the isotopes involved in the reaction, where the lighter isotope reacts faster; leaving the plant tissues enriched in ^{12}C over ^{13}C (Chisholm, 1989; Marshall et al., 2007).

Ultimately, it is the fractionation effect that allows for the application of stable isotopes to dietary reconstruction (Figure 4.1). The ratios differ between plants and atmospheric CO_2 (for carbon studies) in a predictable manner depending on the type of plant. Carbon in the biosphere is mainly determined by the isotopic fractionation that occurs during photosynthesis (Sealy, 2001). There are two major photosynthetic pathways for plants, the Calvin-Benson (C_3 pathway), and the Hatch-Slack (C_4 pathway). In the C_3 pathway, which most plants follow, the first product of photosynthesis is the 3-carbon compound (hence C_3). These plants have very low (negative) $\delta^{13}C$ values because they discriminate strongly against ^{13}C (Sealy, 2001) (see section 4.3, below, for an explanation of the 'delta' notation). C_3 plants include most trees, shrubs, fruits, vegetables and grains (e.g., wheat, barley oats, rye, and rice). Plants utilizing the C_4 pathway include maize, sugarcane, sorghum, and millet. A 4-carbon compound is the first product of C_4 photosynthesis and $\delta^{13}C$ values are higher (more positive), since the plants discriminate less against ^{13}C (Sealy, 2001).

A third photosynthetic pathway is named CAM, for Crassulacean Acid Metabolism. The CAM pathway is found in tropical succulents such as pineapple and various cacti. Plants with CAM photosynthetic pathways have $\delta^{13}C$ values that overlap the values in C_3 and C_4 plants, because they use either a C_3 or C_4 pathway depending on environmental conditions (Larsen, 1997). However, since there are no known CAM plants available in Upper Canada during the 19th century this pathway was not investigated.

The different photosynthetic pathways are what prompted archaeologists to initiate studies on stable isotopes because radiocarbon dates from maize samples always appeared considerably younger than the dates of other samples from the same site (Hall, 1967). In 1973, van der Merwe suggested that these differences might translate into different isotopic values within human bone and therefore provide information on diet.

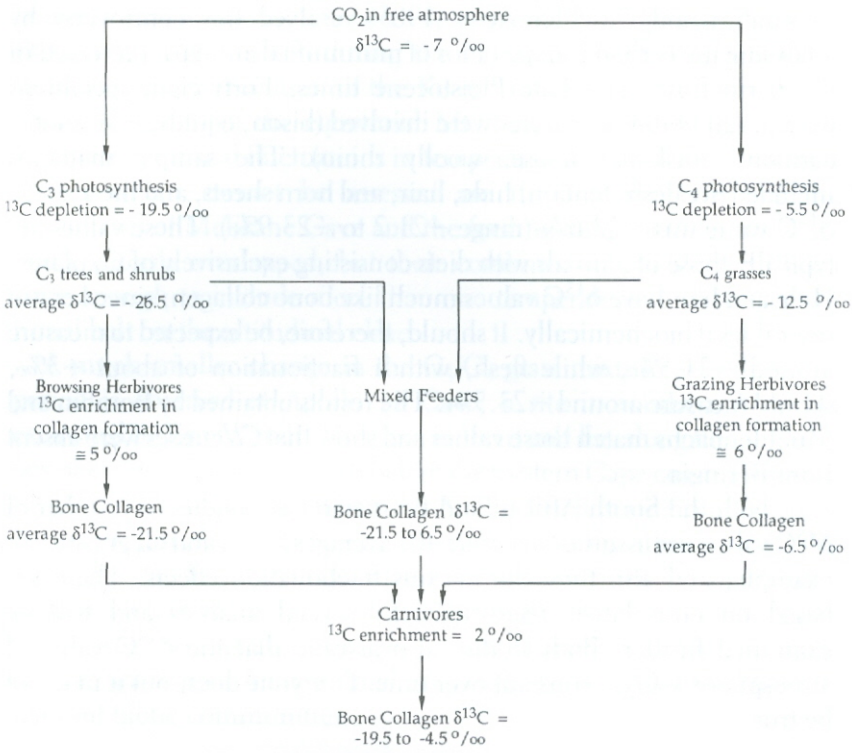

Figure 4.1: Diagram showing the fractionation steps of $\delta^{13}C$ throughout a C_3 and C_4 food web (van der Merwe, 1982: 110).

4.3 Expression of Stable Isotopes Values

Stable isotope analysis of organic substances such as bone collagen or hair keratin is done on a mass spectrometer, which measures the carbon and nitrogen values of the sample by combusting the sample and measuring the CO_2 and N_2 gases (Chisholm, 1989). To quantify the small differences in isotopic compositions between different organic materials, the $^{13}C/^{12}C$ ratio and $^{15}N/^{14}N$ ratio of a given sample are compared with the ratio of a standard in the mass spectrometer. The difference between the samples and the standards are known as the relative ^{13}C and ^{15}N contents, expressed as delta (δ) values. Since the absolute differences in isotopic abundances between sources are relatively

small, these differences are expressed in parts per thousand or per mil (‰) (Katzenberg, 2008). For example, if a sample of carbon dioxide has a $^{13}C/^{12}C$ ratio that is less than that of the standard by 6 per mil, it is said to have a $\delta^{13}C$ value of -6‰. A negative value indicates a sample that is depleted in ^{13}C relative to the standard, that is, it is isotopically 'lighter' (Chisholm, 1989). The equation used to determine the stable isotope ratio in a sample is:

$$\delta \text{ in } ‰ = \{ \frac{R_{sample} - R_{std}}{R_{std}} \} \times 1000$$

R= the ratio of the number of heavier to lighter isotopes (Katzenberg, 2008).

4.4 Reference Standards

The reference used for $\delta^{13}C$ measurements is known as the Chicago PDB marine carbonate standard, and it is derived from a piece of Cretaceous marine fossil, *Belemnitella americanna,* from the Peedee formation in South Carolina (van der Merwe, 1982). This marine carbonate is a convenient standard because it has a higher $^{13}C/^{12}C$ ratio than most other natural carbon-based materials. The PDB standard is assigned a $\delta^{13}C$ value of zero, and consequently most natural materials have negative values. The $\delta^{13}C$ values for natural carbon-bearing materials vary from about +4‰ to -50‰, relative to the PDB standard (van der Merwe, 1982). The reference standard for $\delta^{15}N$ measurements is atmospheric nitrogen gas (AIR) and its value is set at 0‰ (Schwarcz et al., 1985). These standards allow different labs to compare the results of their analysis. Most labs also have internal standards that are used to ensure equipment consistency (Katzenberg, 2008).

4.5 Carbon

Certain categories of food differ predictably in the stable carbon ratios. These differences include: 1) plants with C_3 versus C_4 pathways, 2) animals feeding on those plants and 3) animals in terrestrial versus marine ecosystems. The main source of carbon in plants is atmospheric CO_2. C_3 plants have very negative $\delta^{13}C$ values averaging nearly -26‰. Alternatively, C_4 plants have less negative $\delta^{13}C$ values, averaging about -12‰ (Katzenberg, 2008). Carbon in marine environments is derived from dissolved bicarbonate and the isotopic compositions are intermediate between terrestrial C_3 and C_4 plants (Ambrose et al., 1997; Price and Burton, 2011).

Carbon isotope analysis was originally used to detect the introduction of maize into North America. Maize uses a photosynthetic pathway that results in higher $^{13}C/^{12}C$ ratios in comparison to most terrestrial plants (Katzenberg et al., 1995). Vogel and van der Merwe (1977) studied North American skeletons from New York and determined that pre-maize skeletons all had $\delta^{13}C$ values clustered near the prehistoric Western European average of about -21‰, whereas post-maize skeletons showed a change over time from -16.5‰ to -13.5‰, which correlated with an increase in maize consumption (Vogel and van der Merwe, 1977). This study was one of the first to demonstrate that the stable isotopes of carbon could be used successfully to document the adoption of maize agriculture in eastern North America. Schwarcz and colleagues (1985) reported stable isotope ratios for carbon and nitrogen in human bone collagen from sites in Southern Ontario that dated between 2300 BC to AD 1640 and concluded that while there was significant increase in maize consumption, meat and fish remained the main source of

protein. Katzenberg and colleagues (1995) also analyzed stable carbon and nitrogen for sites in southern Ontario ranging from AD 400 to AD 1100 and determined that the importance of maize in the diet gradually increased over a period of approximately 600 years. These studies used isotope data to demonstrate marked differences in the timing and intensity of maize agriculture in various regions in southern Ontario.

Most native plants in Ontario are categorized as C_3 plants and use a photosynthetic pathway that results in a relatively low $^{13}C/^{12}C$ ratio. In a study by Katzenberg and coworkers (2000), stable carbon values were determined for foods commonly eaten in 19th century Belleville, Ontario, by preparing recipes found in historical documents. An average $\delta^{13}C$ for diet was estimated by multiplying the $\delta^{13}C$ of each food group (e.g., meat, baked goods, and vegetables) by their relative contribution to the diet, as described by Kenyon and Kenyon (1992). The estimated $\delta^{13}C$ for the total diet was -25.0‰ and the spacing between the $\delta^{13}C$ of food and that of bone collagen was 5.6‰ (Katzenberg et al., 2000). Accordingly, the estimated $\delta^{13}C$ for the total diet once it had been consumed by Belleville inhabitants and incorporated into their bone collagen would be -19.4‰, which corresponds to the values that Katzenberg and coworkers found. This study estimated $\delta^{13}C$ values of -20.4‰ for meat, -19.5‰ for baked goods, and -27.6‰ for vegetables and determined that a diet composed of 20% baked goods, 10% meat, and 70% vegetables would result in a human bone collagen $\delta^{13}C$ value of -19.4‰ (Katzenberg et al., 2000). It is important to note that several other combinations of these food groups could result in the same human bone collagen $\delta^{13}C$ value.

Carbon isotopes can also be used to differentiate between marine-based diets and terrestrial-based diets in temperate regions where there are no C_4 plants, because terrestrial C_3 plants are depleted in ^{13}C compared to marine plants and animals (Schwarcz and Schoeninger, 1991; Sillen et al., 1989). Most marine plants use a C_3 photosynthetic pathway, however their seawater source of carbon is enriched in ^{13}C relative to atmospheric CO_2 and this enrichment is reflected in the $\delta^{13}C$ values (Keegan, 1989). Carbon sources in the oceans include dissolved carbon dioxide as well as bicarbonates and carbonates (Katzenberg, 2008). Carbonates have a $\delta^{13}C$ value of zero, which makes it possible for some marine organisms to be isotopically heavier than terrestrial ones. Marine plants have isotopic compositions that are intermediate to those of C_3 and C_4 plants with $\delta^{13}C$ values ranging from -7‰ to -31‰ (Keegan, 1989).

Early stable isotope studies analyzed carbon isotopes from the organic portion of bone (i.e., collagen). A study by Krueger and Sullivan (1981) proposed analyzing isotopes from the inorganic (apatite) portion of bone, but this idea was met with initial criticism due to concerns with diagenesis (Ambrose and Krigbaum, 2003). It was argued that there was no way to control for the amount of post-depositional change and that the isotopic values would be too altered from the original values to reveal any valuable information (Nelson et al., 1986; Schoeninger and DeNiro, 1984). Despite this, Krueger and Sullivan (1981) demonstrated that much of the variability in values from bone apatite was due to trophic level differences and methods used in sample preparation. Since that time other researchers have demonstrated that with proper sample preparation and site specific methods of diagenetic identification, isotopic analysis from apatite is a valid

technique (e.g., Koch et al., 1997; Kohn et al., 1999; Lee Thorp and Talma, 2000; Sponheimer and Lee Thorp, 1999). Since the isotopic values from bone collagen mainly represent dietary protein, some researchers prefer to analyze apatite because it represents the average overall diet (Ambrose, 2003; Katzenberg et al., 2000).

In addition to dietary information, the inorganic portion of bone allows the analysis of older material, as collagen tends to degenerate in very ancient samples (Katzenberg, 2008; Sealy, 2001). Researchers also analyze apatite from tooth enamel because it is less susceptible to diagenesis than bone or dentin and persists longer in the archaeological record (e.g., Balasse, 2002; Katzenberg, 2008; Lee Thorp et al., 1989; Sponheimer and Lee Thorp, 1999). Lee Thorp and van der Merwe (1987) and Sponheimer and Lee Thorp (1999) used this technique successfully to conduct isotopic analysis of *Australopithecus robustus* and *Australopithecus africanus* tooth enamel and found that these early hominids were more enriched in ^{13}C than was anticipated on a C_3 based diet. This suggested that the hominids were either eating C_4 plants (grasses and leaves) or were more likely eating the flesh of animals or insects (e.g., termites) that consumed the grasses (Sponheimer and Lee Thorp, 1999). In addition to paleoanthropological applications, carbon isotopes from tooth enamel allow investigations into diet of fossil animals, and contribute to studies on long-term climate change (Sealy, 2001). One caveat is that tooth enamel only records diet during permanent enamel formation, which starts at birth (first permanent molar crown) and continues into the teen years (third molar crown); therefore teeth generate data about the childhood diet of the individual (Katzenberg, 2008; Koch et al., 1997).

Isotope data from tooth enamel are also valuable in determining the average weaning age at archaeological sites (e.g., Balasse, 2002; Dupras et al., 2001; Richards et al., 2002). Different teeth develop at different ages allowing researchers to gain information about weaning for individuals who survived the weaning process. Wright and Schwarcz (1998) applied this concept in their analysis of stable isotopes of carbon and oxygen from the carbonate of tooth enamel in 35 burials from Kaminaljuyu, an early state in the valley of Guatemala (700 BC to AD 1500). They compared $\delta^{13}C$ and $\delta^{18}O$ values from tooth crowns formed at different ages and found that carbon isotopes reflect the introduction of weaning foods, such as gruel, which resulted in an increase in $\delta^{13}C$ values (Wright and Schwarcz, 1998).

4.6 Nitrogen

Nearly all nitrogen (99%) is bound up in the atmosphere or in ocean water (Larsen, 1997). The ratio of the two isotopes of nitrogen, ^{14}N and ^{15}N was first used to identify legumes in terrestrial diets (Keegan, 1989). Beans, lentils, and other leguminous plants have lower ^{15}N values than other plants because they can fix molecular nitrogen directly from the atmosphere rather than having to rely on nitrates in the soil as is the case for all other plants (Sillen et al., 1989). Therefore, legumes have $\delta^{15}N$ values that are similar to the atmospheric nitrogen value of 0‰, whereas soil nitrogen is enriched in ^{15}N relative to air resulting in plants that rely on soil nitrogen having higher levels of $\delta^{15}N$ (Keegan, 1989).

The first researchers to investigate the stable isotopes of nitrogen were DeNiro and Epstein (1981) who explored the relationship between diet and tissues for nitrogen, and found that nitrogen isotopes vary depending on trophic level. Studies in the ecology of food webs also revealed that there is a progressive increase of 3‰ in nitrogen values in successively higher trophic levels and therefore nitrogen isotope values reflect the position in the food chain (Katzenberg, 1992; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984). As isotopes move through the food chain along the continuum from plants to herbivores to carnivores, $\delta^{15}N$ values become more positive (Keegan, 1989). For example, herbivores such as cattle feeding on grass will have higher $\delta^{15}N$ values by 3-4‰ compared to the $\delta^{15}N$ signature of grass (Meier-Augenstein, 2010). Moving up the food chain from herbivore to carnivore (i.e., from one trophic level to the next), $\delta^{15}N$ values in tissue will increase by 3-4‰ when moving up each trophic level. The manner by which the isotopes are transferred from one trophic level to another is through the process of enrichment known as isotopic fractionation (DeNiro and Epstein, 1981). As mentioned earlier, isotopic fractionation is the enrichment of one isotope relative to another in a chemical or physical reaction (Chisholm, 1989). When either terrestrial or marine herbivores eat plants, their metabolism selects and recombines plant chemicals in the process of tissue formation, resulting in fractionation of the carbon and nitrogen isotopes (Chisholm, 1989). For humans, $\delta^{15}N$ values of structural proteins such as bone collagen or hair keratin reflect the relative level of meat protein in the diet. Hair from vegans exhibits lower $\delta^{15}N$ values than hair from ovo-lacto vegetarians. In fact, hair $\delta^{15}N$ values from ovo-lacto vegetarians are identical to those from omnivores and hair from

individuals who eat a large proportion of meat and/or fish have higher $\delta^{15}N$ values than omnivores and ovo-lacto vegetarians (O'Connell et al., 2001; O'Connell and Hedges, 1999; Petzke, 2010).

Conversely, there is little difference in $\delta^{13}C$ values for the same tissues in successive trophic levels (van der Merwe, 1982). A carnivore feeding on herbivore flesh will consume a diet that has a $\delta^{13}C$ value only 1‰ more positive than the plants from which the herbivore fed (Schoeninger, 1989). Therefore, carnivore bone collagen exhibits a $\delta^{13}C$ value approximately 1‰ more positive than that of the herbivore upon which it fed.

According to Schwarcz and Schoeninger (1991) attempts to use nitrogen isotopes to estimate the amount of meat in human diet have been largely unsuccessful. Delta ^{15}N values show a nonlinear relationship between the amounts of meat versus plant protein in the diet, therefore the percentage of meat versus plant protein cannot be accurately estimated from $\delta^{15}N$ values (Ambrose et al., 2003; Schwarcz and Schoeninger, 1991). It appears that the nitrogen isotopic signal reflects an average of the total pool of nitrogen from the diet, and specific dietary sources of nitrogen cannot be identified (Schwarcz and Schoeninger, 1991).

Stable nitrogen isotope ratios can also be used to differentiate marine from terrestrial protein (Sillen et al., 1989). Generally, the $\delta^{15}N$ values for terrestrial plants are 4‰ lower than for marine plants (Larsen, 1997). Since marine ecosystems have far longer food chains than terrestrial ecosystems, marine foods exhibit significantly higher $\delta^{15}N$ values and the $\delta^{15}N$ values in marine organisms are up to 20‰ higher than terrestrial

organisms (Larsen, 1997; Meier-Augenstein, 2010; Schoeninger and DeNiro, 1984). Typically, terrestrial herbivore flesh has a $\delta^{15}N$ value of about 6‰ while freshwater fish have values around 16‰ (Schwarcz et al., 1985). These differences in marine and terrestrial environments are reflected in human tissue and can be used to determine the relative importance of foods from the respective ecosystems (Larsen, 1997).

Another reason nitrogen isotopes are useful in distinguishing marine from terrestrial diets is that stable nitrogen isotopes are not influenced by the type of terrestrial plants consumed, with the exception of leguminous plants (Katzenberg, 1992). The use of stable carbon isotopes alone to detect marine foods in the diet is limited because in areas where C_4 plants are consumed, the carbon isotope signals for the marine foods and the C_4 plants become indistinguishable (Katzenberg, 1992). For example, in the coastal regions of the New World, where marine food and maize were simultaneously consumed by the local inhabitants, the overlapping carbon isotope values for both foods inhibited the discrimination of diets when trying to assess the relative contribution of marine foods versus maize (Larsen, 1997).

Nitrogen isotopes have also been used to investigate weaning in archaeological samples due to the trophic level effect (e.g., Fogel et al. 1989; Katzenberg and Pfeiffer, 1995; Richards et al., 2002; Schurr, 1997). A nursing infant should show nitrogen values that are about 3‰ higher than its mother. Fogel and colleagues (1989) study used isotopes taken from the fingernails of modern mothers and their nursing infants and found an expected enrichment of the heavier isotope nitrogen in nursing infants. As soon as weaning began and solid foods were introduced, the $\delta^{15}N$ values converged with those of

the adults in the community (Fogel et al., 1989). Other studies have estimated the duration of nursing in past populations (e.g., Herring et al., 1998; Schurr, 1998) as well as the problem of stress and mortality during the weaning process (Katzenberg and Pfeiffer, 1995). Nitrogen values may be elevated in times of stress and this enrichment might make the weaning age difficult to determine. Despite this, Herring and coworkers (1998) determined that breast milk remained the main source of protein in infant diet in the Belleville sample until about 14 months of life. They studied infants buried in the St. Thomas' Anglican Church cemetery and used a combination of historical demographic and stable nitrogen isotope analysis to determine a general pattern of extended nursing of about 14 months. Solid foods other than breast milk were introduced to some infants around five months of age, while others were breast-fed until 14 months resulting in variation in breast-feeding and weaning behaviours for St. Thomas' infants (Herring et al., 1998). There was an increase in $\delta^{15}N$ values as the infant nursed, then a decrease beginning at the time of weaning where $\delta^{15}N$ values returned to levels similar to those of the mother shortly after breast-feeding stopped (Herring et al., 1998).

Geographic variation unrelated to diet may occur in the $\delta^{15}N$ values of bone collagen (Schoeninger, 1989). Climate may affect the $\delta^{15}N$ values of soils, plants, and animals in the ecosystem. Generally, cool forest soils have low $\delta^{15}N$ values, due to higher nitrogen fixation and mineralization rates. Conversely, hot savannah or desert soils have high $\delta^{15}N$ values (Larsen, 1997). Heaton and colleagues (1986) demonstrated a climatic influence on nitrogen isotope ratios, with nitrogen values being negatively correlated with annual rainfall. A decrease in the total amount of precipitation or relative humidity cause

$\delta^{15}N$ values in soils and plants to become enriched (Iacumin et al., 1998). The $\delta^{15}N$ of animals may also vary according to the amount of rainfall; herbivores have higher $\delta^{15}N$ values in regions with less than 400 mm of rain per annum. This is due to the regulation of urea excretion in response to water stress (Iacumin et al., 1998). In some habitats, terrestrial animals living in arid environments have higher $\delta^{15}N$ values than marine animals (Larsen, 1997).

4.7 Nitrogen Balance in the Human Body

There is evidence that factors other than dietary input may influence nitrogen isotope ratios in certain situations. In particular, conditions such as nutritional stress and disease have been shown to increase $\delta^{15}N$ values when an organism is catabolic and in negative nitrogen balance (e.g., Fuller et al., 2004; Katzenberg and Lovell, 1999; White and Armelagos, 1997). The term nitrogen balance refers to the amount of nitrogen the body excretes, as opposed to the amount of nitrogen the body takes in (Tomé and Bos, 2000). All of the macronutrients such as protein, carbohydrate, and fat are made up of carbon, hydrogen, and oxygen molecules (Bingham and Cummings, 1985; Tomé and Bos, 2000). Protein alone also contains an additional nitrogen molecule and when the body digests protein, the nitrogen molecules are generally released into the blood (Bingham and Cummings, 1985). Measuring bodily nitrogen levels can be an accurate way to determine whether the body is receiving adequate, inadequate, or excess protein (Calloway and Spector, 1954).

There are three states of nitrogen balance: negative, positive, and equilibrium. If an individual has a negative nitrogen balance, that person's nitrogen output is greater than their nitrogen input. Because protein is the only macronutrient containing a nitrogen molecule, a negative nitrogen balance can be a sign of inadequate protein consumption, malnutrition or physiological stress (Tomé and Bos, 2000). An individual in negative nitrogen balance loses tissue during stress because less nitrogen is being consumed than is needed to maintain and replace proteins in the body. As a result there is catabolism of existing proteins in the body which is the metabolic breakdown of complex molecules into simpler ones, often needed to release energy (Huelsemann et al., 2009; Tomé and Bos, 2000). The nitrogen that is excreted will show a preferential loss of ^{14}N relative to ^{15}N as amino acids with ^{14}N are broken down more readily than those containing ^{15}N, this leaves ^{14}N to be preferentially excreted (Katzenberg and Lovell 1999; Steele and Daniel, 1978). The remaining tissues are enriched in the heavier isotope resulting in an elevation of $\delta^{15}N$ values.

If an individual has a positive nitrogen balance, it means that person is consuming more protein than the body needs; more nitrogen is ingested than is excreted (Bingham and Cummings, 1985). This results in tissue gain during growth while new tissues are being produced. The expectation is that diet will be reflected by the newly forming tissues in the $\delta^{15}N$ values (Hobson et al., 1993; Katzenberg and Lovell, 1999). Trophic enrichment relative to diet is expected because more ^{14}N than ^{15}N is excreted relative to what has been ingested. Therefore, little or no variation in $\delta^{15}N$ values would be expected (Katzenberg and Lovell, 1999).

If an individual's nitrogen balance has reached a state of equilibrium, it means that an individual is excreting approximately the same amount of nitrogen as they are taking in. This is considered a state of tissue maintenance in healthy individuals, where the person is in nitrogen equilibrium and the protein in the body is being maintained (Tomé and Bos, 2000). The $^{15}N/^{14}N$ values will reflect the ingested protein from the diet ($\delta^{15}N$ plus 3‰) and should show no fluctuation in $\delta^{15}N$ values.

The human body needs more than just protein to maintain appropriate nitrogen levels. The human body also needs an adequate supply of fats and carbohydrates. These macronutrients are considered essential to energy production (Tomé and Bos, 2000). When dietary levels of fat and carbohydrate fall too low or the body is undergoing a stressful physiological event, the body uses protein for energy, which may elevate the body's nitrogen levels and inhibit the body's ability to renew damaged cells (Tomé and Bos, 2000).

Katzenberg and Lovell's (1999) study used the concepts of nitrogen balance in the human body to explore $\delta^{15}N$ and $\delta^{13}C$ variation in autopsy specimens exhibiting several different pathological responses. They investigated seven modern bone samples (three normal, four pathological) with the objective to determine if pathological conditions resulted in variations in stable isotope ratios (Katzenberg and Lovell, 1999). An individual with osteomyelitis as a complication of the AIDS virus had a 2‰ elevation in $\delta^{15}N$ from the diseased section of bone compared to the normal segment (Katzenberg and Lovell, 1999). The authors suggested that elevated nitrogen was a result of the recycling of nitrogen within the body during bone remodeling and tissue repair. This means that

elevated $\delta^{15}N$ values have the potential to be used as an indicator of nutritional or physiological stress in humans.

4.8 Integrating Carbon and Nitrogen Isotopes

The simultaneous use of carbon and nitrogen ratios serves to clarify the potentially conflicting signatures of either element alone, particularly in marine environments (Larsen, 1997). Figure 4.2 represents a plot of typical $\delta^{15}N$ and $\delta^{13}C$ values.

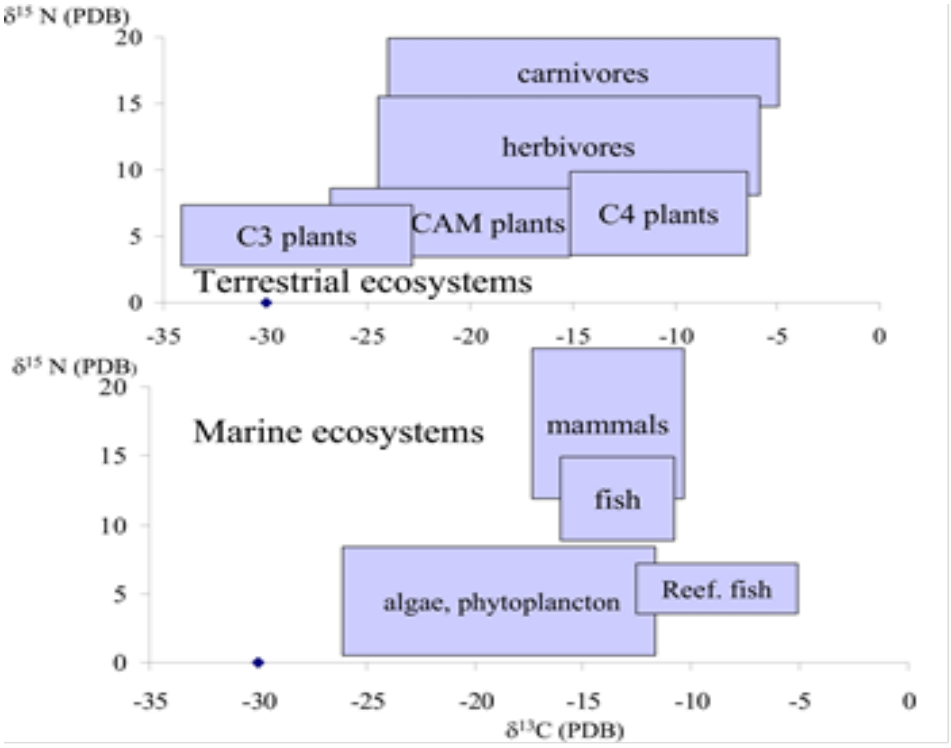

Figure 4.2: Bivariate plot showing the distribution of carbon and nitrogen isotopes in terrestrial and marine food webs (Szostek, 2009:10).

Many studies use both carbon and nitrogen stable isotopes in the analysis of past diet, since the addition of nitrogen reduces the number of possible interpretations (Katzenberg and Harrison, 1997). For example, Larsen's (1992) isotopic analysis of bone collagen specimens from prehistoric foragers, farmers, and contact period Natives in Georgia Bight revealed a distinctive temporal trend showing increasing $\delta^{13}C$ values and decreasing $\delta^{15}N$ values. This trend indicated an increasing consumption of terrestrial plants (especially maize) and animals in conjunction with a decreasing reliance on marine resources (Larsen, 1992).

Other applications of carbon and nitrogen isotopes include exploring differential access to food related to age, sex, and social status. Katzenberg and colleagues (1993) looked at age differences in carbon and nitrogen isotope ratios from an Ontario Iroquoian village (AD 1530-1580). Their samples ranged in age from infants to the elderly and the only significant differences were in the infants due to the enrichment of $\delta^{15}N$ in nursing infants, and enrichment in $\delta^{13}C$ values upon the introduction of weaning gruel (Katzenberg et al., 1993).

Social status and gender differences in diet have also been investigated using carbon and nitrogen isotopes. For example, Ambrose and colleagues (2003b) examined the isotopic values of nine individuals from Mound 72 at Cahokia, a large Mississippian site (AD 1050-1150) in Illinois. By using burial type and amount of grave goods as indicators of status, Ambrose and colleagues (2003b) were able to infer differences in diet between high and low status individuals. They found that low status individuals consumed slightly more maize (10% more) and less animal protein than high status

individuals; however, females found in mass graves consumed 60% more maize than high status individuals and were thought to have originated from another population (Ambrose et al., 2003b).

4.9 Limitations of Stable Isotope Analysis

Stable isotope analysis provides direct evidence of individual diet by reflecting the isotopic ratios of consumed food and may be a reliable method to detect the onset and duration of a stressful event in an individual prior to death. However, stable isotope analysis is limited in the type of dietary information that it can provide, for example the consumption of several different combinations of foods can result in the same stable isotope ratios (Katzenberg, 2008). In cases where the introduction of one isotopically distinct food source to an otherwise isotopically similar diet has occurred, such as the introduction of maize in the New World, stable isotope analysis can be a useful indicator of dietary change. However, more complicated human diets result in an isotopic mixture that can be difficult to decipher from bulk isotopic values, and that is why it is important to identify the foods actually consumed and their isotopic compositions. This study utilized several sources of dietary information such as historic records, archaeological data, published studies, and two books written by women who resided in Belleville during the 19th century (e.g., Moodie, 1853; Parr Traill, 1836). Katzenberg and coworkers (2000) used modern samples of food cooked in the traditional manner employed by 19th century Belleville inhabitants to obtain estimates of the isotopic values of these foods. They also analyzed the stable isotopes of carbon in preserved bone collagen from the St. Thomas'

skeletal collection. The results indicated a diet-to-collagen spacing of 5.6‰ which is very close to the expected fractionation effect between $\delta^{13}C$ of food and that of bone collagen, offering further support to the accuracy of their dietary reconstruction (Katzenberg et al., 2000).

4.10 Summary

This chapter presented a basic introduction to stable isotopes, starting with the physical chemistry of an isotope and progressing to how isotopes have contributed to the study of past diet. By understanding the $\delta^{13}C$ and $\delta^{15}N$ isotope values in consumer tissues and how they correlate with dietary isotopic composition, researchers can make a significant contribution to the reconstruction of past human life. The importance of nitrogen balance in the human body was discussed to provide a background on how nitrogen balance can affect $\delta^{15}N$ values in relation to nutritional or physiological stress. While stable isotope analysis as a methodology can provide invaluable information about diet and health in the past, it also has some limitations that must be kept in mind when conducting stable isotope analysis.

With the literature available on the stable isotopes, it is important to recognize that most research completed to date has been done on bone collagen or tooth enamel. Alternate tissues, such as hair keratin can contribute equally to the growing body of knowledge on the isotopic analysis of past diet and can aid in discerning potential stressful events on an individual level. The specific use of hair keratin for stable isotope studies will be explored further in the next chapter.

Chapter 5

Stable Isotopes of Hair

5.1 Introduction

Carbon and nitrogen isotopes of hair keratin, similar to bone collagen, can be used to distinguish past human diet. Traditionally, research has focused on $\delta^{13}C$ and $\delta^{15}N$ values in human bone collagen and carbonate rather than hair keratin. This chapter will discuss the isotopic analysis of hair keratin specifically focusing on how hair keratin reflects the food consumed. This chapter begins with an overview of the current literature on the use of hair keratin as a medium to examine dietary and physiological conditions in animals, in modern human and archaeological populations, and will include the use of hair in forensic studies. A discussion on the variation in isotopic signals between hair keratin and bone is followed by a consideration of whether the isotopic composition of hair can be used as a biomarker to provide information about nutritional status, metabolism, and disease.

5.2 Hair Keratin Reflects Food Consumed in Animals

A problem in animal ecology is the difficulty in observing and accurately reconstructing an animal's diet and stable isotope analysis of hair keratin has been employed to assess the general nutritional status of an animal (West et al., 2004). Animal studies involve isotopic analyses that concentrate on the relationship between the isotopic values of bone, hair, and diet. For example, Cerling and coworkers (2005) conducted a

sequential analysis of tail hair of Northern Kenyan elephants and $\delta^{13}C$ values provided weekly records of diet and water resources correlating with satellite-based measurements of the vegetation available. The authors were able to identify differences in feeding behaviour and range locations of the elephants by combining the tracking and $\delta^{13}C$ data (Cerling et al., 2005).

Similar work has been done on nitrogen and sulphur isotopes in controlled feeding experiments on llamas, cattle, goats, horses, and alpacas (Richards et al., 2003; Schwertl et al., 2003; Sponheimer et al., 2003). Controlled feeding studies demonstrated the potential for identifying short term changes in diets from $\delta^{13}C$ values in hair (e.g., Ayliffe et al., 2004; Caut et al., 2008; West et al., 2004). For example, Ayliffe and colleagues (2004) conducted a long term controlled feeding trial with horse hair and suggested that the change in $\delta^{13}C$ of hair can be explained by a three-pool exponential-decay model. The three pools are described as 1) a fast pool reflecting amino acids directly from the diet, 2) a fast pool reflecting amino acids from the breakdown of metabolic proteins in the body, and 3) a slow pool representing amino acids from the breakdown of structural proteins in the body (Ayliffe et al., 2004). They stated that although a change in the $\delta^{13}C$ of the hair can be detected rapidly after a change in diet, the time for a body to reach full equilibrium can be a few days to several weeks (Ayliffe et al., 2004).

Isotopic studies conducted on animals fed a controlled diet provide an important framework for understanding isotopic variation in humans, and indicate that information about the chronology of diet can be recorded with high resolution in hair keratin and these

methods can be applied to modern human, forensic, and archaeological samples to enhance the temporal examination of diet.

5.3 Hair Keratin Reflects Food Consumed in Modern Humans

Animal studies have shown that hair can be used as an isotopically representative tissue and suggest that hair from modern humans can also be used to study the effects of different dietary compositions on the isotopic values of the human body. Webb and coworkers (1980) were among the first to examine the effects of animal protein consumption on hair isotopic values from individuals in the same population. The authors measured the $\delta^{13}C$ of hair keratin from omnivores and ovo-lacto-vegetarians (individuals who eat no animal flesh but consume animal protein such as eggs and milk) and found little effect on $\delta^{13}C$ values from the consumption of secondary animal protein as opposed to meat (Webb et al., 1980). In other words, cow's meat and milk are isotopically equivalent to other animal derived protein such as cheese and eggs and there is no difference in $\delta^{13}C$ values between the dietary components of omnivores and ovo-lacto-vegetarians.

Minagawa (1992) also questioned how to reconstruct the dietary pattern of modern humans from the isotopic values given the large range of food sources available to modern individuals. Minagawa (1992) suggested that for archaeological populations the number of dietary sources is usually restricted to two or three food sources, whereas for contemporary humans the food web is more complex than that of prehistoric humans who depended largely on local food. With the expansion of the food trade and

supermarkets in the modern world it is difficult to limit major food to just two or three groups. Minagawa (1992) evaluated the $\delta^{13}C$ and $\delta^{15}N$ compositions of contemporary Japanese scalp hair and estimated the proportions of five food groups (fish, meat/egg/dairy, C_3 plants, C_4 plants, legumes) consumed by Japanese individuals and was able to determine human feeding behaviour, even for a complex contemporary sample.

Isotopic studies on the hair of modern individuals enabled O'Connell and Hedges (1999) to demonstrate that $\delta^{15}N$ values of hair keratin reflect the proportion of animal protein consumed in the diet. There was a relationship between the reported amount of animal protein eaten (either meat or secondary animal products) and the $\delta^{15}N$ values within two groups of omnivores and ovo-lacto-vegetarians, indicating that increasing the proportion of animal protein in the diet resulted in an increase in the $\delta^{15}N$ values of hair keratin (O'Connell and Hedges, 1999). This provided the first independent support for the hypothesis that a diet high in meat equates to elevated nitrogen isotopic values in the body. Huelsemann and coworkers (2009) conducted a study on $\delta^{13}C$ and $\delta^{15}N$ values for segmented hair using controlled diets for modern individuals. The dietary change included a change from a C_3 to C_4 plant diet and the replacement of terrestrial animal food sources by marine sources. All individuals showed significant fluctuation in $\delta^{13}C$ and $\delta^{15}N$ during the dietary change period, and the change in $\delta^{15}N$ was faster than the change in $\delta^{13}C$ in the hair of all individuals, indicating that segmented hair analysis can provide data on any abrupt changes in dietary habits (Huelsemann et al., 2009). Isotopic data from hair keratin and breath samples were used to explore $\delta^{13}C$ and $\delta^{15}N$ isotopic composition in reported diets of modern Fijians with similar results to the previous study

(Hedges et al., 2009). The authors were able to characterize the reported diet, confirming that the isotopic composition of hair accurately reflect individual dietary changes (Hedges et al., 2009).

5.4 Isotopes in Hair Keratin Detect Weaning Patterns

Delta ^{15}N values in hair keratin also have the potential to track the weaning process by signalling the introduction of solid foods into an infant's diet. Fuller and coworkers (1999, 2006) utilized $\delta^{13}C$ and $\delta^{15}N$ values from the hair and fingernails of modern infants to measure trophic level enrichment in a modern population. They determined that exclusively breastfed infants had a dual enrichment in carbon (1‰) and nitrogen (2-3‰) when compared to maternal values (Fuller et al., 2006). Alternatively, the breast and formula fed infants had reduced enrichments compared to the exclusively breastfed infants, and the formula-fed infants showed no increase in $\delta^{13}C$ or $\delta^{15}N$ values (Fuller et al., 2006). This study demonstrates that the trophic level effect produces a detectable and quantifiable signal in the $\delta^{15}N$ values in hair keratin and is valuable in refining breastfeeding and weaning patterns.

5.5 Hair Keratin Use in Forensic Cases

While hair keratin can provide a record of diet through the stable isotope analysis of $\delta^{13}C$, and $\delta^{15}N$, $\delta^{18}O$ and $\delta^{2}H$ reflect the geographic environment by recording the isotopic signatures of drinking water (Dupras and Schwarcz, 2001; Meier-Augenstein, 2010; Neuberger et al., 2013). Isotope signatures can yield information on a person's

place of birth and movement during life and this is of interest for forensic identification. Scalp hair can be analysed for $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ to determine a victim's recent history in terms of diet or geographic location. Studies are now exploring the link between isotopic signals of dietary components and the isotopic composition of hair keratin in an effort to identify individuals from natural catastrophes, murder victims, victims of human trafficking, and cases of intentional starvation (e.g., Fraser et al., 2006; Meier-Augenstein and Fraser, 2008; Mützel (Rauch) et al., 2009; Neuberger et al., 2013). For example, Fraser and coworkers (2006) analyzed the $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$, and $\delta^{18}O$ values of scalp hair and fingernails with the intention of creating a database that can be used to compare samples of unknown origin to aid in the identification of disaster victims. The authors noticed that the $\delta^{15}N$, and $\delta^{18}O$ values of fingernails were noticeably more variable than those obtained from scalp hair from the same individual, suggesting that the faster rate of formation for hair means there is less time for the forming hair to be affected by biochemical processes, such as the different growth rates of tissues, that could alter their isotopic signature (Fraser et al., 2006). A study by Meier-Augenstein and Fraser (2008) used the isotopic signatures of $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ in nail and hair in a modern forensic case. This was one of the first cases in which data from stable isotope analysis of hair and nails established a murder victim's geographic place of birth and life history to confirm the identification of the victim (Meier-Augenstein and Fraser, 2008). A larger study by Mützel (Rauch) and colleagues (2009) utilized the isotopic values in hair samples from 111 individuals from 13 different countries all over the world with the aim of creating a database to aid in provenancing people. The results indicated that individuals

from Costa Rica and Brazil can be differentiated from European individuals by $\delta^{13}C$ values, and Australians by $\delta^{34}S$ and $\delta^{2}H$ in hair samples (Mützel (Rrauch) et al., 2009).

A recent study analyzed the $\delta^{15}N$ and $\delta^{13}C$ values in sequentially segmented hair to diagnose starvation episodes in children and elderly adults to investigate forensic cases of suspected abuse or neglect (Neuberger et al., 2013). Increasing $\delta^{15}N$ values indicated that food deprivation caused the body to catabolize protein and break down body fat deposits, whereas they found less variation in $\delta^{13}C$ values and the authors suggested that these findings could be applied to forensic cases as an unbiased biomarker to identify the time frame of nutritional deprivation. Analysis of stable isotopes in human hair has become another important tool in forensic cases to reconstruct diet and place of origin for unidentified individuals.

5.6 The Use of Hair Keratin in Archaeology

The ability to make quantitative statements about ancient diet has fundamental importance for archaeology, since diet reflects the interaction between demography, economy, environment, social status, and food production (O'Connell and Hedges, 1999). Macko and coworkers (1999a) were the first to perform stable isotope analysis on a wide variety of archaeological hair samples. They investigated $\delta^{13}C$ and $\delta^{15}N$ values in hair from the mummified remains of Egyptian Coptics (1000 BP) and the Chinchorro of Chile (5000-800 BP) to assess whether the isotopic composition of hair was a reliable indicator of ancient diet. The authors were able to determine certain dietary trends, for example the hair analysis of the Coptics of Egypt indicated the influence of both C_3 and C_4 plants in

the diet and they found differences in diet based on socio-economic status with peasants consuming less protein from meat sources (Macko et al., 1999a). The Chinchorro hair samples were from two sites (Morro and Maderas Enco) and appeared to have differing types of meat protein in their diet. The Morro diet consisted of foods from a marine source, and the Maderas Enco people consumed mainly terrestrial foods, suggesting that it was possible to use hair keratin to look at dietary differences to provide information on coastal migration, food cultural practices, and possible exchange or trade between communities (Macko et al., 1999a). Macko and coworkers (1999b) analyzed the hair from the Tyrolean Iceman (5200 BP) discovered in the Oetztaler Alps and found that this individual ate a primarily vegetarian diet which was in agreement with his dental wear pattern.

Roy and coworkers (2005) analysed the $\delta^{13}C$ and $\delta^{15}N$ ratios in small hair segments (less than one cm long) from two Native American historic cultural groups and found distinct dietary profiles between the two indicating a higher consumption of maize (or of animals that had fed on maize or other C_4 plants) in one of the groups, connected to changing residency patterns among Native American (Roy et al., 2005).

White and coworkers (2009) compared $\delta^{13}C$ and $\delta^{15}N$ values in hair and bone from ancient humans for the coastal site of Pacatnamu dating from Moche to Lambayeque periods (AD 450-750 and AD 900-1100). The authors' goal was to determine the cause of isotopic variation in the isotopic composition of hair segments within individuals and assess differences in bone and hair isotopic values from the same individuals. The variation in isotopic data for individual diet indicated that people were

moving from the coast to highland regions and the authors suggested that pilgrimage was a possible explanation for the frequent large-scale mobility (White et al., 2009). A significant finding in this study was that the degree of variation in $\delta^{13}C$ and $\delta^{15}N$ values of hair keratin within individuals was greater than that of bone collagen, indicating that sequential isotopic analysis of hair keratin was useful in revealing dietary variation that was masked in isotopic analysis of bone.

5.7 Patterns in Seasonal Variation and Season of Death using Hair Keratin

Although limited to archaeological sites with exceptional preservation, analysis of human hair to identify seasonal trends in paleodiet studies has become increasingly common (e.g., Macko et al., 1999a, b; O'Connell and Hedges, 1999; Roy et al., 2005; Sandford and Kissling, 1993; Schwarcz and White, 2004; White, 1993). Analysis of archaeological hair can provide a fine-grained resolution of seasonality and White (1993) demonstrated this in a study on Sudanese Nubian mummies representing X-Group (AD 350-550) and Christian (AD 550-1300) periods in the Wadi Halfa region. The study compared hair segments taken next to the scalp with those further along the hair shaft to determine diet just prior to death and any shift in food intake and established that the most common season of death was summer which was a time of climatic and nutritional stress for the Nubian population (White, 1993). Following this, Schwarcz and White (2003) performed a re-analysis of sequential segments of hair using samples from the same Wadi Halfa region and showed that $\delta^{13}C$ values of hair varied throughout the year in accordance with two models of subsistence practices: 1) immediate consumption where

seasonal crops were consumed when harvested, and 2) stored/pooled consumption, where crops were harvested and stored in granaries until needed. A similar study analysed the $\delta^{13}C$ and $\delta^{15}N$ for sequential segments of human hair from two Chilean sites, Chiribaya Alta and El Yaral (AD 1000–1450) and demonstrated seasonal variation in the consumption of marine products and C_4 plants such as maize (Knudson et al., 2007). The authors noted that the most seasonal variation was found for individuals buried at Chiribaya Alta and suggested that this developed from the community's access to food resources from a variety of ecological zones (Knudson et al., 2007).

Williams and Katzenberg (2012) also observed that the pattern of isotopic data from sequential segmented hair keratin exhibited variation that could be linked to season of death. The authors reconstructed the season of death in an archaeological sample of 500 year old Peruvian mummies and investigated variation in mortality between males and female and between summer and winter months. For most of the individuals the $\delta^{13}C$ values showed a variation in diet from month to month while the $\delta^{15}N$ values displayed only minor variation, therefore the season of death was assigned based on the carbon values. If the $\delta^{13}C$ values decreased towards the proximal end (towards the scalp) the individual died during the winter/spring; alternatively, if the $\delta^{13}C$ values tended to increase towards the proximal end, the individuals died during the summer/fall (Williams and Katzenberg, 2012). The fluctuations of the $\delta^{13}C$ values indicated that these individuals were consuming different foods prior to death that could be linked to seasonality in food sources.

In archaeological contexts where tissues such as hair are preserved, they can provide an important source of information about short-term diet that allows us to explore larger issues such as food storage and subsistence strategies. Tissues that grow incrementally such as hair are a particularly important source of data to explore questions about reconstruction of seasonal variation in diet, season of death, and morbidity and mortality in past populations.

5.8 Isotopic Comparison of Hair and Bone

Unlike most body proteins that are remodelled throughout life, hair is unusual in that the protein is not reabsorbed (O'Connell and Hedges, 1999). The $\delta^{13}C$ and $\delta^{15}N$ values in tissues with a slow turnover rate, such as bone collagen, reflect an average of the body's protein dietary intake over a long period of time, whereas the $\delta^{13}C$ and $\delta^{15}N$ values of hair record the most recent diet because hair fibers retain isotopic information from the time it was produced (Williams et al., 2011). Human hair grows continuously from its base in the hair follicle and does not change after formation; therefore hair provides a linear record of diet that varies in time according to its length (White et al., 2009). Bone collagen and hair keratin have very different turnover rates, with bone reflecting the average diet consumed over a period of 10-25 years, whereas hair keratin reflect more recent diet (in months) (O'Connell and Hedges, 1999; Stenhouse and Baxter, 1979). However, bone is not always readily available for study, so other biological tissues may need to be used.

O'Connell and Hedges (1999) compared the $\delta^{13}C$ and $\delta^{15}N$ values of 23 pairings of hair keratin and bone collagen in both archaeological and modern samples in the United Kingdom. Their first objective was to compare the isotopic signatures of various diets in archaeological hair samples to determine if a diet high in meat equated to elevated $\delta^{15}N$ values; their second objective was to examine whether archaeological isotopic data could be directly compared to modern individuals (O'Connell and Hedges, 1999). The authors concluded that both archaeological and modern samples displayed bone collagen $\delta^{13}C$ values that were enriched relative to hair keratin $\delta^{13}C$ values by between 0 and +2‰, while nitrogen bone collagen $\delta^{15}N$ values were enriched by between 0 and +1‰ (O'Connell and Hedges, 1999). One explanation for the consistent $\delta^{15}N$ value enrichment between collagen and hair keratin was that individuals may have died after an illness and this may have been recorded in the hair, but not the bone. O'Connell and Hedges (1999) also showed a clear association between the proportion of animal protein in the diet and increased $\delta^{15}N$ values in hair keratin.

A second study by O'Connell and Hedges (2001) compared the major proteins or amino acids in bone collagen, hair keratin, and nail keratin in 12 pairings taken from living individuals in the United Kingdom. With the similar goal to determine if modern human isotopic values can be directly compared to archaeological isotopic values, results showed that bone collagen was enriched relative to hair keratin within the same individual by +1.4‰ for $\delta^{13}C$ and +0.9‰ for $\delta^{15}N$, with a small degree of variability (O'Connell et al., 2001). These two studies showed that both archaeological hair and bone appeared to be almost isotopically equivalent for $\delta^{13}C$ and $\delta^{15}N$, but the degree of the

difference between hair keratin and bone collagen has not yet been quantified on a large scale. Ultimately, the results from the modern individuals and archaeological samples were broadly similar, but not identical.

As mentioned earlier, Katzenberg and coworkers (2000) examined dietary indicators in preserved bone and hair from St. Thomas' Anglican Church in Belleville. Stable isotopes of carbon were analyzed in bone collagen and in foods prepared following recipes in historical documents with the aim to estimate the spacing between the $\delta^{13}C$ values of food and those of bone collagen (Katzenberg et al., 2000). The authors also looked at $\delta^{13}C$ and $\delta^{15}N$ values for nine hair samples and determined that the offset between $\delta^{13}C$ in bone collagen and hair was 1.8‰, while the difference between collagen and hair for $\delta^{15}N$ was 0.9‰ (Katzenberg et al., 2000). They concluded that hair and bone collagen reflect similar dietary information but there was a difference in the diet-hair and diet-collagen spacing for both carbon and nitrogen stable isotopes (Katzenberg et al., 2000). Bone collagen is 1.8‰ heavier than hair for $\delta^{13}C$ and bone collagen is 0.9‰ lighter than hair for $\delta^{15}N$ (Katzenberg et al., 2000). To date, no studies have been published that explain the slight variation in $\delta^{13}C$ and $\delta^{15}N$ values between bone collagen and hair keratin, however O'Connell and Hedges (2001) suggested that it is the different amino acid composition of collagen and keratin that account for the relative differences in both $\delta^{13}C$ and $\delta^{15}N$ values.

5.9 Nutritional and Metabolic Status Detection in Modern and Archaeological Samples

According to Jackson and coworkers (1999) serious illness or injury results in increased protein turnover. In fact, critical illness or nutritional deprivation is associated with altered metabolism characterized by an increased catabolic rate, negative nitrogen balance, wasting of lean body mass, immune-suppression, and compromised wound healing (Jackson et al., 1999; Neuberger et al., 2013). A seminal study by Hobson and coworkers (1993) investigated the effect of ^{15}N enrichment in association with the loss of body mass as a result of fasting in birds. The authors found that as starvation episodes in Japanese Quail (*Coturnix japonica*) produced declines in body muscle mass, the birds' tissues produced a corresponding increase in $\delta^{15}N$ values (Hobson et al., 1993). They hypothesized that under conditions of fasting and nutritional stress, a greater proportion of the nitrogen compounds available for protein synthesis are derived from catabolism and since this source of nitrogen has already been enriched in ^{15}N relative to diet, additional enrichment in the metabolic nitrogen pool must occur (Hobson et al., 1993). A consequence of this process is the increase in $\delta^{15}N$ values in all body tissues during stress, relative to periods without stress.

Only a small number of isotopic studies have examined metabolic state and diseases in humans. One of the first archaeological studies to look at the use of stable isotopes to reveal information about dietary habits and specific nutrient intakes in human bone was by White and Armelagos (1997) in which they examined whether isotopic compositions could be used as a biomarker to provide information about disease. White

and Armelagos (1997) provided stable isotope data from individuals who appeared to have an already compromised health status or were highly susceptible to metabolic disease such as osteopenia. Osteopenia is an abnormal reduction in bone mass due to poor mineralization where there are no fractures present. White and Armelagos, (1997) used $\delta^{13}C$ and $\delta^{15}N$ values in bone collagen of Sudanese Nubians from the X-Group period (AD 350-550) to evaluate the high frequency of osteopenia in this sample. Stable carbon isotope ratios indicated that both normal and osteopenic individuals consumed the same mixed diet of C_3 and C_4 sources; however, females with osteopenia had significantly elevated nitrogen values, particularly in the third and fifth decade of life (White and Armelagos, 1997). This early study demonstrated that enrichment of $\delta^{15}N$ values in human tissue can be used as a biomarker to provide information about disease such as osteopenia.

Following this, Petzke and coworkers (2005) investigated the bulk analysis of $\delta^{13}C$ and $\delta^{15}N$ values versus amino acids in modern human hair keratin to predict the dietary intake of animal-derived food. Results were similar to previous studies, that is $\delta^{13}C$ and $\delta^{15}N$ values accurately predicted the proportion of protein consumption in the diet; however they found no evidence that amino acids could better predict the proportion of animal protein intake than those from bulk isotopic values of hair (Petzke et al., 2005). A second study by Petzke and coworkers (2006) analyzed hair keratin to investigate the effects of a known physiological condition on $\delta^{13}C$ and $\delta^{15}N$ values and amino acids. The authors measured the $\delta^{13}C$ and $\delta^{15}N$ values in patients with cirrhosis of the liver and compared the results with those of healthy individuals and determined that $\delta^{15}N$ values

were significantly higher in cirrhotics than in healthy patients, whereas the $\delta^{13}C$ values and amino acids did not differ between ill and healthy patients (Petzke et al., 2006). The authors postulated that elevated $\delta^{15}N$ values in cirrhotic patients occurred for the same reason fasting or low protein diets caused a discrimination of ^{15}N against ^{14}N resulting in a relative enrichment of ^{15}N in plasma proteins and a depletion in urinary urea and ammonia (Petzke et al., 2006).

In a third study, Petzke and coworkers (2010) reviewed the literature on the use of stable isotope ratios and amino acids in hair keratin across many disciplines such as archaeology, nutrition, and medicine, to examine whether hair isotopic compositions could be used routinely as unbiased biomarkers to provide information about nutritional status, metabolism, and diseases. Their review of the literature indicated that hair is isotopically representative of dietary protein sources and that atmospheric contamination, cosmetic treatments, and graying were shown to not affect stable isotope values in hair if washed properly before sample preparation (Petzke et al., 2010). Based on the previous studies, the authors found that δ-values of hair could be used as unbiased markers to evaluate nutritional intake but they extended their inquiry to suggest that bulk hair δ-values are sensitive enough to be used as potential biomarkers for individuals with metabolic disorders such as diabetes (Petzke et al., 2010). The authors suggested that $\delta^{13}C$ and $\delta^{15}N$ values in hair keratin could be used to track changes in nitrogen balance during the transition from an anabolic state to a catabolic state during physiological stress and concluded their literature review by stating that $\delta^{13}C$ and $\delta^{15}N$ values may fractionate in unique patterns that can be used to better understand and diagnose variability in human

nitrogen balance (Petzke et al., 2010). This suggests that measuring stable isotope ratios in hair may have the potential for not only describing diet but also diagnosing diet-related diseases.

Williams and O'Connell (2002) used hair keratin as a unique way to test whether a diet rich in fish protein may protect against Alzheimer's disease. As mentioned earlier, $\delta^{15}N$ values can be used to distinguish marine-sourced foods from terrestrially-sourced foods, where $\delta^{15}N$ is significantly higher for marine foods. The authors analyzed $\delta^{15}N$ values in bulk in hair samples combined with a dietary questionnaire to assess an individual's consumption of meat, fish, dairy products, maize, rice, fruits, and vegetables. They used a control group not diagnosed with Alzheimer's disease and patients with Alzheimer's disease and found that individuals who reported eating fish frequently had higher $\delta^{15}N$ values, and that these individuals were less likely to have Alzheimer's disease (Williams and O'Connell, 2002). This study suggested that a diet rich in fish may protect against Alzheimer's disease as indicated by the direct correlation of individuals with lower $\delta^{15}N$ values (less fish in the diet) with cognitive deterioration (Williams and O'Connell, 2002).

Fuller and coworkers (2004, 2005) conducted two seminal studies using the sequentially segmented hair keratin of pregnant women to investigate $\delta^{13}C$ and $\delta^{15}N$ values during gestation. The first study explored the $\delta^{13}C$ and $\delta^{15}N$ values of pregnant women before and after giving birth and found that although there were no significant changes in $\delta^{13}C$ values, all of the subjects showed a decrease in $\delta^{15}N$ values (-0.3 to -1.1‰) during gestation (Fuller et al., 2004). The authors did not know the mechanisms

causing the decrease in hair $\delta^{15}N$ values, however since the $\delta^{15}N$ values of dietary nitrogen and urea nitrogen were significantly lower compared to maternal tissues, they hypothesized that the increased use of dietary and urea nitrogen for tissue synthesis during pregnancy resulted in a reduction of the steady state diet to a body trophic level effect by approximately 0.5-1.0‰ (Fuller et al., 2004). The authors also found an inverse relationship between hair $\delta^{15}N$ values and weight gain, suggesting that positive nitrogen balance results in a reduction of $\delta^{15}N$ values independent of diet (Fuller et al., 2004). These results led to the second study in which the hair samples of pregnant women who were experiencing nutritional stress associated with nausea and vomiting due to morning sickness (hyperemesis gravidarum) were measured for $\delta^{15}N$ and $\delta^{13}C$ values (Fuller et al., 2005). Again, the $\delta^{13}C$ values showed no change during morning sickness or pregnancy when compared with pre-pregnancy values; however $\delta^{15}N$ increased during periods of weight loss and or restricted weight gain associated with morning sickness (Fuller et al., 2005). Fuller and coworkers (2005) found that with normal weight gain and recovery from nutritional stress, the hair $\delta^{15}N$ values showed a decrease over the course of gestation towards birth. The results of these studies indicate that $\delta^{15}N$ values have the ability to monitor not only dietary intake, but also the nitrogen balance of an individual. In particular, the $\delta^{15}N$ values appear to be influenced by the nitrogen balance of an individual such that anabolic states can cause a decrease in $\delta^{15}N$ values and catabolic states can increase the $\delta^{15}N$ values.

Recent research has also found a means of diagnosing anorexia nervosa and bulimia nervosa by using $\delta^{13}C$ and $\delta^{15}N$ values in hair. Using only five hairs per

individual and measuring body mass index, Hatch and colleagues (2006) could distinguish between individuals with an eating disorder and those without (controls) in over 80% of subjects by detecting a change in $\delta^{15}N$ values over time. The authors found that the hairs of individuals with anorexia and bulimia were more variable in $\delta^{15}N$ values along the length of the hair than those from the normal control group, while the $\delta^{13}C$ values appeared to be less variable and the authors suggested that $\delta^{13}C$ values were less useful in distinguishing among individuals with an eating disorder and the controls (Hatch et al., 2006). A similar study by Mekota and coworkers (2006) conducted a serial analysis of $\delta^{13}C$ and $\delta^{15}N$ values in hair to monitor starvation and recovery phases in individuals diagnosed with anorexia nervosa. These authors found that $\delta^{15}N$ values were inversely related to body mass index, and during therapy administered in hospital to increase weight it was possible to observe dietary change and $\delta^{15}N$ value variability within days (Mekota et al., 2006). The elevated $\delta^{15}N$ values were thought be a result of a severe lack of dietary protein which causes an individual to metabolize their skeletal muscle tissue and such recycling of an individual's own body protein will produce the trophic level effect observed within the individual's body (Mekota et al., 2006). These studies demonstrate that a chronology of dietary intake can be reconstructed by the isotopic analysis of hair and that the isotopic values of hair can be clearly altered by the metabolic and physiological changes in the body.

Changes in diet related to anorexia, low protein intake, chronic iron deficiency, or pregnancy can produce fluctuations between growing hair (anagen) and resting hair (catagen/telogen), and subsequently increase the number of hair fibers in the

catagen/telogen phase (Hatch et al., 2006; Williams et al., 2011). Although the timing of growth phases tends to be constant in a healthy individual, factors such as intrinsic or hormonal changes can influence the duration of each phase in an unhealthy individual (Williams et al., 2011). Healthy individuals have an average ratio of anagen to telogen phase of approximately 10:2; however discrepancies in this ratio could be indicative of a pathological condition or a nonspecific stressor (Williams et al., 2011). Williams and coworkers (2011) were the first to use the growth phases of hair to determine the effects of age, sex, and health status on carbon and nitrogen isotopic ratios. This study selected hair samples from mummified individuals from the Kellis 2 cemetery, Dakhleh Oasis, Egypt dating from AD 40-450. Some of these individuals had identified pathological conditions determined through skeletal analysis, and the isotopic data for sequentially segmented hair identified the timing of the metabolic reaction of an individual to a stressful event (Williams et al., 2011). This separation of hair fibers into growth phases as a preliminary step is important because bulk analysis of hair typically assumes that approximately 80% of scalp hairs are in anagen (actively growing) phase at any given time and 20% of hairs are inactive. However, it has been found that individuals undergoing a physiological or nutritional stressor may have up to 60% to70% of their hair in the catagen/telogen phase (resting) (O'Connor and Hedges, 1999). Because of this, the isotopic values of hair in telogen phase (resting) can lag behind current diet; there can be as long as four to six month delays. Therefore, the growth phase analysis of hair provides a means for a finer resolution of the isotopic values and enables for more exact timing of a stressful event. The study by Williams and colleagues (2011) demonstrated the

importance of understanding the positional-temporal relationship for accurate interpretations of isotopic analysis of sequentially segmented hair and suggests that the analysis of variability in $\delta^{13}C$ and $\delta^{15}N$ values in hair has the potential to aid in interpreting the metabolic balance of an individual close to the time of death.

5.10 Summary

Research on the use of hair keratin in stable isotope analysis in both animals and humans has clearly demonstrated the feasibility of using hair not only to reconstruct short-term dietary change but also to monitor an individual's metabolic reaction to stress. Numerous studies have demonstrated the applicability of using isotopic analysis of hair keratin in both modern and archaeological populations to track patterns of diet, seasonality, and weaning. Evidence from recent research has shown that hair isotopic compositions can be used as biomarkers to provide information about nutritional status, metabolism, and disease. While the studies discussed in the previous sections have established that $\delta^{13}C$ and $\delta^{15}N$ in hair keratin reflect diet in individuals they have not always accounted for possible confounding factors such as physiological conditions that can influence the body's overall nitrogen balance (Meier-Augenstein, 2010). In addition to the primary effect of the diet, $\delta^{15}N$ values in human hair are also likely to be affected by the metabolic state of the individual where $\delta^{15}N$ may be elevated if the body is undergoing stress, so the seminal research by Williams and colleagues (2011) using trichogram analysis has provided a means to control for the potential lag in isotopic values due to metabolic stress.

Chapter 6

Material and Methods

6.1 Introduction

Archaeological context is essential in the analysis of skeletal remains as it provides the framework for the interpretation of results. This chapter describes the excavation of the St. Thomas' cemetery, and the techniques employed for sex and age estimations of the Belleville samples, followed by a description of the methods used to identify pathology in the skeletal remains. A detailed explanation of sample preparation and the process of data acquisition using the trichogram analysis and mass spectrometry will also be described below.

6.2 Excavation of the Belleville Sample

During the summer of 1989, a total of 579 grave shafts were excavated from an area of 1.75 acres, representing one-third of the original grounds of the St. Thomas' cemetery (Saunders et al., 1993). The well drained, sandy soil allowed for excellent preservation such that over 60% of the adult burials were in the range of good to almost perfect preservation (Jimenez, 1991). Fully 87% of all adult skeletons were sufficiently complete to evaluate trauma and infection on all of the bones (Saunders et al., 1994). A total of 604 individuals were analyzed from the 579 grave shafts, indicating that some burials contained more than one individual, often a mother and child or infant. It was determined that 282 individuals were immature or under the age of 15 years (subadults),

while the remainder were older adolescents or adults. A total of 149 infants (<1 year) were identified among the 282 subadults (Saunders et al., 1995). The remains were transferred to McMaster University for analysis and were retained for a period of one year after which they were reburied at St. Thomas' new church cemetery in September, 1990 (Herring et al., 1991). Following excavation, researchers from 15 different universities studied the skeletal remains and collected morphological, histological, genetic, and chemical data for subsequent analysis (Herring et al., 1991). Rib samples were obtained from 439 individuals and preserved hair samples were obtained from 72 individuals and are curated at McMaster University (Saunders et al., 1995). There are no cemetery plans indicating who was buried in each plot, but there are burial records indicating who was buried in the cemetery.

6.3 Sex Estimation

Of the 604 individuals who were documented using coffin plates and burial records, sex estimation for 245 individuals from the Belleville sample was determined from intact morphological features of the bony pelvis and cranium (Rogers, 1991). Rogers (1991) examined six features on the pelvis (true pelvis shape, sacrum shape, subpubic concavity, pubis shape, ventral arc, obturator foramen) and eight features on the cranium (supraorbital ridges, mastoid size, malar size and shape, occipital markings, chin form, general size, zygomatic root extension, mandibular shape) to estimate sex. Tests of accuracy of morphologically determined sex from the pelvis using the documented

sample were highly reliable yielding an accuracy rate of 99% (Rogers and Saunders, 1994).

The lack of sexually dimorphic traits in individuals who have not yet undergone puberty made sex estimation of subadults difficult and there are no widely accepted standards for determining sex in subadults. For this reason, there was no attempt to establish the sex of subadult skeletal remains (Saunders et al., 1995).

6.4 Age Estimates

Macroscopic evaluation of age-at-death was completed by Rogers (1991) using several morphological methods (e.g., Lovejoy et al., 1985; Suchey, 1988). Observations of changes to the pubic symphysis and the auricular surface of the innominates, metamorphosis of the sternal rib ends, dental wear, and dental changes were recorded. Age-at-death for adults was also estimated using histological analysis of bone thin sections (Saunders et al., 1995). Average age-at-death of adults in the skeletal sample was 42.5 years and the average age-at-death for the total burial sample in the cemetery taken from the parish records was 47.9 years (Saunders et al., 1997).

Subadult age was estimated using dental development, dental eruption, diaphyseal length, and degree of epiphyseal fusion (Saunders, 2000). Dental development was used as the primary method of age estimation for subadult skeletons because the formation of tooth crowns and roots is much less affected by hormonal influences, as well as environmental, nutritional, and social factors (vs. skeletal development) (Saunders, 2000). A total of 266 subadults were assigned a dental age based on Moorrees and colleagues

(1963a, 1963b) standards for tooth formation for three deciduous and twelve permanent teeth. Sixteen subadult individuals were assigned ages from long bone diaphyseal lengths (Saunders et al., 1997).

The age and sex of the individuals used in this study (n=10) included one subadult, five females, and four males and their ages ranged from 7 years to 57.5 years old. Table 6.1 below summarizes the age and sex distribution of the sample, as well as the reported pathology associated with each individual in the sample. All but one sample (B251) had burial records that were used to corroborate the individuals age and sex.

6.5 Methods Used to Identify Pathology

Macroscopic examination by Jimenez (1991) recorded the specific locations of pathological changes to bone (e.g., distal, proximal). Jimenez (1991) also recorded the type of pathology (e.g., lytic, sclerotic) and the extent of the pathological process (e.g., size and number of lesions). Fragmentary remains that exhibited signs of pathological change were also included in the overall assessment. Jimenez (1991) used a magnifying glass to determine the specific region of bone involvement (i.e., diploe, cortical bone) and the presence and degree of healing was recorded. Utilizing complete or nearly complete skeletons, it was possible to reach a differential diagnosis based on pathological changes in 146 cases (Jimenez, 1991). The overall preservation of the skeletal material was also recorded (excellent, good, fair, poor) in order to assess whether or not the data obtained were reliable, and preservation was generally good to excellent (Jimenez, 1991). Skeletal elements demonstrating evidence of either trauma or infectious disease were

photographed and recorded. For the purpose of this study the results obtained from Jimenez' (1991) palaeopathological analysis were compared to the burial records in order to correlate sex, age, and possible cause of death with the hair specimens.

6.6 Description of Pathology of Individuals Chosen for This Study

Ten individuals were chosen for this study, six had evidence of pathology in the form of non-specific infections or healed/unhealed fractures, and four displayed no osteological evidence of pathology (Table 6.1). Non-specific infection, including periostitis, occurred within the sample (Jimenez, 1991). Periostitis is an inflammation of the periosteum and is characterized by fine pitting, longitudinal striation, and plaque-like new bone formation on the cortical surface of the affected bone (Roberts and Manchester, 2005). In one case, a subadult (B364) exhibited a ridge of active periostitis along the superior edge of the right mandibular alveolar border and evidence of a dental abscess. The sinus or hole around the tooth root developed to allow pus to escape due to the accumulation of micro-organisms from periodontal disease (Roberts and Manchester, 2005). Individual B262 had active periosteal reactions on the mandibular ramus and left and right parietals. The other case (B442) demonstrated possible areas of mild, active periostitis on the left and right parietals (Jimenez, 1991).

Traumatic injuries were present in the St. Thomas' skeletal sample (76/250 or 30.4%) (Jimenez, 1991). Perimortem fractures are manifested in three of the individuals chosen for this study and there was evidence of healed fractures (B151, B339, B260), and one unhealed (B339) fracture within the sample.

Table 6.1: Summary Table Age, Sex, and Pathology in the Belleville Samples Used in This Study

Burial No.	Age-at Death	Sex	Pathology (Jimenez, 1991)
B76	27	M	-No reported pathology
B92A	37	F	-No reported pathology -Buried with infant
B 151	57.5	F	-Healed rib fractures: Left 5, 6, 7, 11 Right 2, 5 -Healed fracture 3rd left metatarsal -Healed fracture right clavicle
B251	Unknown	F	-No reported pathology
B260	35.4	M	-Schmorl's nodes on most vertebrae -Healed fracture on left ribs 5,6,8,9,10,11 -Healed fracture on left humerus
B262	40	F	-Active periostitis on ramus and left and right parietals -Active periostitis right inner mandible
B339	55	M	-Misaligned unhealed fracture on right humerus -Healed rib fracture, right 9 -Suspected rheumatoid arthritis, 2nd , 3rd proximal phalanges fused to intermediate phalanges with no bone growth in left hand
B 343	42.6	M	-No reported pathology
B 364	7.34	Unknown	-Infection in left mandible: ridge of active periostitis along the superior edge of the right mandibular alveolar border -Evidence of a dental abscess -Skull infection (location unknown)
B442	36.6	F	-Possible zones of active periostitis on left and right parietals -No other reported pathology

6.7 Inventory of Hair Samples

The Bellville collection contains 72 preserved hair samples associated with complete skeletons that were assigned burial numbers. All of the hair specimens were examined visually and the length of the hair fibers was estimated (in cm) to determine if the hair was long enough for a sequential stable isotope analysis (Appendix A). Ten individuals were chosen based on the length and preservation of the hair fibers (four males, five females, one subadult). Generally, any hair specimen over 7 cm (representing seven months growth) was considered for further analysis. There was an attempt to balance equal number of males and females; however more females were ultimately included in the sample because females tended to have longer hair prior to death. When necessary the hair specimens were gently cleaned using distilled water to separate the hairs fibers from each other and to remove excess dirt, dried fluids, and insects. After choosing the ten individuals, Jimenez's (1991) original pathological records were examined and an inventory of the exact type and location of the pathology or trauma found on the individual skeleton was recorded. This information was then correlated with the individual's hair specimen using burial numbers as a reference.

6.8 Sample Preparation and Trichogram Analysis

A trichogram is a method of hair analysis that involves plucking 50 to 100 hairs from different parts of the scalp, placing them on a glass slide, and examining them under a microscope. The aim is to determine the rate of hair loss progression by establishing the percentage of hair in each of the three stages of the hair growth cycle, anagen (growth),

catagen (transition to resting) and telogen (resting). Approximately 10-20% of the hairs should be in the resting phase; any more may indicate a potential health problem (Dhurat, 2006).

Each hair fiber was washed with distilled water and examined with a digital microscope and photographed. Every hair root was photographed under 120x magnification using a Nikon SMZ 1000 digital microscope. The hair roots were compared to microscopic archetypes of each stage as described in Petraco and Kubic (2003). Anagen phase hairs have an epithelial sheath attached to the root and a pointed or cup-shaped root tip (Figure 6.1). Telogen phase hairs have an enlarged root bulb or rounded root tip, and catagen hairs have a slightly enlarged root with some epithelial tissue attached (Figure 6.2) (Van Neste et al., 2007; Williams et al., 2011). Two groups of hair strands per individual were selected for isotopic analysis and stored temporarily in labelled tin foil pouches. One group consisted of hairs in the anagen phase (growing) and the other group contained hairs in the catagen (transitional) or telogen (fully resting) phase. There were, therefore, two sets of samples for each individual chosen for stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$.

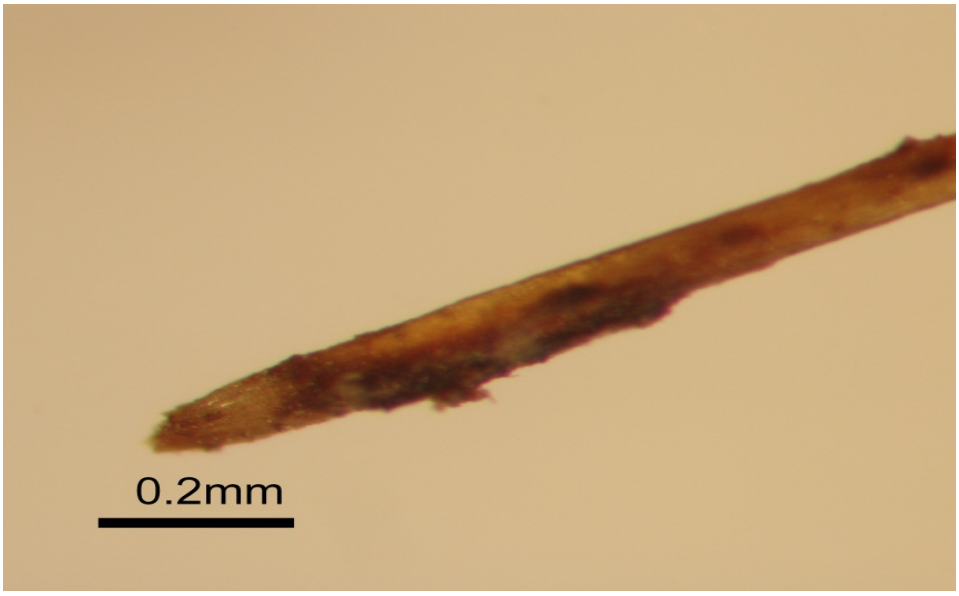

Figure 6.1: Anagen (growth) Phase (note pointed tip), Sample B343, 120x magnification.

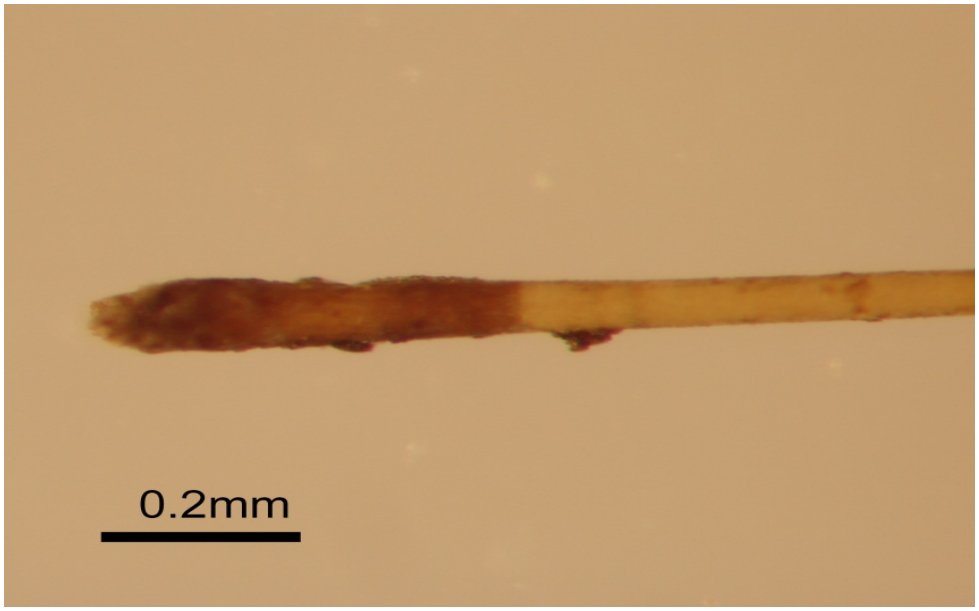

Figure 6.2: Telogen (resting) Phase (note rounded tip), Sample B343, 120x magnification.

6.9 Sample Preparation for Stable Isotope Analysis

The trichogram analysis was based on the methodology described by Williams and colleagues (2011). After separating 50 hair strands into anagen (growing) and catagen/telogen (resting) groups for each individual, the two bundles of hair fibers were laid out on a template of metric graph paper that had been laminated in plastic. A glass sheet from a picture frame was then placed over the graph template that was cleaned with methanol. The hair sample was placed on the graph template and distilled water was dribbled onto the hair sample in order to straighten and align the hair fibers. Using tweezers the hair was gently aligned along the graph template with the proximal (root) end facing left or on the zero line. The sample fibers were pinched together at the centimeter mark, and a stainless steel scalpel blade was carefully rolled across the hair fibers to cut the segment. Following this procedure, the rest of the hair sample was cut into sequential one cm segments and stored separately in labelled tin foil pouches. The sequence of hair segments were labelled from proximal to distal ends on the foil. The one cm segments were ultrasonicated first with distilled water for 10 minutes then soaked in methanol and chloroform (2:1 v/v) to remove surface contamination (after O'Connell and Hedges, 1999; Williams et al., 2011). The methanol-chloroform solution was changed after 10 minutes and hair segments were rinsed again with distilled water and utrasonicated for 10 minutes after the change of fresh solution. Detergents were not used because these have been known to damage the hair surface (Taylor et al., 1995). Segments were rinsed three times with distilled water and air dried for 24 hours to remove any remaining water. Hair colorants (hydrogen peroxide or henna) could elevate

the C:N ratios, however O'Connell and Hedges (1999) determined that most dyed hair falls within the accepted C:N range of 2.9 to 3.8. If hair strands had been treated with colouring agents they were eliminated from the sample set, however, there is no difference between the C:N ratios or the isotopic values of grey and non-grey hair (O'Connell and Hedges, 1999). For this study, no samples were included that contained grey hair or evidence of colouring agents. The hair segments were stored in labelled foil pouches and placed in a sequentially labelled ice cube tray to maintain proper order during storage. The hair segments were weighed into high purity tin capsules using a microbalance. This was done by flattening the tin cups (that contain the hair samples in the mass spectrometer) into a disc, tarring the tin disc on the microbalance, and gently placing the 1cm hair segments onto the tin disc until the required weight was obtained. The ideal weight for carbon and nitrogen values for hair tissue is between 0.4-0.5 milligrams and each sample required approximately 7 to 8.5 individual 1cm hairs to reach that weight. The $\delta^{13}C$ and $\delta^{15}N$ values for the specimens were determined using a mass spectrometer.

6.10 Mass Spectrometer

To analyze the $\delta^{13}C$ and $\delta^{15}N$ in each sample, measurements were obtained using a Thermo Finnigan Delta Plus XP, TC-EA continuous flow isotope ratio mass spectrometer (CF-IRMS) in the MRSI laboratory at McMaster University. Each sample was analyzed for percentages of carbon and nitrogen as well as the ratio of carbon to nitrogen or C:N. In addition, the data included the amplitude for both carbon and nitrogen

in each sample. This measured the strength of the signal that the mass spectrometer was receiving from each sample. Amplitudes that were too high or too low may indicate unreliable results. The mass spectrometer was calibrated to operate best within a particular amplitude range, which for both carbon and nitrogen was between 2,300 and 3,000 millivolts. With each run of samples, standardized samples were included for which the amplitude was already known. This ruled out equipment error and allowed for comparison with unknown samples. The results for the C:N ratios were given in weight percents (C wt% and N wt%). To calculate the atomic C:N ratios the data set was converted using the formula C_w/N_w x 1.16616 (see Appendix B).

The atomic C:N ratios from the hair samples were used to evaluate diagenesis and the results were compared to laboratory standards to assess accuracy. The stable isotope data were reported as $\delta^{13}C$ (‰) relative to VPBD and $\delta^{15}N$ (‰) relative to AIR. Precision of analysis for $\delta^{13}C$ was ±0.1‰ and $\delta^{15}N$ was ±0.2‰ and based on the reference samples, the mass spectrometer was correctly calibrated; therefore any questionable data would be the result of the samples themselves.

6.11 Statistical Analysis

Statistical tests were used to determine whether any significant differences exist within the Belleville samples when comparing paired isotopic values of anagen phase and catagen/telogen phase hair. Non-parametric statistics were applied to the Belleville data because of the small sample size (n=10 individuals) and because the data were not normally distributed. Non-parametric statistics make no assumptions about the parameters

of the population from which the sample was obtained (Madrigal, 2012). Statistical tests were performed using Microsoft Excel statistiXL Version 1.8 (add in package) and all graphs were completed using Microsoft Excel 2010. The mean differences in $\delta^{13}C$ and $\delta^{15}N$ values were conducted using Wilcoxon matched pairs signed rank test and linear regression was used to independently regress the two paired samples where a lag appeared to determine the best match or optimal shift to identify growth cycle error (Schwertl et al., 2003; Williams et al., 2011).

Chapter 7

Results

7.1 Introduction

This chapter begins with the results of the mean $\delta^{13}C$ and $\delta^{15}N$ values to provide a general estimate of the diet that the individuals used in this study were consuming. Next, the results of the trichogram analysis are presented and when used in conjunction with stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$, provide a means to assess the metabolic state of Belleville individuals in the months prior to death. The results of the $\delta^{13}C$ and $\delta^{15}N$ values are presented graphically in order to observe variability over time for each individual and fluctuation of $\delta^{15}N$ values greater than 1‰ may indicate that an individual was potentially in a catabolic or anabolic state resulting from physiological stress or pregnancy.

7.2 General Isotope Data

A total of 216 hair samples were run in the mass spectrometer and results for the isotopic analysis are provided in Appendix B. The atomic C:N ratio was used to measure the effects of diagenesis and to determine the quality of preservation of the hair keratin samples. Atomic C:N ratios for all hair keratin samples measured had a mean of 3.5 ± 0.1. O'Connell and colleagues (2001) consider contamination likely when values fall outside the range observed for modern hair samples (2.9-3.8). All of the hair samples (n=216) were well within the range with one exception. One of the hair segments from individual B262 (C6) had a C:N ratio of 3.94, which was marginally outside the range so the data

from this sample was discarded. The range of isotopic values as represented by the minimum and maximum values for $\delta^{13}C$ were -20.8‰ and -17.9‰ respectively, and for $\delta^{15}N$ were 8.4‰ and 13.0‰. The mean isotopic values were calculated by summing the data for all individuals and dividing by the number of samples (Appendix B); the overall mean for the entire sample for $\delta^{15}C$ was -19.7 ± 0.3‰ and the mean for $\delta^{15}N$ was 10.6 ± 0.5‰.

7.3 Diet for the Belleville Sample

The mean $\delta^{13}C$ value (-19.7 ± 0.3‰) indicates that these individuals were consuming predominantly C_3 plants and meat from herbivores on a C_3 diet. Although maize and sugarcane were available to the inhabitants of Belleville, they did not consume significant quantities of these C_4 plants. Delta ^{15}N values are linked to the consumption of proteins from terrestrial animals, fish, or legumes and the mean $\delta^{15}N$ values of 10.6 ± 0.5‰ indicates that these individuals consumed principally herbivore meat and that fish and legumes did not play a significant role in the diet (Iacumin et al., 1998). Katzenberg and coworkers' (2000) study on $\delta^{13}C$ and $\delta^{15}N$ values for bone collagen used to reconstruct the diet of Belleville inhabitants found similar results with the $\delta^{13}C$ and $\delta^{15}N$ values for bone collagen averaging -19.4 ± 0.7‰ and 11.1 ± 1.4‰, respectively.

7.4 Trichogram Results

The trichogram analysis examined the form and structure of the hair fibers, and quantified the number of individual hair roots in each of the different growth phases for each individual and was used as an indicator of stress. Results of the trichogram analysis are provided in Table 7.1 and the percentage of catagen/telogen (resting) hairs was calculated by dividing the number of catagen/telogen hairs by the total number of hairs observed (n=50).

Table 7.1: Trichogram Analysis for Belleville Hair Samples

Burial ID	Anagen (Growing) Hairs (n)	Telogen/Catagen (Resting) Hairs (n)	% Telogen/Catagen (Resting)
B76	40	10	20%
B92A	33	17	34%
B151	31	19	38%
B251	36	14	28%
B260	30	20	40%
B262	35	15	30%
B339	30	20	40%
B343	41	9	18%
B364	36	14	28%
B442	40	10	20%

In normal healthy individuals, it is assumed that approximately 80-90% of scalp hairs are in anagen phase (growth) at any given time and 10-20% of hairs are inactive (catagen/telogen phase) (Van Neste et al., 2007). Results of the trichogram analysis indicate that seven individuals had catagen/telogen rates greater than the commonly accepted 10-20% average for normal healthy individuals. Of the ten individuals sampled, only three had catagen/telogen (resting) rates within the accepted 10-20% range (B76,

B343, B442, indicated in red in Table 7.1). In three cases (B151, B339, B260) the catagen/telogen rates were double the 'normal' range of 10-20%.

Combined results from the trichogram analysis and evidence of skeletal pathology or injury indicated that individuals with the highest rate of hair fibers in the catagen/telogen (resting) phase exhibited pathological changes to their skeleton (Table 7.2). One exception was B251 who had relatively high catagen/telogen rates (28%), but with no reported pathology. Another interesting exception to the expected growth phase rate was B92A, an adult female who displayed no evidence of pathology or trauma on her skeleton, but had a high catagen/telogen rate (34%) and burial records report that she was buried with an infant.

Table 7.2: Combined Results from Trichogram and Pathology for the Belleville Samples (pathology component adapted from Jimenez, 1991)

Burial No.	**Age**	**Sex**	**Telogen/Catagen (Resting) %**	**Pathology (Adapted from Jimenez, 1991)**
B76	27	M	20%	-No reported pathology
B92A	37	F	34%	-No reported pathology -Buried with infant
B151	57.5	F	38%	-Healed rib fractures: Left 5, 6, 7, 11, Right 2, 5 -Healed fatigue fracture 3rd left metatarsal -Healed fracture right clavicle
B251	?	F	28%	-No reported pathology
B260	35.4	M	40%	-Schmorl's nodes on most vertebrae -Healed fracture on left ribs 5,6,8,9,10,11 -Partially healed fracture on left humerus

Burial No.	Age	Sex	Telogen/Catagen (Resting) %	Pathology (Adapted from Jimenez, 1991)
B262	40	F	30%	-Active periostitis on ramus and left and right parietals -Active periostitis right inner mandible
B339	55	M	40%	-Misaligned unhealed fracture to right humerus -Healed rib fracture, right 9 -Suspected rheumatoid arthritis, 2^{nd} , 3^{rd} proximal phalanges fused to intermediate phalanges with no bone growth in left hand
B343	42.6	M	18%	-No reported pathology
B364	7.34	?	28%	-Infection on left mandible: ridge of active periostitis along the superior edge of the right mandibular alveolar border -Evidence of a dental abscess -Skull infection (location unknown)
B442	36.6	F	20%	-Possible zones of active periostitis on left and right parietals -No other reported pathology

7.5 Isotopic Results for Pathological vs. Non-pathological Individuals

The mean $\delta^{13}C$ value for non-pathological individuals was -19.8 ± 0.3‰ and the mean $\delta^{13}C$ value for pathological individuals was -19.6 ± 0.9‰. The mean $\delta^{15}N$ value for pathological individuals was 10.9 ± 0.6‰ and for non-pathological individuals, the mean $\delta^{15}N$ value was 10.5 ± 0.7 ‰ (Appendix C). Although there was a slight elevation for the pathological $\delta^{15}N$ values (0.4‰ variation), the means for $\delta^{13}C$and $\delta^{15}N$ values were expected to be approximately equal for pathological and non-pathological individuals because it is not until the values are plotted versus distance along the hair shaft (in one cm segments) that one can establish intraindividual variation.

7.6 Variation of Carbon and Nitrogen Isotopic Values between Anagen and Catagen/Telogen Growth Phase Hairs

Comparison of paired isotopic values for anagen (growing) and catagen/telogen (resting) phase hairs indicate there is a mean difference of -0.04 ± 0.3‰ in $\delta^{13}C$ values and a mean difference of 0.14 ± 0.4‰ in $\delta^{15}N$ values (Appendix D)**.** Table 7.3 summarizes the mean values of the anagen (growth) and catagen/telogen (resting) phases along the hair fiber for each individual.

Table 7.3: Mean Isotopic Values between Anagen and Catagen/Telogen Hair Phases

Burial ID	$\delta^{15}N$ (‰) Anagen	$\delta^{15}N$ (‰) Catagen/Telogen	$\delta^{13}C$ (‰) Anagen	$\delta^{13}C$ (‰) Catagen/Telogen
B76	11.1	11.3	-20.2	-20.2
B92A	9.1	9.0	-19.7	-19.8
B151	11.0	11.0	-18.4	-18.4
B251	11.1	10.6	-19.8	-19.6
B260	10.9	10.7	-20.5	-20.5
B262	10.3	9.6	-20.5	-20.4
B339	10.8	10.9	-19.6	-19.7
B343	10.8	10.8	-19.7	-19.6
B364	11.3	11.4	-20.6	-20.6
B442	11.3	11.3	-19.5	-19.5

The null hypothesis states that there is no difference between the mean $\delta^{13}C$ and $\delta^{15}N$ values in the hair fibers for the same individual. The overall mean difference in both $\delta^{13}C$ and $\delta^{15}N$ values was not statistically significant ($p>0.05$). When comparing the means of two sample sets a paired *t* test can be used, but this test assumes that the data are normally distributed and randomly selected. In this case, the hair fibers were from 10 individuals

resulting in a data set that is very small, not randomly generated, nor normally distributed. Because the basic assumptions for the *t* test were not met, the Wilcoxon signed rank test was used. Wilcoxon signed rank test is more powerful in detecting the mean difference between two sample sets that are not random or normally distributed (Madrigal, 2012). To calculate the mean differences between two samples, the data are ranked and the sum of the ranks is called T. The T statistic is compared with the critical values; if the sample T is less than or equal to the critical value then the null hypothesis is rejected (Madrigal, 2012). Table 7.4 displays the T statistic and the critical value for this data set. N represents the number of hair segments in each of the samples. Table 7.4 shows that the T statistic for all of the samples was greater than or equal to the critical value, indicating that the null hypothesis was not rejected (i.e., there is no difference between the mean $\delta^{13}C$ and $\delta^{15}N$ values for hair in the anagen phase versus hair in the catagen/telogen for the same individual). The T statistic for B251 was much higher than the others because of the larger sample size (n=30). Overall, this means that there was little to no difference between the mean $\delta^{13}C$ and $\delta^{15}N$ values between the hair fibers in the anagen phase versus hair fibers in the catagen/telogen phase for the same individual. This outcome was not unexpected as paired $\delta^{13}C$ and $\delta^{15}N$ isotopic values along the hair fiber should be representative of the same relative time periods in an individual and should therefore reflect the same overall mean values.

Table 7.4: The Wilcoxon Signed Rank Test Summary Statistics

Identifier	N (number of hair segments)	T-Statistic	Critical Value
B76 Carbon	7	3.0	2
B76 Nitrogen	7	2.0	2
B92A Carbon	17	49.5	34
B92A Nitrogen	16	61.0	29
B151 Carbon	11	10.0	10
B151 Nitrogen	11	29.0	10
B251 Carbon	29	186.5	126
B251 Nitrogen	29	148.0	126
B260 Carbon	5	7.0	0
B260 Nitrogen	5	0.0	0
B262 Carbon	5	3.0	0
B262 Nitrogen	5	0.0	0
B339 Carbon	8	3.0	3
B339 Nitrogen	8	13.0	3
B343 Carbon	7	7.0	2
B343 Nitrogen	7	11.0	2
B364 Carbon	4	4.0	0
B364 Nitrogen	4	4.0	0
B442 Carbon	4	4.0	0
B442 Nitrogen	4	3.0	0

7.7 Graphs of Variation in Carbon and Nitrogen Values along the Length of Hair

Another way to explore variation in the data was to plot $\delta^{13}C$ and $\delta^{15}N$ isotopic values versus distance from proximal (hair root) to distal hair ends (left to right). Meier-Augenstein (2010) stated that variability of $\delta^{15}N$ values in hair keratin ~1‰ is indicative of physiological factors such as nutritional or physiological stress and are thought to be the result of muscle protein catabolism that makes up for the lack of dietary protein (Meier-Augenstein, 2010). Under conditions of physiological stress there will be larger variation (>1‰) in $\delta^{15}N$ values coinciding with less change (<1‰) in $\delta^{13}C$ values, and the

smaller variation in $\delta^{13}C$ values may indicate a lack of change in diet. If $\delta^{15}N$ values varied (increased or decreased) by 1‰ or more, accompanied by less variability in $\delta^{13}C$ values, this was considered a potential indicator of physiological stress. Recent research by Neuberger and coworkers (2013) isotopically analyzed hair keratin from malnourished individuals or individuals who experienced starvation prior to death, and considered $\delta^{15}N$ shifts less than 1‰ to be significant. For example, their study treated a nitrogen elevation of only 0.54‰ as significant and they suggested that this (smaller) nitrogen enrichment was an indicator of malnutrition and starvation (Neuberger et al., 2013).

The following provides a detailed description of the study samples and a graphic display of the $\delta^{13}C$ and $\delta^{15}N$ isotopic values plotted for each individual to determine if variation existed between the anagen (growing) and catagen/telogen (resting) values.

7.7.1 Sample B151:

Individual B151 was a 57 year old female with a healed clavicle and rib fracture and a healed fatigue/stress fracture on her third left metatarsal (Jimenez, 1999). Results of her trichogram analysis indicated that individual B151 had catagen/telogen rates outside the commonly accepted 10-20% average for normal healthy individuals (Van Neste et al., 2007). At 38%, her rate of hairs in the catagen/telogen phase was double the assumed 'normal' range of 10-20% (Table 7.1). Variability greater than 1‰ was not evident in the $\delta^{15}N$ values for B151 (Figures 7.1 and 7.2). The $\delta^{15}N$ values ranged from 10.6‰ to 11.3‰ which showed a variation of 0.7‰ and based on Meier-Augenstein's (2010) minimum value of 1% this was not considered significant. The $\delta^{13}C$ values ranged from

-18.1‰ to -18.6‰ (0.5‰ variation) which showed slightly less variation than $\delta^{15}N$. Delta $\delta^{13}C$ values show a slight decrease in the months prior to death that may indicate a shift to a more terrestrial C_3 plant based diet.

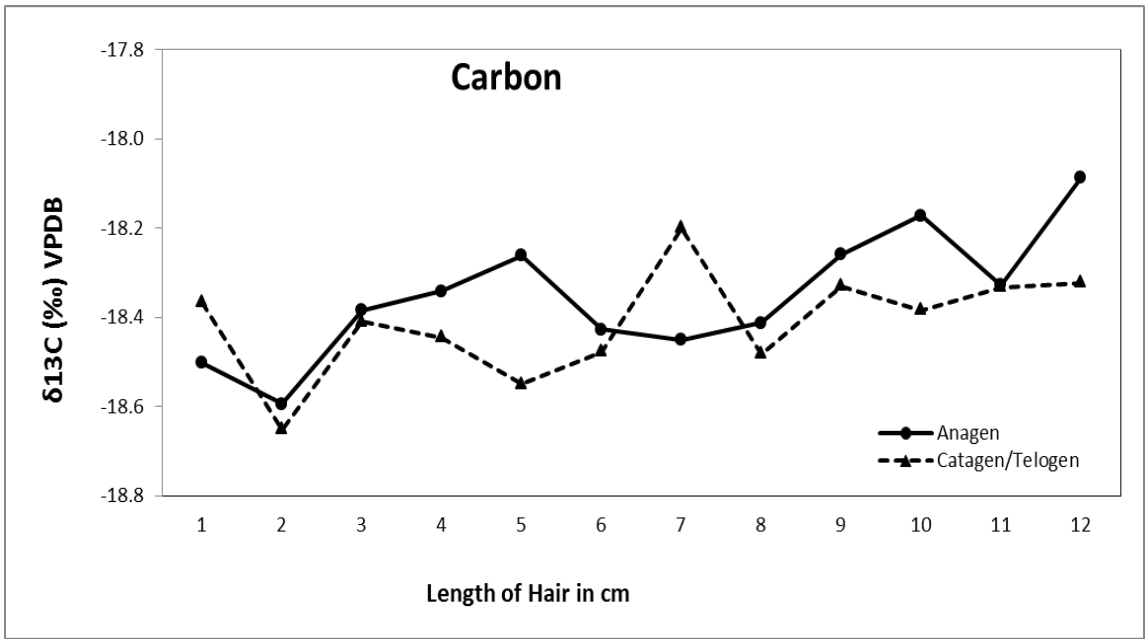

Figure 7.1: Carbon isotope variation for sample B151 (Note: 1cm represents the value closest to the scalp or just prior to death)

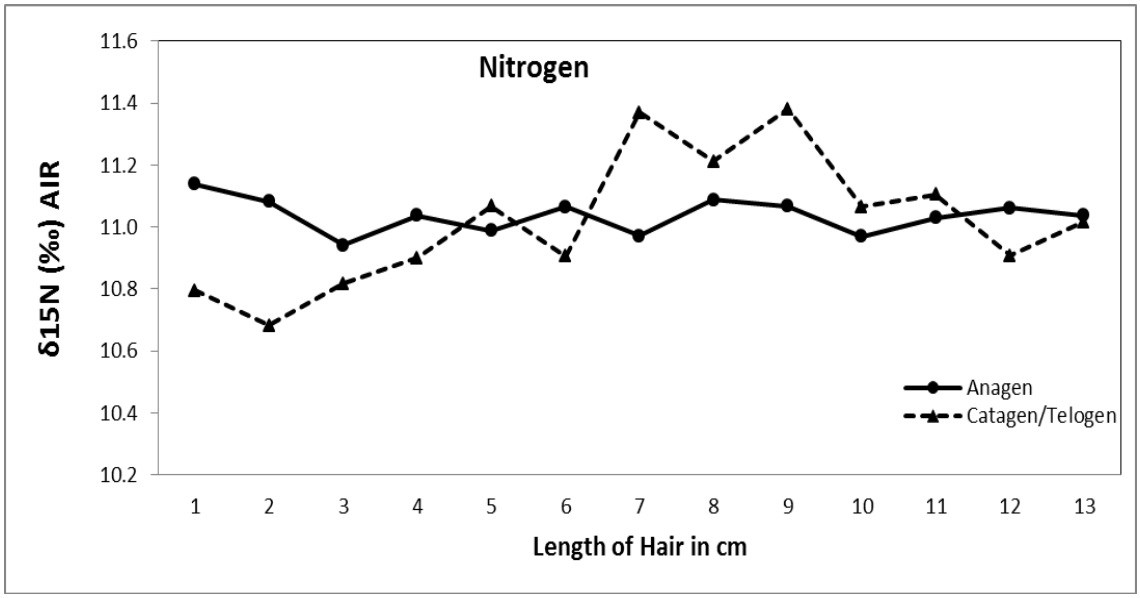

Figure 7.2: Nitrogen isotope variation for sample B151. (Note: 1cm represents the value closest to the scalp or just prior to death).

7.7.2 Sample B364:

Individual B364 was a seven year old subadult of undetermined sex who had evidence of infection on the left mandible in the form of a ridge of active periostitis along the superior edge of the right mandibular alveolar border, evidence of a dental abscess, and a skull infection (location unknown) prior to death (Jimenez, 1999). Results of the trichogram analysis indicate that 28% of this individual's hair was in the catagen/telogen phase, which suggests that this person was undergoing stress. Delta ^{13}C values ranged from -20.3‰ to -20.8‰ (0.5‰ variation) and $\delta^{15}N$ values ranged from 10.7‰ to 12.0‰ (1.3‰ variation) (Figures 7.3 and 7.4). The $\delta^{15}N$ values showed pronounced variability and there was clear elevation of $\delta^{15}N$ values (1.3‰) starting at two and six months prior to death and peaking in months one and five (shown by the red arrows in Figure 7.4), whereas the $\delta^{13}C$ values only show slight decrease. Similar to B151, there is a general

decline in $\delta^{13}C$ values closer to death, however the lack of pronounced variability in the $\delta^{13}C$ values suggest that there was no dramatic change in this individual's diet prior to death, whereas the elevated $\delta^{15}N$ values may be indicative of a catabolic response to the infectious process or the body's metabolic reaction to a febrile illness associated with the dental abscess, skull and mandibular infections. The presence of two $\delta^{15}N$ value elevations (at one and five months) helps support the hypothesis that this may have been a repeated febrile illness, possibly associated with the infections (Figure 7.4).

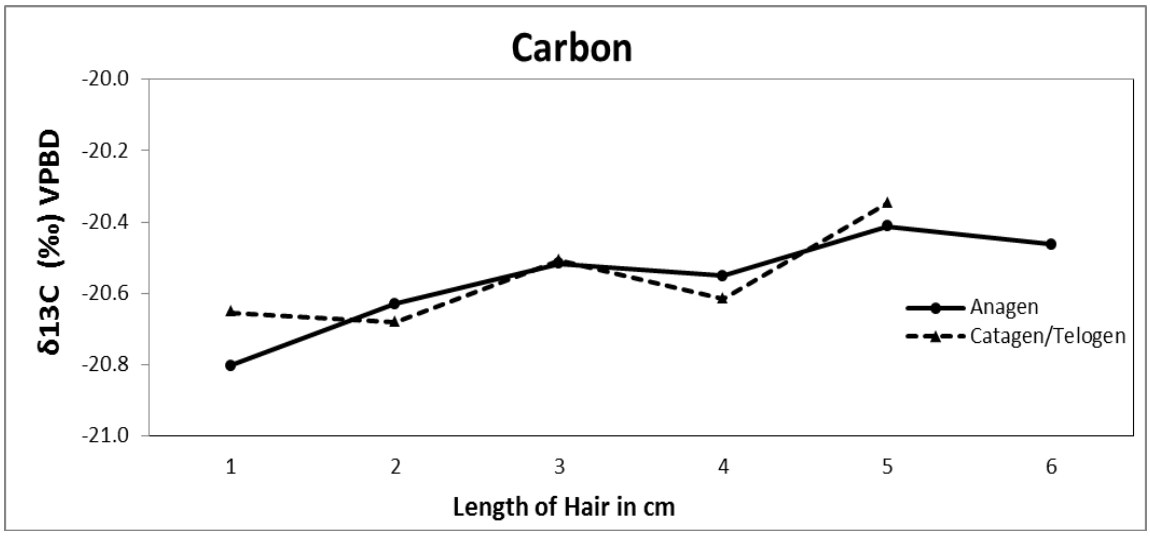

Figure 7.3: Carbon isotope variation for sample B364.

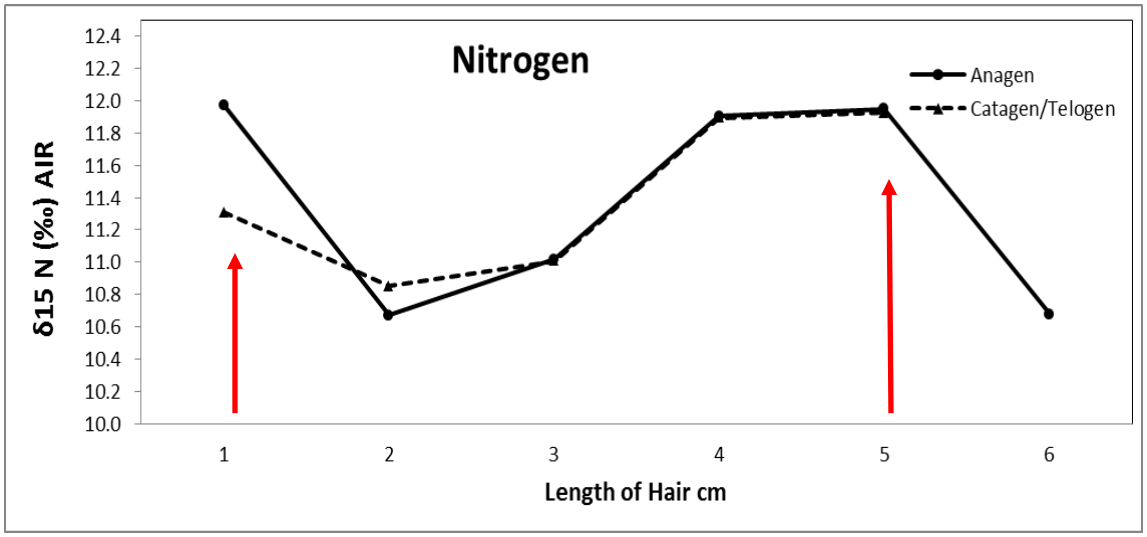

Figure 7.4: Nitrogen variation for sample B364.

7.7.3 Sample B76:

Individual B76 was a 27 year old male with no osteological evidence of trauma, infection, or reported pathology. The trichogram analysis indicated that only 20% of his hair was in the catagen/telogen phase suggesting that he was not undergoing physiological or nutritional stress prior to death. His $\delta^{13}C$ values range from -20.1‰ to -20.5‰ (0.4‰ variation) and the $\delta^{15}N$ values ranged from 11.1‰ to 11.4‰ (0.3‰ variation) (Figures 7.5 and 7.6). There is little variability in both $\delta^{13}C$ and $\delta^{15}N$ values that suggest there was little metabolic alteration in association with injury, disease, or a change in dietary input. One can hypothesize that the lack of evidence for stress combined with his relatively young age suggests the possibility that he died as a result of an accident, acute illness, or injury. The isotopic and trichogram evidence appear 'normal', yet he died at the age of 27 years.

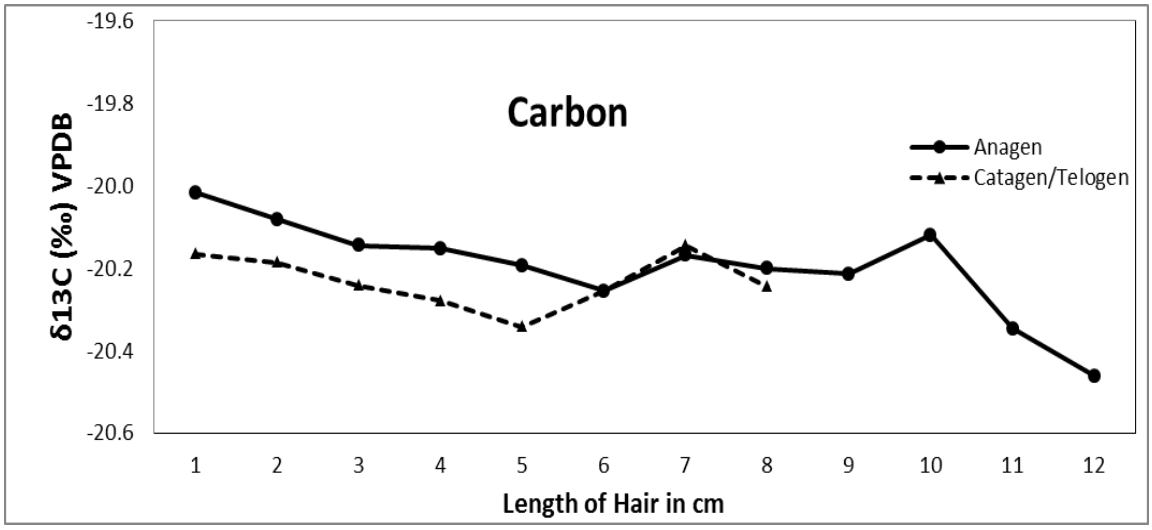

Figure 7.5: Carbon isotope variation for sample B76.

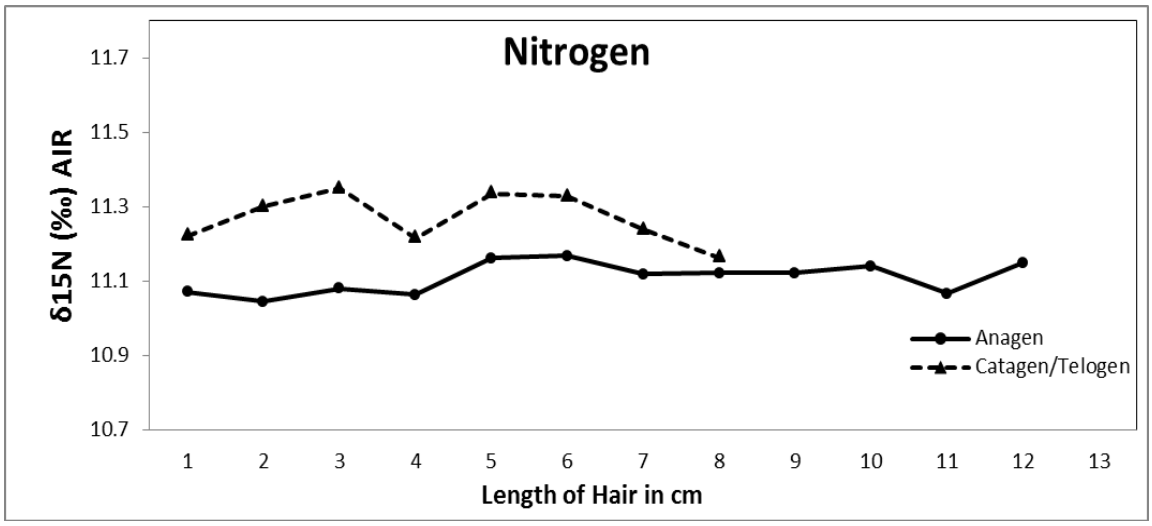

Figure 7.6: Nitrogen isotope variation for sample B76.

7.7.4 Sample B339:

Individual B339 was a 55 year old male with osteological evidence of arthritis (i.e., evidence of lipping, erosion of bones on the medial phalanges of the right hand, with no new bone growth), a misaligned unhealed fracture to his right humerus, and a healed rib fracture (Jimenez, 1999). His trichogram analysis showed that 40% of his hair was in the catagen/telogen phase (resting) which is indicative of stress. Delta ^{13}C values ranged from -19.4‰ to -19.7‰ (0.3‰ variability) and $\delta^{15}N$ values ranged from 10.5‰ to 11.7‰ (1.2‰ variability) (Figures 7.7 and 7.8). Approximately four months prior to death, his $\delta^{15}N$ values start to increase and peak in the second month by1.2‰ and this suggests an extended period during which his nitrogen values were increasing (shown by red arrow in Figure 7.8). His $\delta^{13}C$ values show less variation indicating that his diet did not change drastically (as shown by the small variation in $\delta^{13}C$ values over the same time frame), however, the combined evidence of the $\delta^{15}N$ values with the trichogram results is highly suggestive of physiological or nutritional stress four months prior to his death.

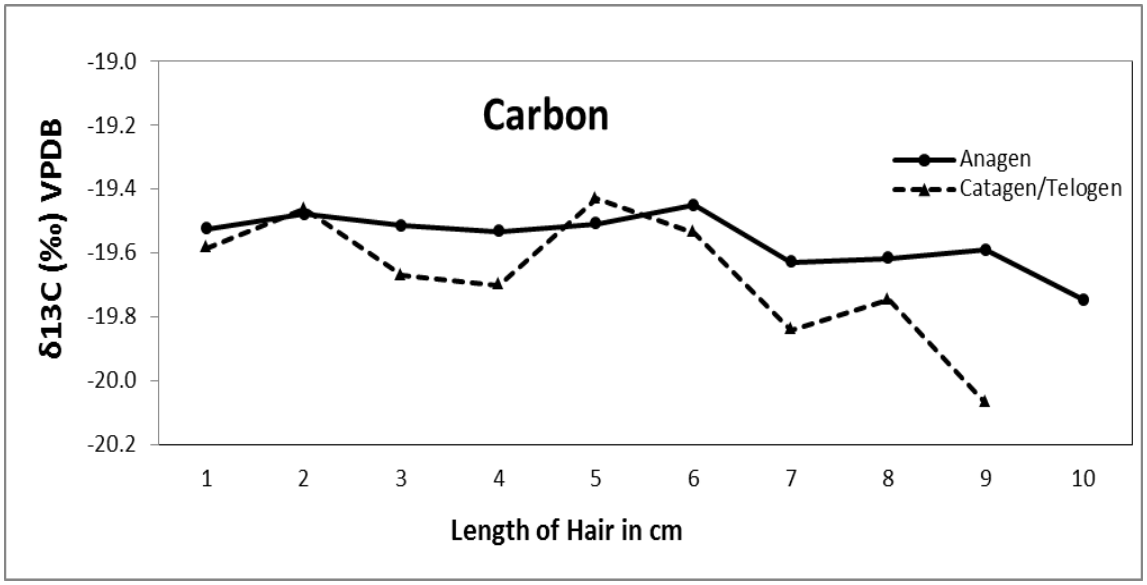

Figure 7.7: Carbon isotope variation for sample B339.

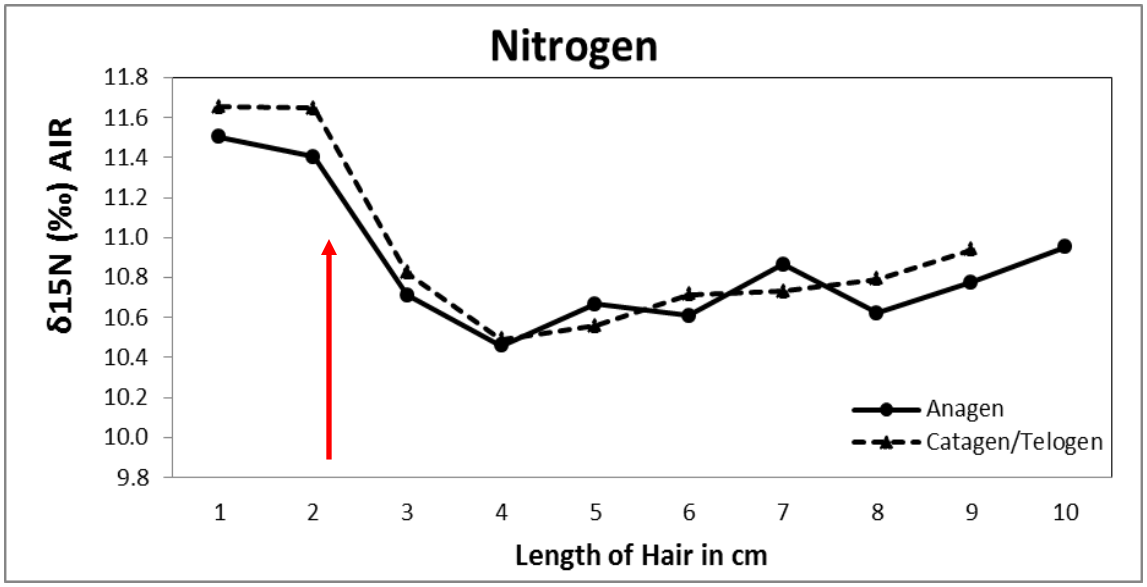

Figure 7.8: Nitrogen isotope variation for sample B339.

7.7.5 Sample B260

Individual B260 was a 35 year old male with evidence of Schmorl's nodes on most vertebrae, healed fractures on his left ribs (5, 6, 8, 9, 10, 11), and a partially healed fracture on his left humerus (i.e., evidence of remodeling) (Jimenez, 1999). His trichogram analysis showed that 40% of his hair was in the catagen/telogen phase (resting) which indicated this individual was undergoing stress in the 10 month period before he died. Figures 7.9 and 7.10 show that $\delta^{13}C$ values ranged from -20.4‰ to -20.7‰ (0.3‰ variation), whereas $\delta^{15}N$ values ranged from 10.2‰ to 11.5‰, showing an elevation of 1.3‰ between months three and four prior to his death (shown by the red arrow in Figure 7.10). This individual exhibited an increase in $\delta^{15}N$ values that corresponded with a slight increase in the $\delta^{13}C$ values at the three to four month mark, so there appears to be notable increase in nitrogen, which is suggestive of a metabolic reaction to stress or a possible dietary shift prior to death. This implies that the combination of $\delta^{13}C$ and $\delta^{15}N$ values can aid in discerning stress from a dietary shift.

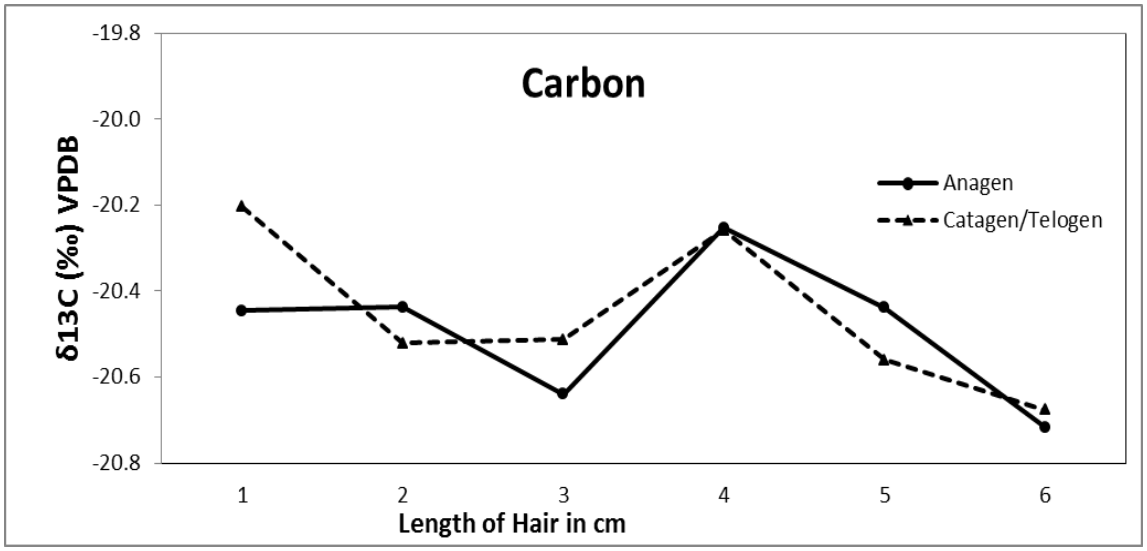

Figure 7.9: Carbon isotope variation for sample B260.

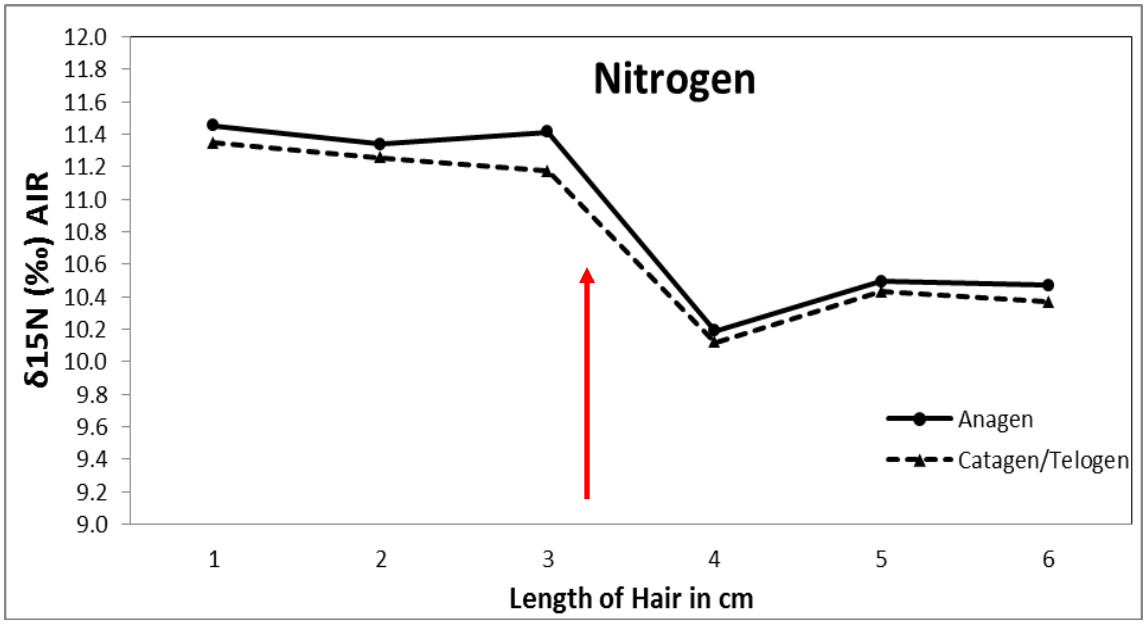

Figure 7.10: Nitrogen isotope variation for sample B260.

7.7.6 Sample B92A

Individual B92A was a 37 year old female with no reported pathology or skeletal lesions visible; she was buried with an infant that was approximately eight months old (0.87 years) (Jimenez, 1991). The trichogram analysis showed that 34% of her hair was in catagen/telogen phase, which is indicative of stress and $\delta^{13}C$ values ranged from -19.4‰ to -20.3‰ (0.9‰ variation). The $\delta^{15}N$ values ranged values showed considerable variation ranging from 8.3‰ to 10.0‰ (1.7‰ variation). Contrary to the expected $\delta^{15}N$ value enrichment, this individual showed a decrease in $\delta^{15}N$ values (1.3‰) for a nine month period between months 8 and 17 (shown by red arrows in Figure 7.12); the $\delta^{13}C$ values also showed a similar decrease. Given her age-at-death, the absence of any indicators of chronic illness or injury, the presence of an infant, and the significant variation in $\delta^{15}N$ values between sequential segments 8 to 17 which represents a nine month interval, it is hypothesized that individual B92A may have been pregnant in the year before her death. During pregnancy, the body becomes anabolic and enters a positive nitrogen balance that may result in the $\delta^{15}N$ depletion exhibited by this individual (Duggleby and Jackson, 2002; Williams et al., 2011). Since there is also a slight increase, followed by a decrease in $\delta^{13}C$ values, one cannot exclude the possibility that her diet changed in association with pregnancy or was altered to support subsequent breastfeeding after the birth of the infant.

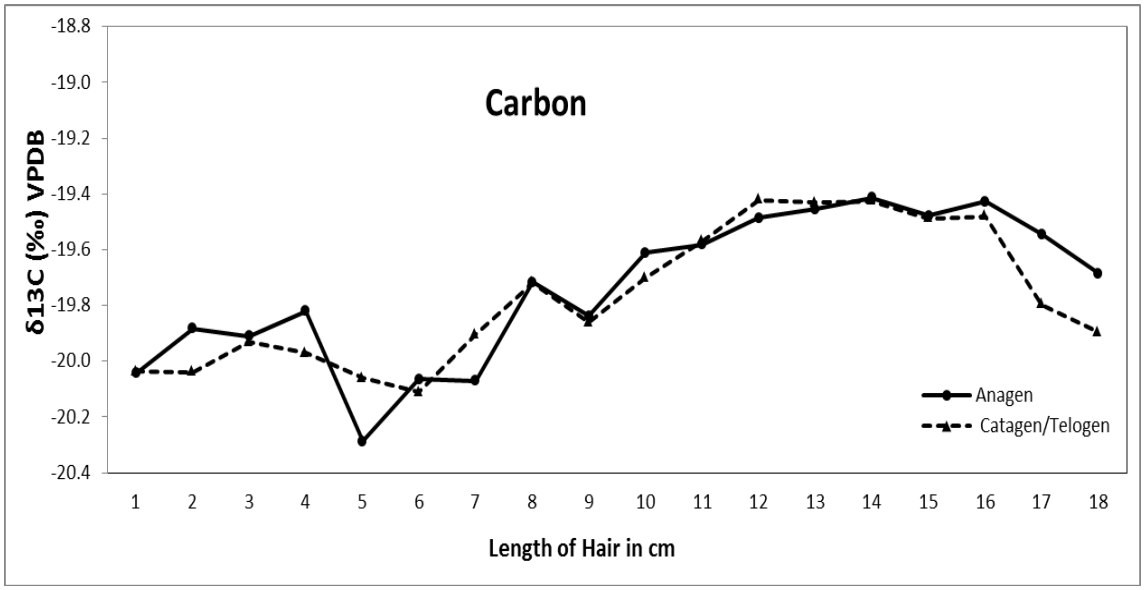

Figure 7.11: Carbon isotope variation for sample B92A.

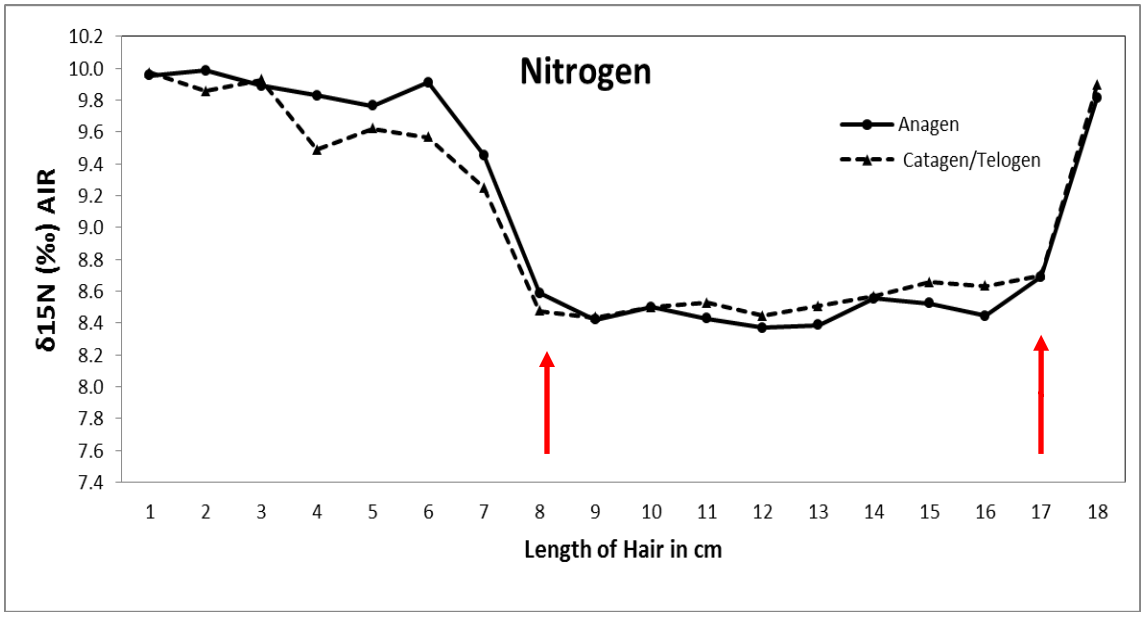

Figure 7.12: Nitrogen isotope variation for sample B92A.

7.7.7 Sample B442

Individual B442 was a 36 year old female who displayed possible areas of active periostitis on the left and right parietals although this finding was not definitive, and there was no other reported pathology or injury (Jimenez, 1999). The trichogram analysis showed that 20% of her hair was in catagen/telogen which was within the range of a normal healthy individual (Van Neste et al., 2007). The $\delta^{13}C$ values ranged from -19.4 to -19.6‰ (0.2‰ variation), and the $\delta^{15}N$ values ranged from 11.1‰ to 11.4‰ (0.3‰ variation) indicating modest variation (Figures 7.13, 7.14). Despite the evidence of parietal periostitis there appeared to be no evidence of significant stress in the five months prior to death as exhibited by her low trichogram rate and low variation in both her $\delta^{15}N$ and $\delta^{13}C$ values. This suggests that the parietal periostitis may have occurred earlier in her life (longer than five months prior to death).

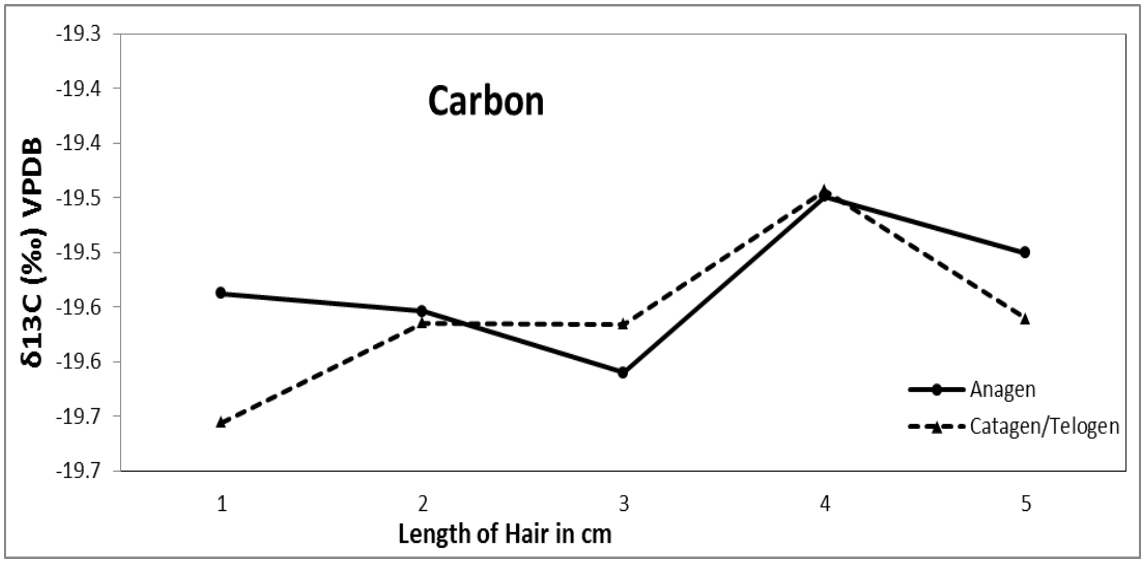

Figure 7.13: Carbon isotope variation for sample B442.

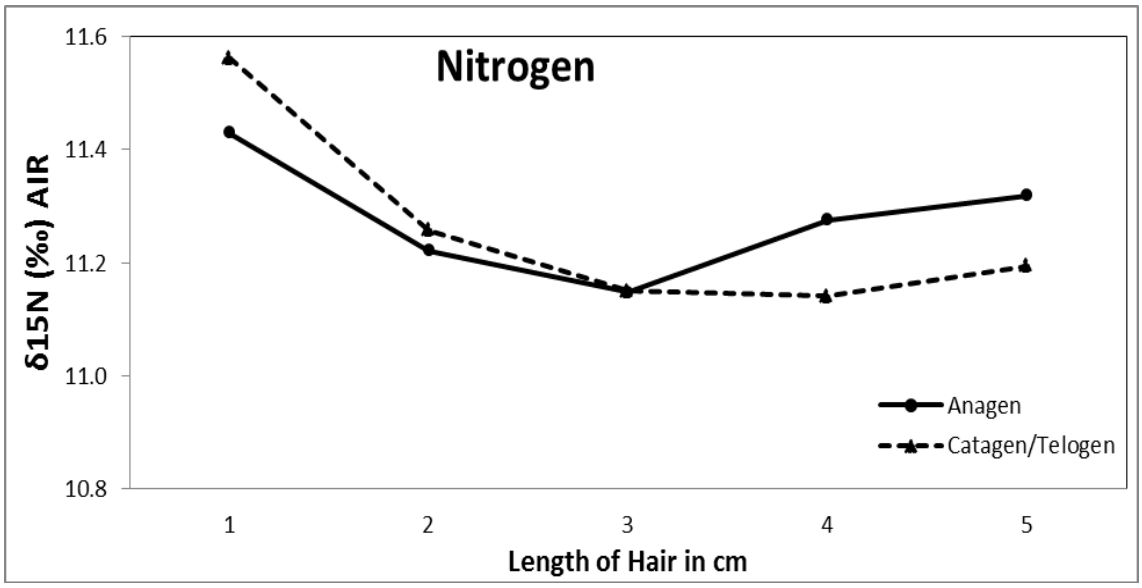

Figure 7.14: Nitrogen isotope variation for sample B442.

7.7.8 Sample B251

There were no burial records for individual B251, therefore sex was inferred by the length of the hair (over 35 cm) that had been formed into a chignon and held in place with metal clips in a style that was fashionable for women in the 19th century. The trichogram analysis showed that 28% of her hair was in catagen/telogen phase, which suggest that she was undergoing stress prior to death, and the $\delta^{13}C$ values were between -19.1‰ and -20.3‰ (1.2‰ variation), while $\delta^{15}N$ values ranged from 9.2‰ to 13.0‰ (3.8‰ variation). Both her $\delta^{13}C$ and $\delta^{15}N$ values exhibited pronounced variability with the $\delta^{15}N$ elevating significantly (1.9‰) at around nine months prior to death (Figures 7.15, and 7.16). Similar to B92A, this individual displayed lower $\delta^{15}N$ values during a nine month period between months 10 to 19, which suggests that this person may have

been pregnant in the two years before her death (shown by the red arrows in Figure 7.16). There is considerable variability in $\delta^{13}C$ values prior to conception (~21 months) as well as pronounced variability in $\delta^{15}N$ values 10 months prior to death which suggests possible dietary shift associated with breastfeeding or possible illness prior to death. One hypothesize is that an infection from childbirth resulted in a febrile illness that ultimately led to her death.

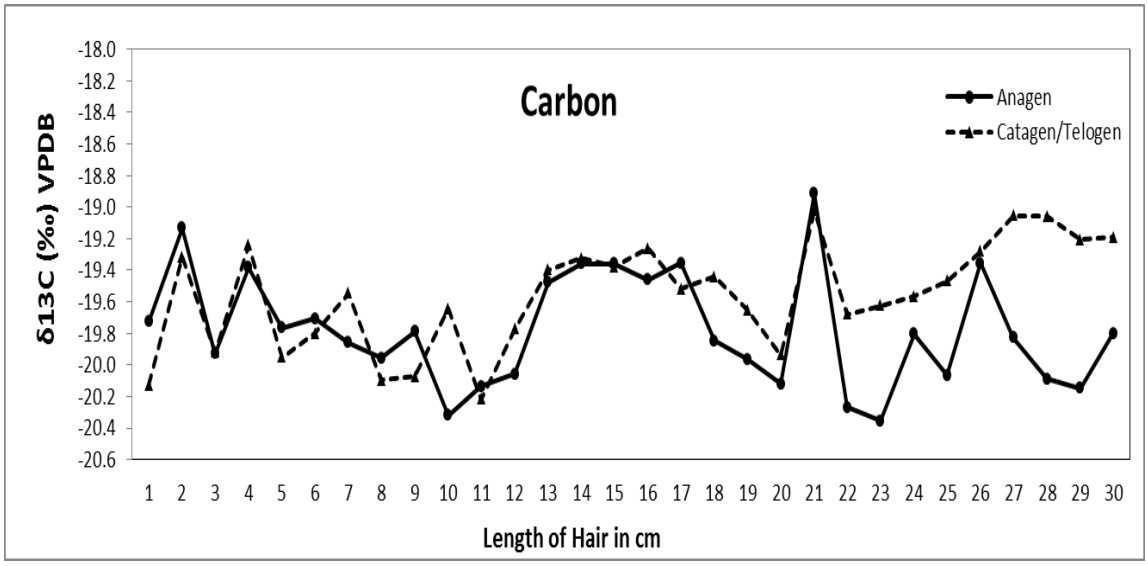

Figure 7.15: Carbon isotope variation for sample B251.

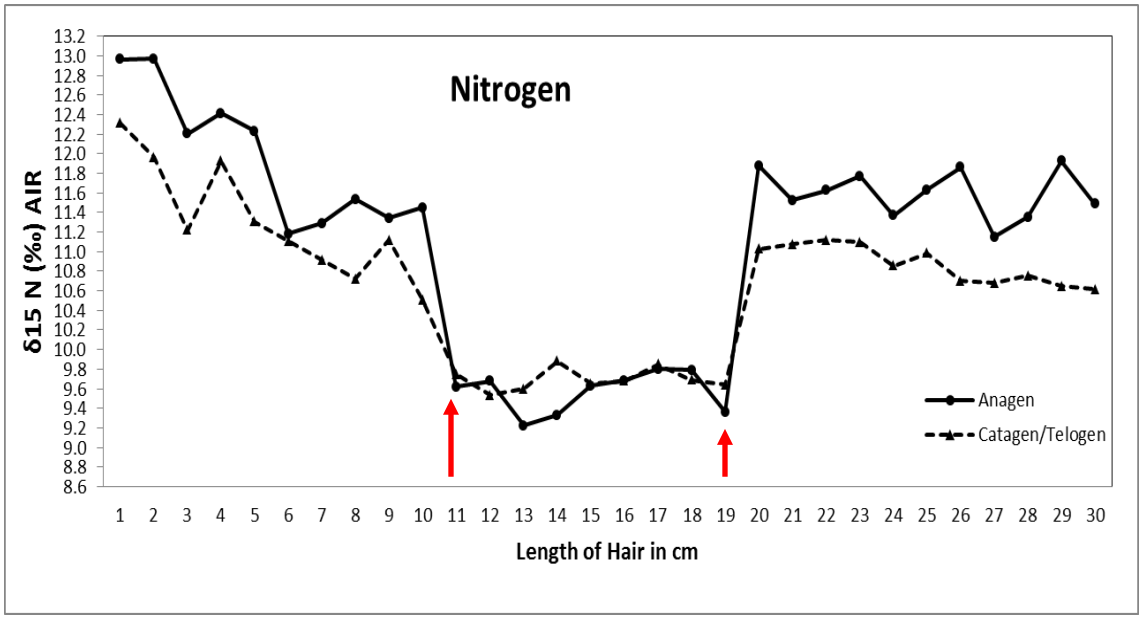

Figure 7.16: Nitrogen isotope variation for sample B251.

7.8 Samples with a Growth Cycle Error Requiring Optimal Shifts

Of the 10 individuals sampled two (B262 and B343) had paired trichogram values that indicate a possible growth cycle error. In some cases, when the catagen/telogen (resting) hair is sampled, it may contain more than just the most recently formed hair tissue and as a result, the isotopic data for these samples may not be representative of the isotopic data for the comparative time interval (Williams et al., 2011). The catagen/telogen hairs could be considered older than anagen hairs because they rest in the hair follicle for a varying length of time before they are shed. In some cases, the isotopic values for catagen/telogen (resting) hairs may therefore lag behind the values for anagen (growing) hairs (O'Connell and Hedges, 1999; Williams et al., 2011). The consequence of this is that if stable isotope analyses are conducted on bulk hair samples, without

determining the proportion of hairs in anagen versus catagen/telogen, then an incorrect estimation of the timing of dietary shifts and/or stress events will occur due to the potential lag. Schwertl and coworkers (2003) developed a method to identify growth cycle error in animal hair segments in which isotope data for the catagen/telogen growth phase hair segments were aligned with anagen phase segments in one cm steps until the best match between hairs in the anagen phase and those in the catagen/telogen phase was obtained. Williams and coworkers (2011) took this a step further by statistically testing for the optimal correspondence by independently regressing $\delta^{13}C$ and $\delta^{15}N$ values of the paired samples while the position of one sample was sequentially shifted relative to the other. The optimal shift was defined as the one that produced the highest correlation coefficient or r^2, between the $\delta^{13}C$ and $\delta^{15}N$ values (Schwertl et al., 2003; Williams et al., 2011). High correlation coefficients calculated for the $\delta^{13}C$ and $\delta^{15}N$ values indicate how well the regression line approximates the data (Madrigal, 2012). This number determines what proportion of the one variable's variance (hair in the catagen/telogen phase) is best matched by the other variable's variance (hair in the anagen phase) (Madrigal, 2012). In other words, the higher correlation coefficient helps to guide the number of centimeters needed to backtrack the catagen/telogen hair fibers to match the anagen phase hairs in order to determine the optimal match. The original mismatch between the two samples is most likely caused by a growth cycle error and in most cases the optimal shift needed is between one and three centimeters (Schwertl et al., 2003; Williams et al., 2011). No research to date explains why a growth cycle error occurs in some hair samples and not others, but the individuals with the growth cycle error had long hair (over 30 cm). Long

hair is an indicator of a very long growth cycle (over seven years in the anagen phase); therefore long hair has the potential to have more hairs lagging behind in the catagen/telogen phase.

7.8.1 Sample B262:

Individual B262 was a 42 year old female with osteological evidence of active periostitis on the mandibular ramus and left and right parietals (Jimenez, 1999). Periostitis refers to new bone formation on the external cortex resulting from inflammation of the periosteum, which is caused by various types of infection or trauma (Ragsdale, 1993; Roberts and Manchester, 2005). Because osseous reactions to tissue inflammation look similar, regardless of the original cause of the inflammatory response, it is not possible to ascribe a single cause to the pathological changes observed on individual B262. The trichogram results indicate some form of physiological or nutritional stress as 30% of her hair was in the catagen/telogen phase. Delta^{13}C values ranged from -20.3‰ to -20.5‰ (0.2‰ variation) and the δ^{15}N values ranged from 9.6‰ to 12.0‰, resulting in a 2.4‰ elevation in nitrogen approximately one month before death.

For individual B262 the optimal shift was one cm, indicating that isotopic signals for this individual were approximately one month behind in their reflection of diet. Table 7.5 records the r^2 values for B262 and the highest r^2 value correspond to a one cm optimal shift (shown in red). This can be seen graphically (in Figures 7. 17, 7.18, 7.19, 7.20) where the δ^{13}C and δ^{15}N values are shown first with original data and secondly with the

one cm shift. To obtain the r^2 values the data is shifted manually and the Excel program calculates the correlation.

Table 7.5: r^2 Values of Carbon and Nitrogen for Sample B262

B262 Optimal shift	r^2
δ^{13}C: no shift	0.08
δ^{13}C: 1 cm shift	0.65
δ^{13}C: 2 cm shift	0.27
δ^{15}N: no shift	0.73
δ^{15}N: 1 cm shift	0.98
δ^{15}N: 2 cm shift	0.28

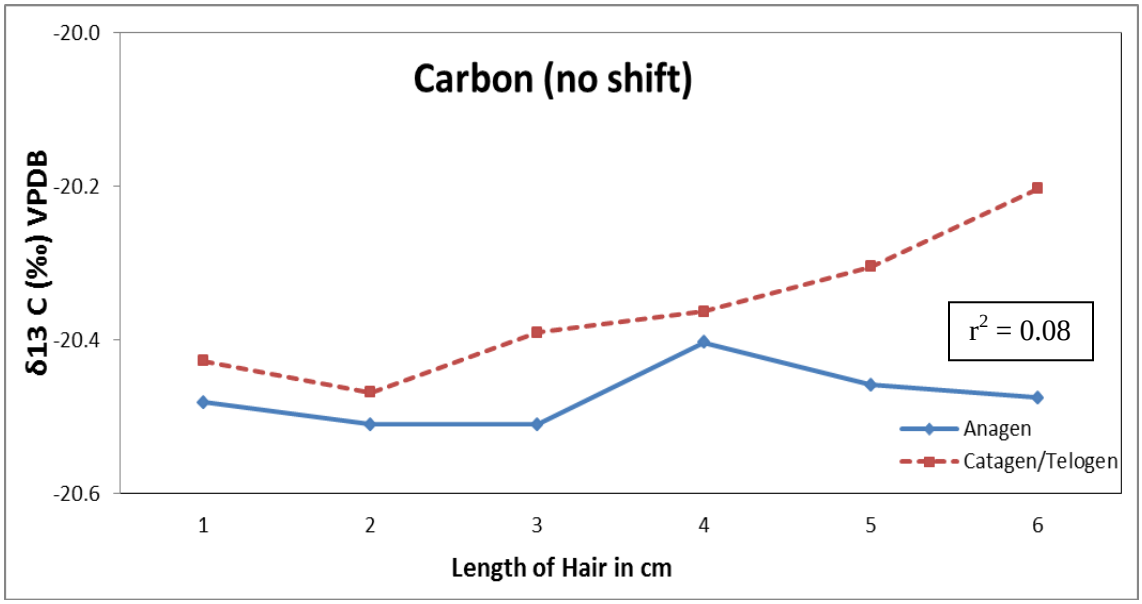

Figure 7.17: Carbon isotope variation for sample B262 with no shift (does not account for growth cycle error).

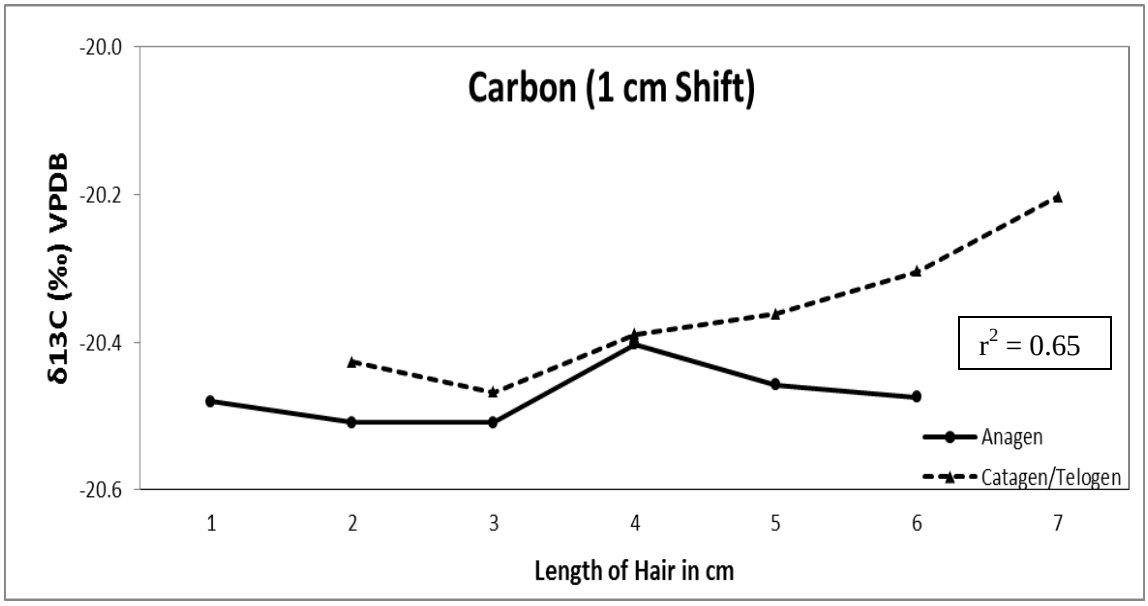

Figure 7.18: Carbon isotope variation for sample B262 with a one cm shift.

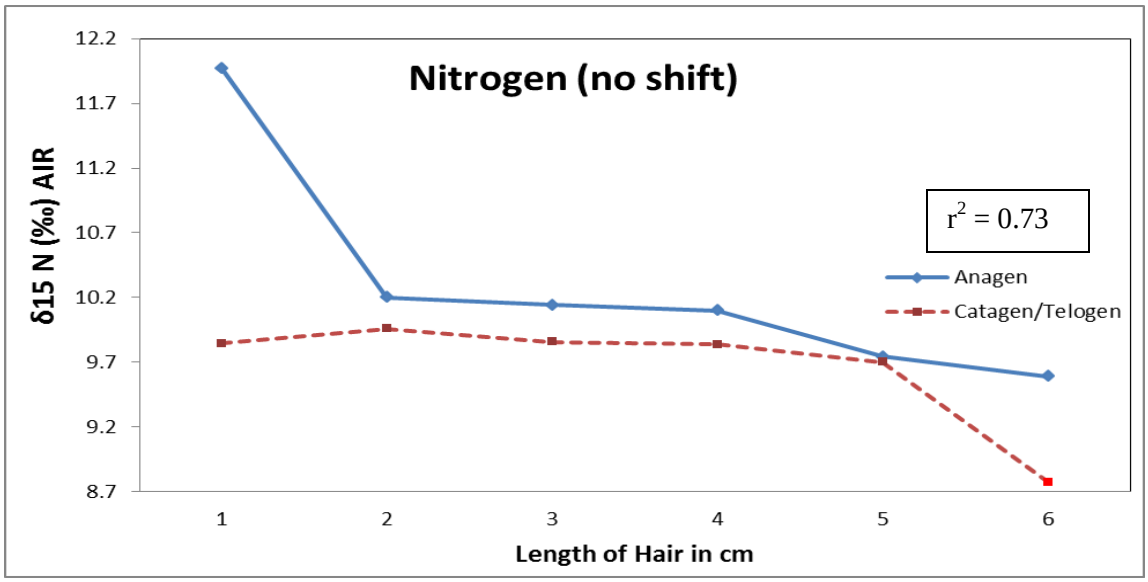

Figure 7.19: Nitrogen isotope variation for sample B262 with no shift (does not account for growth cycle error).

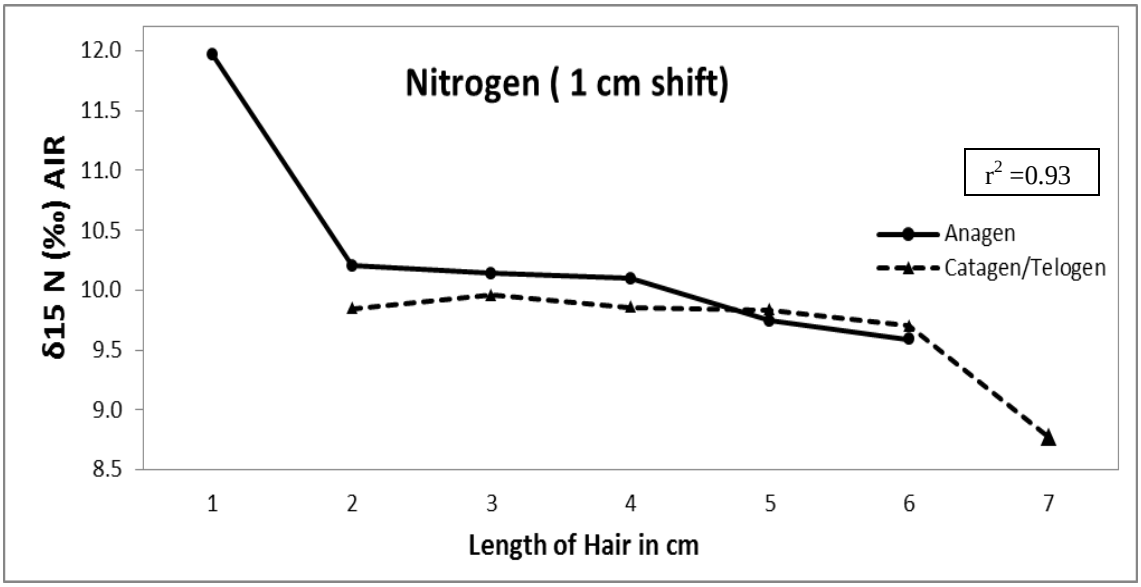

Figure 7.20: Nitrogen isotope variation for sample B262 with a one cm shift.

7.8.2 Sample B343:

Individual B343 was a 40 year old male with no observable osteological evidence of pathology or trauma (Jimenez, 1999). The trichogram was within the range of a healthy individual with 18% of the hair in the resting phase. It is important to note that the first sample (one cm) of hair in the catagen/telogen phase was lost by the mass spectrometer resulting in no $\delta^{13}C$ and $\delta^{15}N$ data for that sample. Delta ^{13}C values ranged from -19.5‰ to -19.8‰ (0.3‰ variation), displaying little variation, but the $\delta^{15}N$ values ranged from 10.4‰ to 12.0‰ (1.6‰ variation) showing a drop around 12 months that remained relatively constant until death. There is a slight decrease in $\delta^{13}C$ values around 12 months that may indicate a dietary shift. The optimal shift for B343 was two to three cm for $\delta^{13}C$ and three cm for $\delta^{15}N$ indicating that isotopic signals lagged behind by two to three months. Table 7.6 shows the r^2 values (highest in red) that correspond to the shift

required to obtain the best match of paired hairs. This can be seen graphically in Figures 7.21 to 7.24.

Table 7.6: r^2 Values of Carbon and Nitrogen for Sample B343

B343 Optimal shift	**r^2**
δ^{13}C: no shift	0.19
δ^{13}C: 1 cm shift	0.01
δ^{13}C: 2 cm shift	0.49
δ^{13}C: 3 cm shift	0.45
δ^{13}C: 4 cm shift	0.31
δ^{15}N: no shift	0.20
δ^{15}N: 2 cm shift	0.48
δ^{15}N: 3 cm shift	0.81
δ^{15}N: 4 cm shift	0.65

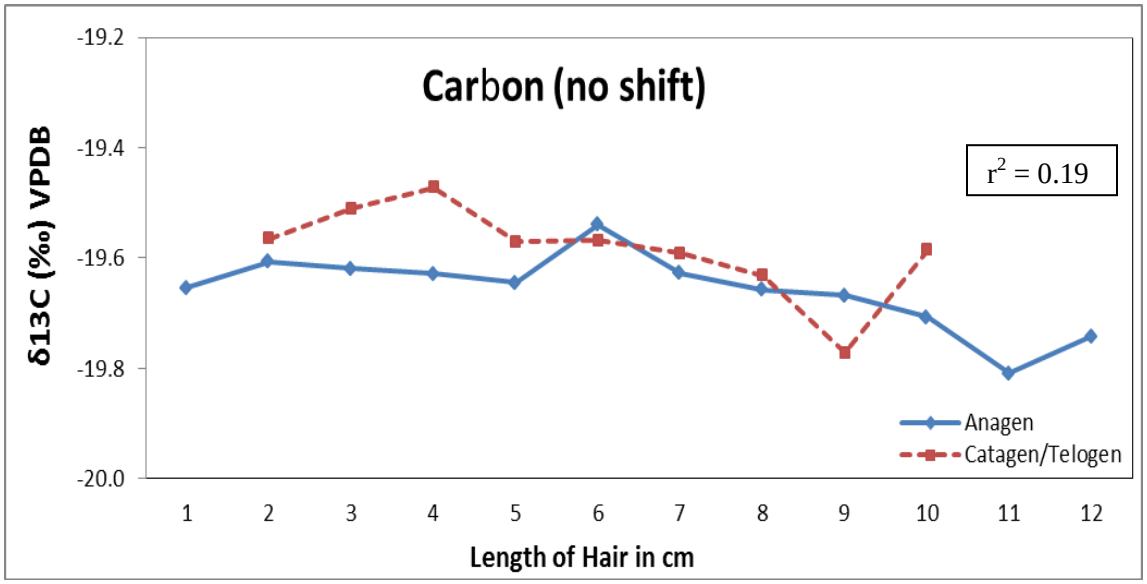

Figure 7.21: Carbon isotopic variation for sample B343 with no shift (does not account for growth cycle error. Note: first cm of Catagen/Anagen phase is missing data).

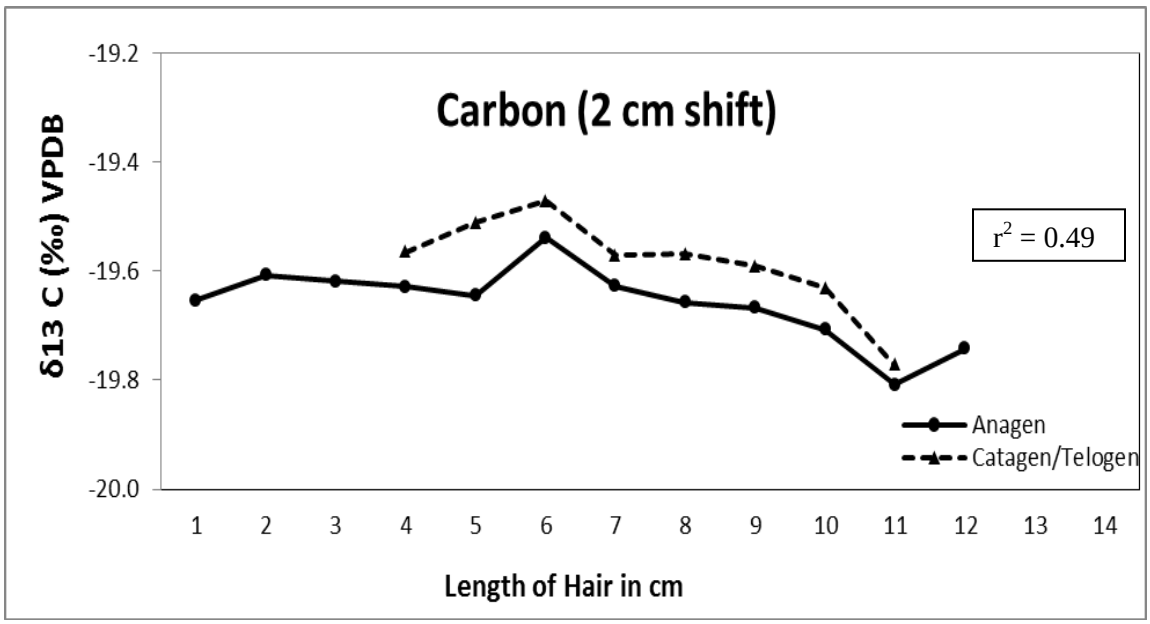

Figure 7.22: Carbon isotope variation for sample B343 with a two cm shift (Note: first cm Catagen/Telogen phase is missing data).

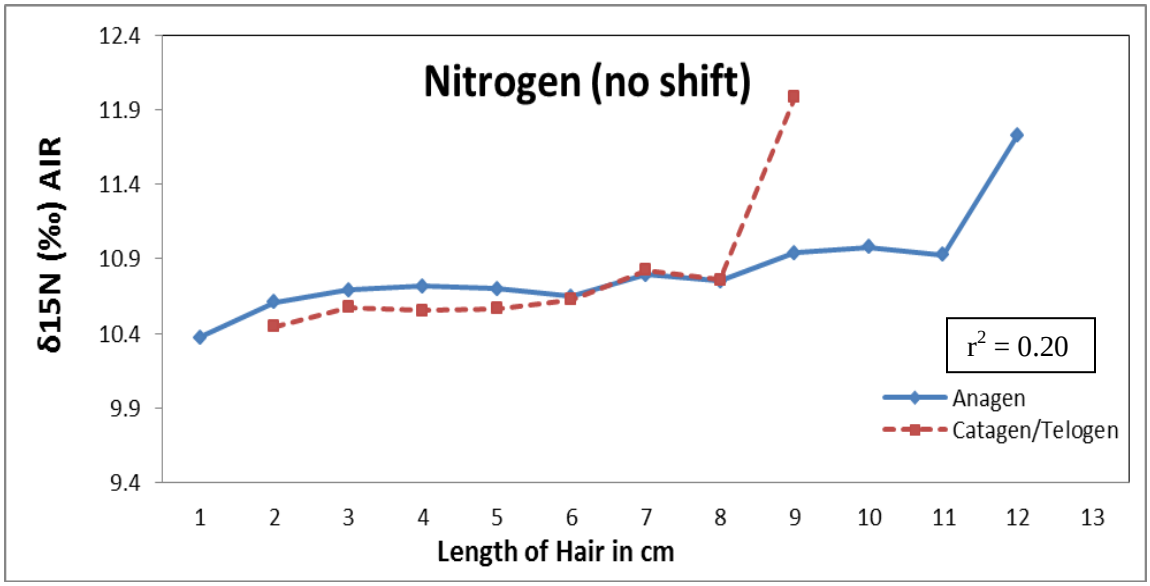

Figure 7.23: Nitrogen variation for sample B343 with no shift (does not account for growth cycle error. Note: first cm of Catagen/Anagen is missing data).

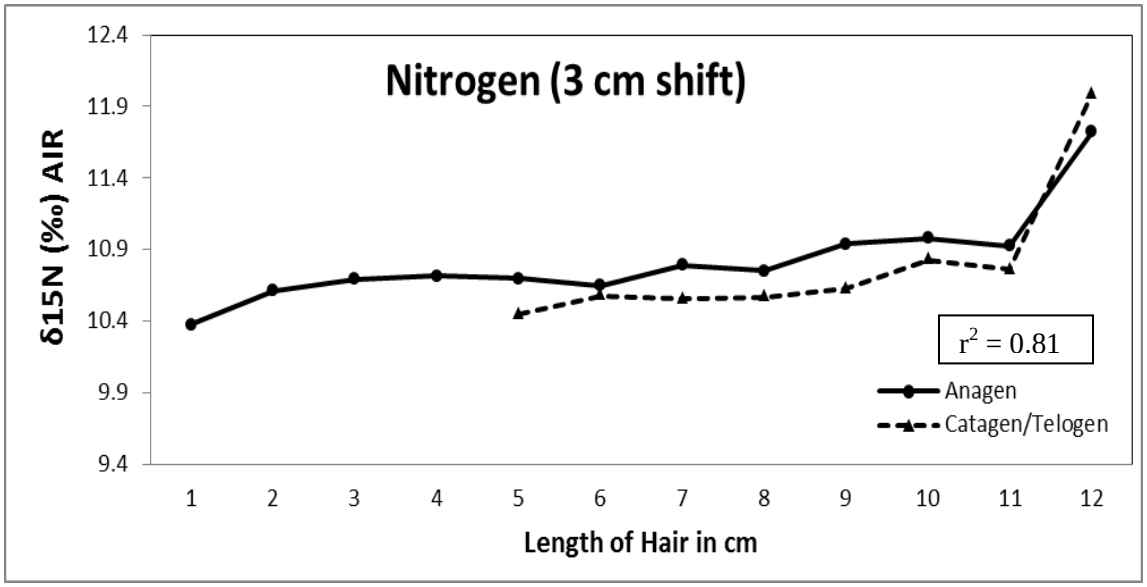

Figure 7.24: Nitrogen isotope variation for sample B343 with a three cm shift (Note: first cm of Catagen/Telogen is missing data).

7.9 Summary

The results presented here demonstrate that trichogram analysis used in conjunction with stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ proves highly effective as a means of investigating the metabolic status of an individual prior to death and the capacity to differentiate between changes in isotopic values due to diet versus 'stress'. Combined results of the trichogram analysis and reported skeletal pathology or trauma indicated that individuals with the highest rate of hair fibers in the catagen/telogen (resting) phase exhibited pathological changes to their skeleton (with the exception of B92A). The data for the paired hair samples showed no significant difference in the mean $\delta^{13}C$ and $\delta^{15}N$ values between hairs in the anagen phase versus the catagen/telogen phase for the same individual. The $\delta^{13}C$ values exhibited minor variation indicating little change in diet prior to death for eight individuals and the δ^{15} N values showed a consistent

elevation in individuals with osteological evidence of pathology. In those individuals with no clear evidence of pathology, the $\delta^{15}N$ values exhibited minor variability (with the exception of B151). Because of a growth cycle error, two of the samples were regressed to find the optimal shift or the number of centimeters that were backtracked in order to find the best match between anagen and catagen/telogen hair. Following the optimal shift the two samples displayed a pattern similar to the other samples used in this study. Two individuals (B92A, B251) exhibited depleted $\delta^{15}N$ values over a nine month period prior to death that may indicate pregnancy. In general, there was $\delta^{15}N$ elevation associated with a high trichogram rate in four individuals (B364, B339, B262, B260), and these individuals also exhibited evidence of pathological changes to the skeleton (i.e., infection, fractures, or periosteal activity), although some of the pathological conditions occurred earlier in life (i.e., B442). These isotopic patterns suggest that the higher $\delta^{15}N$ values are in response to the body's tissue repair process and that $\delta^{15}N$ values in hair keratin may be used as an indicator of physiological stress.

Chapter 8

Discussion

8.1 Introduction

When interpreting the results of the isotopic analysis of the Belleville sample, it is necessary to address the potential biases inherent in the study of a small historic skeletal collection. This chapter will first discuss how archaeological samples do not necessarily represent healthy individuals in a population and will also describe possible sources of error when using stable isotope data of hair keratin. The results presented in the previous chapter will be examined in the following sections; a discussion on the general dietary patterns found in the Belleville samples using the stable isotope data will be followed by a discussion of the $\delta^{13}C$ and $\delta^{15}N$ results for each of the individuals sampled to gain an understanding of the cause of variation observed in the isotopic composition of hair segments. The question of whether pathology, pregnancy, or trauma affects isotope ratios will be addressed as well as the potential implications of these results.

8.2 Sample representativeness

The Belleville hair collection consists of 72 hair specimens with an excellent degree of preservation such that the hair survived to modern times and were recovered during the 1989 excavation of the St. Thomas' Anglican Church cemetery. The hair samples of 10 individuals used in this study represent less than 14% of the total hair available for study, and one limiting factor for stable isotope analysis selection was the

length of the hair available. Archaeological hair keratin samples are uncommon in the archaeological record and are preserved by complex environmental conditions, but hair samples (and all skeletal samples) do not necessarily represent the normal healthy individuals in a population but rather those who died to become part of a biased mortality sample (Johnson, 1962; Wood et al., 1992). The transition from living to dead is inherently selective, which limits the ability of hair samples to represent the community from which they were derived. The population from which the hair samples was drawn was made up of an unknown mixture of individuals who vary in their underlying frailty or susceptibility to disease, trauma, or death due to genetic causes, socioeconomic differences, environmental variation, or temporal trends in health (Wood et al., 1992). Since individual variation in frailty and risk of death can remain concealed in archaeological samples, it has the potential to present a biased picture of population health and disease (Larson, 1995). However, Saunders and coworkers (1993) argued that although mortality bias exists in archaeological skeletal samples, the effects are small and far outweighed by errors introduced by other factors such as aging methodology, sample size, and preservation status. We must acknowledge that the use of the archaeological record to infer the health of past populations is far more difficult than is commonly thought (Wood et al., 1992). Attempting to make broad generalizations based on the results of the analysis of the Belleville hair sample is limited by the small sample size chosen and its ability to represent the inhabitants of 19th century Belleville. Although the problems associated with sample representativeness cannot be ignored, the combination of various sources of information, including information found in burial records (i.e., age,

sex of the individuals) and stable isotope analysis used in conjunction with a trichogram analysis can be used to mediate some of these sample biases and can inform interpretations about health status and diet in 19th century Belleville.

8.3 Possible Sources of Error when Analyzing Stable Isotopes in Hair

The cause of variation observed in the isotopic composition of hair segments within the Belleville individuals was the key question addressed in this research. Interpretation of these data was based on the following conditions: 1) diagenesis was not a factor in creating isotopic variation within individuals, 2) the isotopic values used for diet-tissue differences were within the expected range based on Katzenberg and coworkers' (2000) study, and 3) the lag time between anagen (growing) and telogen/catagen (resting) hair was not a systematic source of error.

The impact of diagenesis was assessed by examining the C:N ratios in the hair samples. The atomic C:N ratios used to measure diagenesis for all but one of the hair samples (n=216) were within the acceptable range of 2.9-3.8, which indicates that the samples have not been diagenetically altered (O'Connell et al., 2001). The mean $\delta^{13}C$ and $\delta^{15}N$ values derived from the bone and hair in Katzenberg et al.'s (2000) study are comparable to the average $\delta^{13}C$ and $\delta^{15}N$ values for hair from this study, however it is important to remember that bone and hair represent different time periods in an individual's life, therefore caution is required for assuming preservation of these samples.

According to Boyce (1967) Belleville inhabitants had access to dietary meat, allowing for sufficient protein in the diet, therefore there is no reason to suspect that

normal tissue-diet differences in isotopic composition would be affected for either carbon or nitrogen (Ambrose and Norr, 1993; Sare et al., 2005). The average offset between $\delta^{13}C$ in bone collagen and hair was 1.8‰, while the difference between collagen and hair for $\delta^{15}N$ was 0.7‰ for the Belleville sample (Katzenberg et al., 2000). This offset is similar to the 1.4‰ in $\delta^{13}C$ and 0.9‰ in $\delta^{15}N$ offset found in the controlled feeding study by O'Connell and coworkers (2001), which implies that the diet from hair closely approximates diet from bone collagen (Katzenberg et al., 2000; White, 2009).

Another potential source of error in detecting change in the isotopic composition of sequential hair samples is created by the three phases of the growth cycle. The anagen or growing phase lasts two years or more, the short transitional catagen phase where hair stops growing lasts two weeks to two months, and the telogen (fully resting phase) lasts three to four months (Valkovich, 1977; Williams et al., 2011). Individual hairs on the scalp will, therefore, be at different stages in their growth phase. Williams and coworkers (2011) demonstrated that variability introduced by sampling hairs in different growth phases can produce an error in some cases, but this error could be much greater if the trichogram had not been implemented. A trichogram completed before stable isotope analysis acts as a screening mechanism and can gauge whether stress is present or absent in an individual before conducting the isotopic analysis and also acts as a signal to determine if calibration or an optimal shift is required. The optimal shift is important for cases such as B262 and B343 because hair in different phases of growth can introduce a growth cycle error that has the potential to conceal or confound the isotopic data resulting in a lag in $\delta^{13}C$ and $\delta^{15}N$ values (Williams et al., 2011).

8.4 Belleville Dietary Patterns Using Stable Isotope Data

Although one can only make generalizations about 19th century Canadian diet based on isotopic analysis of a small number of Belleville hair keratin samples, studies of animals raised on monotonous diets have demonstrated that differences due to sex and individual variation are minimal (Hobson et al., 1993; Minagawa and Wada, 1984; Sponheimer et al., 2003; West et al., 2004). These studies suggest that differences in the isotopic composition of animal tissues between individuals are a reflection of differences in their diet; however metabolic status may be one variable that can alter the isotopic results.

The analysis of $\delta^{13}C$ and $\delta^{15}N$ values in hair keratin samples can identify food groups in the diet whose isotopic signatures are sufficiently similar to permit their distribution into a discrete category that is suitably different from other food groups (e.g., C_3 versus C_4 plant consumers) in order to distinguish between these groups (Keegan, 1992). The isotopic signatures of consumers are used to generate consumption profiles that reflect the relative contributions to the diet of different food groups. To control for the effects of fractionation and to make the isotopic values in hair keratin comparable to those of plants and animals, a value of +5‰ was added to the $\delta^{13}C$ values of the food groups available for human consumption and a value of approximately +3‰ was added to the $\delta^{15}N$ values of the food groups (Schoeninger, 1989).

Katzenberg and colleagues (2000) isotopic study of the Belleville rib samples reported a mean $\delta^{13}C$ value of -19.4 ± 0.7‰ and a mean $\delta^{15}N$ value of 11.1 ± 1.4‰, and the $\delta^{13}C$ value for hair keratin used in this study was -19.7 ± 0.3‰ and 10.6 ± 0.5‰ for

$\delta^{15}N$, which are similar, even though bone and hair keratin provide information on dietary intake from different periods in an individual's life. As mentioned earlier, there is a consistent offset between hair and bone; therefore the isotopic values were not expected to be identical. Individual variation in $\delta^{13}C$ and $\delta^{15}N$ values was small in the Belleville hair sample, which supports historical evidence that while food was plentiful in 19th century Upper Canada, the inhabitants of Belleville had little variation in their diet (Katzenberg et al., 2000). This is consistent with dietary patterns observed by historians, who have shown that historic urban households generally exploited a small range of domestic animals in their diet (e.g., James, 1997; Stewart-Abernathy, 1986). Stewart-Abernathy's (1986) study on urban homesteads from 19th century Arkansas suggest that this was because city ordinances gave each family space to raise domestic animals and gardens that might have reduced the need to hunt wild game animals.

The mean $\delta^{13}C$ value for hair keratin of -19.7 ± 0.3‰ indicates that the individuals used in this study were consuming mainly C_3 plants and meat or by-products from herbivores on a C_3 diet (Chisholm, 1989). The $\delta^{13}C$ values suggest that these individuals were reliant on the consumption of Old World grains such as wheat, barley, oats, and rye. Although historical sources suggest that maize and sugar cane were available in 19th century southern Ontario, the individuals in this study apparently did not consume significant quantities of these C_4 plants. The Belleville isotopic data are similar to those of aboriginal populations living in southern Ontario prior to the widespread adoption of maize agriculture (e.g., $\delta^{13}C$ ranged from -19.2 to -21.1‰ and $\delta^{15}N$ ranged 11.9 to 12.3‰) and for which C_4 plants accounted for less than 10% of the diet

(Schwarcz et al., 1985). Although maize was an important dietary staple for historic aboriginal populations in southern Ontario, where up to 50% of the diet was comprised of maize, the diet of British loyalists living in 19th century Ontario did not appear to be heavily influenced by the maize-reliant populations (Schwarcz et al., 1985). This appears to contradict historical sources that suggest maize was one of the most widely used grains in Canada (Boyce, 1967; Kenyon and Kenyon, 1992).

Delta^{15}N values are linked to the consumption of proteins from terrestrial animals, fish, or legumes and the mean δ^{15}N value of 10.6 ± 0.5‰ indicates that the Belleville sample consumed predominantly herbivore meat such as pork and cattle, and that fish and legumes were not a significant component of the diet. For Canadian settlers, hunting was an unfamiliar practice and although there is evidence to suggest that some settlers consumed wild game and fish, these appear to have been supplemental to a diet based on domesticated livestock (Boyce 1967; James, 1997). The Belleville sample consumed herbivore livestock such as cattle and chickens (Figure 8.1) and because it is unlikely that the inhabitants of Belleville were consuming significant quantities of meat from carnivore animals, it appears that the δ^{15}N data point towards the consumption of omnivorous livestock such as pigs that were fed the butchering offal from chickens and rabbits (Boyce, 1967; James, 1997). The historic records also emphasize the importance of pork for early pioneer populations in Canada (Boyce, 1967; Kenyon and Kenyon, 1992).

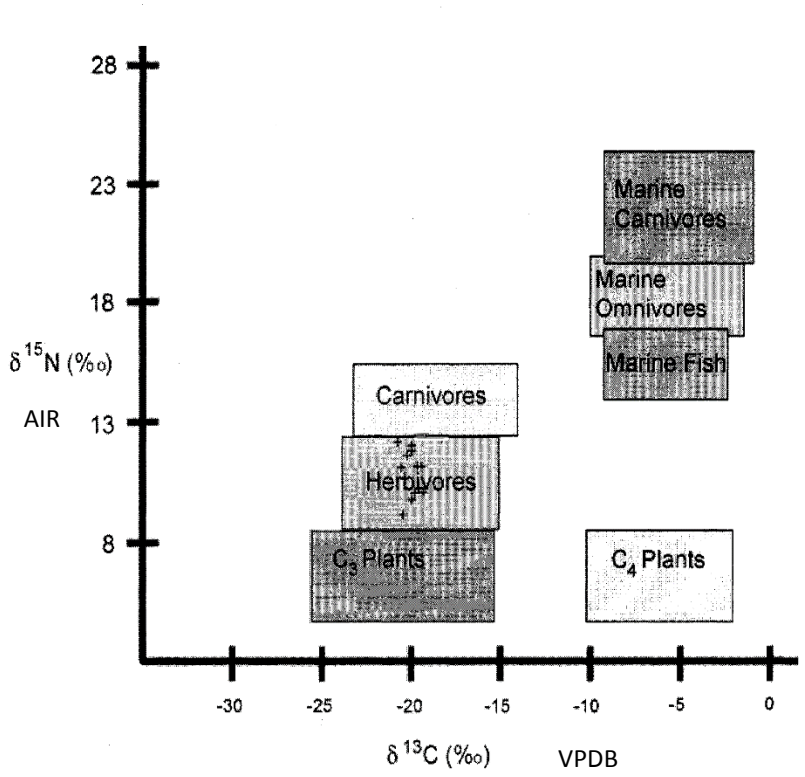

Figure 8.1: Scatter plot of the mean $\delta^{13}C$ and $\delta^{15}N$ values for each individual showing the consumption of herbivore livestock (adapted from Blackbourn (2005) and Prowse (2001).

The $\delta^{13}C$ and $\delta^{15}N$ values also support historical sources that suggested that Belleville inhabitants did not incorporate fish into their diet, despite the abundance of fish and other aquatic food sources available in the Moira River located near Belleville (Parr Traill, 1855). James (1997) examined the faunal remains from four 19th century farmsteads in Upper Canada and found that all of the faunal assemblages analyzed indicated a preference for domesticated livestock. Although most of the rural households investigated by James (1997) supplemented their diet with wild game such as goose, duck, pigeons, fish, and rabbit, the faunal remains were primarily composed of domesticated pig, chicken, cow, sheep, and turkey. Kenyon and Kenyon (1992) emphasized the importance of pork in the early Canadian diet, while James' (1997) study

demonstrated that the diet in Upper Canada often included a wide variety of domesticated livestock. Nonetheless, the isotopic values for wild game versus domesticated animals are similar despite of the type of meat consumed; therefore one cannot clearly distinguish isotopically between the differing terrestrial meat sources that the individuals used in this study preferred.

8.5 Metabolic and Physiological Relationships

The presence of hair in the Belleville archaeological sample provided an opportunity to add more information to the isotopic record about the metabolic status of individuals in the months prior to their death. The results of this study indicate that for Belleville sample the $\delta^{13}C$ values for hair show a fairly narrow 2.9‰ variation across the data (-20.8 to -17.9‰), while the $\delta^{15}N$ values show a larger degree of variation of 4.6‰ (8.4 to 13‰). The level of ^{15}N enrichment and depletion suggest the $\delta^{15}N$ values appear to be influenced by the nitrogen balance of an individual such that anabolic states (building tissue) cause a decrease in $\delta^{15}N$ values, while catabolic states (tissue breakdown) increase $\delta^{15}N$ values. It is important to note that it is the change in $\delta^{15}N$ values combined with the lack of change in the $\delta^{13}C$ values that point to metabolic stress and not dietary shift. It is likely that something other than diet or environmental conditions played a critical role in the high telogen/catagen rates seen in the individuals associated with osteological evidence of trauma or infection and in the timing of the fluctuation in $\delta^{15}N$ values reported here.

8.5.1 *Infection and Periosteal Activity in Relation to Stable Isotope Variation*

Changes in the body that occur in response to infections, inflammatory processes, or underlying nutritional stress may be indirectly measured on the basis of the metabolic reactions (Tomé and Bos, 2000). Metabolic reactions begin immediately following the onset of any clinical symptoms of illness and prompt catabolic processes also occur after the initiation of an illness or injury (Beisel, 1977). Normally, healthy adults are in nitrogen equilibrium, that is, their nitrogen intake equals their nitrogen output. In other words, protein intake from the consumption of food balances with the nitrogen excretion in the urine, feces, and sweat (Beaton et al., 1954; Duggleby and Jackson, 2002). Delta ^{15}N values become more elevated during periods of physiological stress and this change in $\delta^{15}N$ values can be seen in the hair of individuals B364 and B262 starting around two months prior to death. The catabolism (breakdown) of muscle protein causes a redistribution of amino acids required for the synthesis of protein in other parts of the body; therefore the body appears to sacrifice large quantities of muscle protein in order to provide the materials needed to meet the requirements to metabolize energy (Beisel, 1975; Bingham and Cummings, 1985; Tomé and Bos, 2000). Since the body does not store large amounts of free amino acids, a negative nitrogen balance implies a net loss of body protein during an injury or infection and these losses of body nitrogen appear to be discernible in the increased $\delta^{15}N$ values in hair keratin (Beisel, 1975; Tomé and Bos, 2000).

Individual B364 was a seven year old juvenile with evidence of a dental abscess, and infection in the cranium and mandible with 28% of the hair in the catagen/telogen

(resting) phase and individual B262 was a 42 year old female with periosteal activity on the mandibular ramus and parietals, whose trichogram analysis indicated that 30% of her hair was resting. The trichogram analysis for both of these individuals indicated stress and their nitrogen elevations were considered significant (i.e., >1‰ variation along the length of the hair fiber). For individual B262, an optimal shift of $\delta^{13}C$ and $\delta^{15}N$ values was performed in order to better discern the isotopic pattern (Figures 7.18, 7.20), and there is an onset of elevation of 2.4‰ two months prior to death. There was no notable variation in $\delta^{13}C$ values, indicating that the shift in $\delta^{15}N$ was likely not a dietary change (Figure 7.18). Individual B262 was a case where the periosteal reaction may have produced the formation of new bone in response to injury or other stimuli of the periosteum surrounding the bone (Roberts and Manchester, 2005). A periosteal reaction can result from a large number of causes, including injury and chronic irritation due to a medical condition such as bone healing in response to fracture, chronic stress, injuries, subperiosteal hematomas, osteomyelitis, or cancer of the bone (Roberts and Manchester, 2005). Because of the difficulty in assigning a specific medical condition that caused the periosteal bone response in individual B262, one can only speculate that the elevated $\delta^{15}N$ values (2.4‰) were in response to injury or disease where newly formed tissues were the result of recycled nitrogen derived from the breakdown of the existing proteins in the body (Katzenberg and Lovell, 1999). Despite not knowing the cause of the nitrogen elevation, the metabolic response of individual B262 suggests that there was an alteration in the rate of protein synthesis and the metabolic processing of amino acids that could

have been a response to excess nitrogen loss in the body as a result of an illness or injury process within the body (Beisel, 1975; Tomé and Bos, 2000).

Individual B364 had a $\delta^{15}N$ value elevation of 1.3‰ (Figure 7.4) starting at two and six months prior to death, which may have resulted from a febrile illness that initiated a catabolic response resulting in a negative nitrogen balance due to increased urinary loss of nutrients from the body (Beisel, 1975). Given the child's age (7 years) the probability of the nitrogen levels being affected by the weaning process is small, as breastfeeding would have been unlikely. According to Williams and coworkers (2011) a negative nitrogen balance continues until an illness resolves or the individual dies. The recovery process from a negative nitrogen balance brought on by a febrile illness or infection in children may require as long as one month to replenish depleted body stores and the rapid changes in routing of nitrogen and protein synthesis are detectable in hair and may indicate possible timing of physiological depletion and recovery (Beisel, 1977; Mekota et al., 2006). For individual B364, the recovery process may be indicated by the decrease in $\delta^{15}N$ values between months two and four, followed by a second initiation of increased $\delta^{15}N$ values at two months prior to death (possibly another febrile episode) (Figure 7.4). In addition, many nonspecific metabolic responses to infection are modulated by hormone activity (Williams et al., 2011). Glucocorticoids, which are known to interrupt the beginning of the anagen phase in the hair growth cycle, have also been shown to greatly increase at the onset of fever and during acute infection, later reverting to normal levels with recovery (Beisel, 1975, 1977; Kligman, 1961; Paus and Costarelis, 1999). This

hormonal change during febrile illness or periods of acute infection may account for the increased catagen/telogen rate (28%) detected in B364.

8.5.2 *Trauma and Stable Isotope Variation*

One difficulty in the analysis of skeletal samples is determining the age at which an individual may have suffered a traumatic injury, such as a fracture (Jimenez, 1991; Lovejoy and Heiple, 1981). Knowing the age-at-death of the individual does not provide us with information about the time period that has passed since the injury. Murphy and colleagues (1990) examined surgical records of 800 individuals from a Civil War anatomical collection and concluded that bone remodeling began approximately two weeks from the time of the injury. To date there is no technique to determine precisely when a fracture occurred prior to death. For this reason individuals with pathological conditions such as fractures may not always produce a strong pattern of nitrogen isotopic variation, particularly if the fracture event did not occur during the time period represented by the hair sample as was the case for B151. However, results indicate that for two samples (B339, B260) there was a correlation between the occurrences of traumatic injuries with the stable isotope data within the specific time available by the length of the hair sample.

Clinical studies have shown that after a variety of insults to the body (e.g., trauma, shock, or burns) patients develop a systematic inflammatory response that resolves as the patient recovers, however if the systemic inflammatory response is continued then severe disturbances in protein metabolism can occur resulting in catabolism (i.e., tissue

breakdown) (Beretta et al., 2010; Bull, 1958; Clifton et al., 1984; Long et al., 1979). Sequential analysis of hair keratin allows investigation of nitrogen loss, its relationship to the severity of injuries, and the possible role of tissue breakdown and repair during trauma. Individual B339 (55 year old male) displayed osteological evidence of an unhealed humeral fracture, while B260 (35 year old male) and B151 (57 year old female) displayed evidence of healed fractures; all three of these individuals had high catagen/telogen rates (ranging from 38% to 40%). Individual B151 had six healed rib fractures, a healed fracture to the clavicle, and healed fatigue/stress fracture on her third left metatarsal in conjunction with a high (38%) trichogram rate; however she did not exhibit a notable elevation in $\delta^{15}N$ values (0.7‰) or considerable variation in her $\delta^{13}C$ values (0.5‰). The sample consisted of 12 cm of hair; therefore I was able look at the past year of her life to determine isotopic variability. If her fractures occurred during the last year of her life, the hypothesis was that there might be a notable decrease followed by an elevation in nitrogen values possibly associated with the fracture event. Since no notable elevation in $\delta^{15}N$ values occurred, this suggests that the fractures occurred well before the last year of her life or that the nitrogen balance effects associated with those fractures were no longer evident in the isotopic signals from the hair. Many variables can affect the rate and degree of fracture healing such as the age and nutrition of the individual, the degree of immobilization of the injury, hormones, and the state of the immune system (Singh, 2008). It is important to note that the location and type of bone also affects the rate of healing. For example, smaller bones such as metatarsals heal more quickly (~4 to 6 weeks) versus long bones such as humeri (~8 to 12 weeks) (Frost, 1989).

Cancellous (spongy) bone, such as that found in vertebrae or ribs, are usually more stable, involve greater surface areas, and have a better blood supply than do cortical (compact) bone, therefore cancellous bone heals faster than cortical bone (Frost, 1989). This suggests that individual B151's fractures to her ribs and metatarsal may have healed relatively quickly. The lack of significant changes in $\delta^{15}N$ values led to the conclusion that for B151, her injuries did not occur in the last year of her life; however she was still experiencing unspecified stress prior to death as indicated by the high catagen/telogen rate.

Individual B260 had evidence of Schmorl's nodes on most vertebrae, healed fractures on his ribs and a partially healed (i.e., evidence of remodeling) fracture on his left humerus. Individual B339 had osteological evidence of arthritis (evidence of lipping, erosion of bones on the medial phalanges, with no new bone growth) and a misaligned unhealed fracture to his humerus and a healed rib fracture. Both of their trichogram analyses showed that 40% of their hair was in the catagen/telogen phase (resting) which was indicative of stress. In both B260 and B339, $\delta^{15}N$ values were elevated (1.3‰ and 1.2‰, respectively) around four months prior to death and this could represent the recycling of nitrogen derived from the breakdown of existing proteins in the body and subsequent tissue repair in cases of injury or illness (Clifton et al., 1984; Long et al., 1979; Williams et al., 2011). Clinical studies have shown that for patients with fractures, tests revealed that an increased rate of whole-body protein catabolism with a slight increase in protein synthesis leads to negative nitrogen balance (e.g., Beretta et al., 2010; Bull, 1958; Clifton et al., 1984; Long et al., 1979). Even when there is a provision of

adequate amount of amino acids in the form of increased protein in the diet, this partially improves the synthesis rate, but the extent of protein catabolism does not improve quickly in response to change in dietary protein (Beretta et al., 2010; Tomé and Bos, 2000). The metabolic rate and nitrogen excretion are related to the extent of the injury. In cases of injury such as the fractures seen in individuals B260 and B339, one can speculate that the metabolic alterations allowed lean body mass to be catabolized as an energy source to meet the increased energy needs required for tissue repair. When there is excessive muscle wasting associated with increased whole-body energy expenditure, as in severe fractures, whole-body nitrogen balance is notably negative, probably because of changes in the protein breakdown in different tissues in the body (Beisel, 1975; Tomé and Bos, 2000). It has been documented that in severe traumatic conditions, urinary excretion of nitrogen may reach 35-40 grams per day, as the nitrogen balance reflects the difference between whole-body protein synthesis and breakdown (Beretta et al., 2010; Bull, 1958). The timing of the fracture events and period of tissue repair appear to be evident in the $\delta^{15}N$ value elevation (Figures 7.8, 7.10) seen in these two individuals and once again their high catagen/telogen rates could be an indication of the response to the body's repair process.

8.5.3 *Malnourishment and Stable Isotope Variation*

It is not possible to rule out the prospect that the $\delta^{15}N$ value elevations observed in Belleville hair specimens were the result of alterations in metabolic and physiological changes associated with starvation or malnourishment. Despite the historic records

indicating that Belleville inhabitants had access to an abundance of food sources (e.g., Boyce, 1967; Moodie, 1853; Parr Traill, 1836), the St. Thomas' inhabitants experienced more childhood stress than other Canadian populations during the same time period, as indicated by the prevalence of linear hypoplasias found in the skeletal sample (Saunders and Keenleyside, 1999). Although Belleville residents were probably the most economically advantaged, they had the highest rate of enamel hypoplasias of all the Canadian samples studied by Saunders and Keenleyside (1999). Dental enamel hypoplasias are pits or furrows on the enamel surface and are an indicator of stress from birth to about 12 years of age (Lewis, 2002). Numerous conditions have been shown to cause these defects including fever, starvation, infections, and low birth weight (Lewis, 2002). High rates of enamel hypoplasia are often associated with increasing industrialization and urbanization and it is possible that the high rate of enamel hypoplasia was a reflection of poor sanitary conditions in the town of Belleville (Saunders and Keenleyside, 1999). The enamel hypoplasias present in the Belleville skeletons may point to a low-level nutritional deficiency during childhood because it is feasible that the consumption of a diet reliant on pork, potatoes, and bread with little variation could result in nutritional deficiencies in the Belleville individuals.

One cannot exclude the possibility that the individuals in this study were not eating a nutritionally adequate diet due to illness, particularly B364, the seven year old with evidence of infection. A common adverse consequence of infection is a reduction in food intake due to a child's refusal to eat (Beisel, 1977; Williams et al., 2011). This could be intensified by the tendency to reduce consumption of protein-rich foods during

episodes of acute infection and febrile illness (Beisel, 1977). Given the child's age, the probability that the child was going through potentially two episodes of febrile illness, these data suggest cumulative stress that eventually led to this child's death. One cannot rule out the possibility that dietary variation could produce a pattern of $\delta^{15}N$ value variation similar to the one seen here, however, the $\delta^{13}C$ values appear to indicate little variation in the diet (Figures 7.3, 7.4). The degree of nutritional deficiency necessary to cause an alteration in the $\delta^{15}N$ values has been demonstrated by Mekota and coworkers (2006). They recorded weekly weight changes and $\delta^{15}N$ and $\delta^{13}C$ values in the hair of anorexic patients during starvation periods, where catabolism results in the recycling of body protein, producing a trophic level effect within the patient's own body (Mekota et al., 2006). They found that high $\delta^{15}N$ values were inversely related to body mass index and concluded that there needed to be a significant reduction in weight and body mass in order to produce increased $\delta^{15}N$ values (Mekota et al., 2006). For example, the heaviest adult at the beginning of this study weighed less than 31.8 kg (70 pounds) by the end of the study and this individual's $\delta^{15}N$ values elevated less than 1‰ (Mekota et al., 2006).

Despite the difficulty of distinguishing starvation episodes from trauma, infection, or disease in an individual, the historic records indicate that although there may have been nutritional deficiencies present, food was plentiful. This suggests that an individual would not starve to the extent where they could lose significant body mass and produce an alternation of their $\delta^{15}N$ values.

8.5.4 *Pregnancy and Stable Isotope Variation*

Pregnant women are in a positive nitrogen balance or anabolic state because they are adding new blood, bone, and muscle cells to their bodies. Maternal metabolism changes substantially during pregnancy where gestation can be perceived as an anabolic state of the mother because of an increase in maternal fat stores where nutrients are stored in order to meet fetal demands (Duggleby and Jackson, 2002). Individual B92A was a 37 year old female with no reported pathology who was buried with an infant (0.87 years), and B251 was presumed to be female by the length of her hair, but lacked burial records to confirm this. Their trichogram analyses showed that 34% (B92A) and 28% (B251) of their hair were in catagen/telogen phase and both exhibited distinct nine month depletions in $\delta^{15}N$ values followed by a significant elevation of 1.7‰ and 3.8‰, respectively (Figures 7.12, 7.16). The $\delta^{13}C$ values also decreased during this nine month period, possibly related to a change in diet to support pregnancy, but variability was less pronounced (Figures 7.11, 7.15). Fuller and coworkers (2004) suggested that this pattern of $\delta^{15}N$ value fluctuation in hair relates to increased utilization of dietary and urea nitrogen for tissue synthesis during pregnancy, resulting in a greater than 1‰ reduction in the steady state diet-to-body trophic level effect. Consistent with Fuller and coworkers' (2004) findings for modern pregnant women, B92A and B251 showed nitrogen shifts of over 1.7‰ and 3.8‰, and combined with the distinct nine month nitrogen depletion; this suggests that these individuals may have been pregnant in the two years before their deaths.

Most women experience a dramatic shift in hormonal modulation of the hair growth cycle during the post-partum period of about three to four months and the effects of this may have been seen in these two individuals (Lynfield, 1960; Williams et al., 2011). Typically women experience very high anagen (growing) rates during pregnancy, which is a result of estrogen elevation, followed by a notable increase in telogen rates (resting), brought on by estrogen withdrawal and increased levels of prolactin needed for lactation (Ebling, 1990; Lynfield, 1960). At around three to twelve months postpartum, most women experience post-partum effluvium, or extreme shedding of hair, which may explain why individuals B92A and B251 both had high telogen rates (34% and 28%) that were not associated with skeletal evidence of trauma or pathology. The high telogen rates may be an indicator of post-partum effluvium.

Huelsemann and coworkers (2009) suggest that carbon equilibrium is slower and less sensitive due to a larger body carbon pool, requiring greater proportion of exchange before steady state is reached and this may account for the similar but not significant trends seen in the $\delta^{13}C$ values for these two individuals (Figures 7.11, 7.15). Nevertheless, the depletion in $\delta^{15}N$ values may be linked to an increased demand for maternal nitrogen by the fetus during pregnancy (Duggleby and Jackson, 2002; Williams et al., 2011).

8.6 Individuals Lacking Significant Stable Isotope Variation

Individuals B76 (27 year old male), B343 (42 year old male), and B442 (36 year old female) exhibited no observable osteological evidence of trauma, infection, or disease and their trichogram rates were within the range of a healthy individual, at less than 20%

of their hair resting (Van Neste et al., 2007). There was little variability in both $\delta^{13}C$ and $\delta^{15}N$ values that suggested that there was little to no metabolic alteration in association with injury, disease, or a change in dietary input in the months prior to death (Figures 7.5, 7.6, 7.13, 7.14, 7.22, 7.24).

It is possible that individuals B76, B343, and B442 had achieved nitrogen balance in the months prior to death, which means that their dietary intake was balanced by the excretion of urea wastes (Tomé, 2011). If nitrogen excretion was greater than the nitrogen content in the diet, these individuals would have been in a negative nitrogen balance and their $\delta^{15}N$ values may have exhibited elevation because this is an indicator of tissue destruction (Tomé and Bos, 2000; Tomé, 2011). One can also speculate that possible causes of death for these individuals may have been accidental or traumatic deaths, as there were a great many accidents in Belleville (especially drowning) during this time period (Jimenez, 1999). These individuals may have died from a soft tissue injury, chronic illness, or a disease that left no trace on their skeletons (i.e., smallpox, cholera), however if they did, it was not evident in their trichograms nor their $\delta^{15}N$ values.

8.7 Does Pathology or Metabolic Status Affect Isotopic Values?

Katzenberg and Lovell (1999) found an elevated level of $\delta^{15}N$ values around the site of an osteomyelitis infection in a modern autopsy sample. The authors concluded that the elevated $\delta^{15}N$ values were due to new bone formation at the site of the infection, however they surmised that because the individual died from HIV/AIDs, the elevated $\delta^{15}N$ value might be a result of the overall systematic wasting of the individual's body

(Katzenberg and Lovell, 1999). Additionally, White and Armelagos' (1997) study found elevated $\delta^{15}N$ values in the bones of Nubian mummies suffering from osteopenia, which provided a basis for the possibility that there might be a similar elevation in the hair of individuals with known osteological evidence of fractures, periostitis, or infections. Based on the results of this stable isotope analysis, there does appear to be a consistent alteration of $\delta^{15}N$ values associated with infection and trauma that is also correlated with high trichogram rates. Four individuals with osteological evidence of fractures, periostitis, and infection showed an increase in $\delta^{15}N$ values (i.e., >1‰) regardless of age or sex, whereas three individuals with no reported pathology and low trichogram rates (i.e., healthy trichograms) exhibited less variability (<1‰) in their $\delta^{15}N$ values. Injuries or infections lead to an increased nitrogen loss from the body and the catabolic response of an individual may affect their $\delta^{15}N$ values. Episodes of infection or injury can result in negative nitrogen balances that are modulated by hormones and other inflammatory mediators, and it is reasonable to suggest that the loss of body protein or catabolism can be observed in the elevation of $\delta^{15}N$ values along sequentially segmented hair (Kurpad, 2006).

Two individuals were unique because their $\delta^{13}C$ and $\delta^{15}N$ values exhibited a large (>1‰) depletion over a nine month period prior to death, that would not have otherwise been revealed had the sequential hair analysis not been conducted (Figures 7.11, 7.12, 7.15, 7.16). In particular, the $\delta^{15}N$ value depletion could have been the result of an anabolic state produced during pregnancy. Pregnancy is a time when the mother is in an anabolic state which increases the capacity of the mother's body to deliver nutrients to the

fetus (Duggleby and Jackson, 2002; Lain and Catalano, 2007). In these instances, the nine month $\delta^{15}N$ value depletion may be evidence that these two women had given birth prior to their deaths and the dangers of giving birth in the 19th century were serious and sometimes fatal due to the lack of medical aid and high infection rates (e.g., puerperal fever). According to Roland (1983) child-bearing was considered a health hazard in Upper Canada during this time period (1821-1874).

8.8 Implications of this Research

The patterns observed in the isotopic signatures of the hair samples from Belleville are in agreement with the concept of the body 'living on its own meat' and one can observe the isotopic phenomenon of 'enrichment in loss' (Fuller et al., 2005; Waterlow, 1968). The $\delta^{15}N$ values were higher among those individuals with evidence of infection and unhealed fractures and lower during suspected pregnancy, and the trichogram corroborated these findings with clear indicators of hair loss during stress. These results are surprising since one would expect that when exposed to physiological stress an organism would try to conserve nitrogen reserves (Huelsemann et al., 2009). However, the shifts in nitrogen excretion appear to be caused by changes in the body's metabolic status. The results of this project are important as a number of studies that investigated the influence of stress on isotope signatures in a variety of animal tissues found inconsistent results (e.g., Kempster et al., 2007; Williams et al., 2007). The question as to why the pattern of changes in isotopic signatures of $\delta^{15}N$ values were pronounced in this study could have been because of the tissue used. Usually in cases of

severe starvation or trauma leading to a breakdown of energy reserves only a fraction of tissue is metabolized at any given point in time, leading to slower changes in isotopic signatures that are difficult to quantify in tissues such as bone (Hobson 1993; Williams et al., 2007). However, in rapidly growing tissue such as hair keratin, a more pronounced shift in isotopic signatures can be observed since a reduction of energy leads to rapid changes in the proportion of hair compounds produced from these energy reserves (Fuller et al., 2005; Hobson 1993). During periods of physiological stress an increasing amount of the hair keratin compounds originate from metabolized internal tissues (i.e., muscle) that are nitrogen enriched compared with the diet and this, in turn, causes an increase in the $\delta^{15}N$ values of the hair (Huelsemann et al., 2009). Therefore, the isotopic variations appear to be more pronounced in hair compared with the slow and gradual changes that take place in tissues such as bone.

The analysis of hair stable isotope ratios has the potential to become a valuable tool to assess how physiological stress changes the nitrogen balance in past populations. This is of particular importance in providing a more nuanced picture of how the residents from Belleville lived and died. While their nutrient intake appeared to have been relatively stable over time physiological trauma, infection, or pregnancy caused significant intra-individual differences in nitrogen balance. The detection of fluctuations in energy as used in this study on hair keratin may be highly relevant and applicable to medical research in the form of diagnosing and monitoring conditions marked by changes in nitrogen balance such as infection, trauma, or malnourishment in modern humans.

8.9 Summary

The stable isotope data indicated that 19th century Canadian settlers retained the dietary practices common to their European heritage and the $\delta^{13}C$ and $\delta^{15}N$ results for the individuals sampled indicate a trend of $\delta^{15}N$ value elevation for individuals with osteological evidence of pathology. Moreover, the combination of the trichogram analysis with the stable isotope analysis should become standard practice for analyzing archaeological hair samples from other time periods in order to accurately distinguish between dietary shifts and metabolic process. Additionally, there was significant nine month $\delta^{15}N$ value depletion in two women that may be an indicator of pregnancy, which also would not have been detected without the sequential analysis of hair keratin. The fluctuation in $\delta^{15}N$ values may be the result of physiological stress that can cause a pattern of metabolic changes in individuals that included negative or positive nitrogen balances that can be observed in the isotopic analysis of sequentially segmented hair fibers.

Chapter 9

Conclusions

Isotopic analysis of Belleville hair samples provided information about the diet and physiological stress of settlers in 19th century Upper Canada and demonstrated the usefulness of an integrated methodology employing both microscopic and isotopic analysis of hair. The purpose of this thesis was to expand knowledge concerning how pathology or trauma alters isotopic signals in human hair keratin. Specifically, this research investigated the correlation between observed pathological conditions, microscopic evidence of stress through trichogram analysis, and $\delta^{13}C$ and $\delta^{15}N$ values found in scalp hair samples from Belleville, Ontario.

Data from the stable isotopes confirmed previous isotopic analysis by Katzenberg and colleagues (2000) that the diets of Belleville inhabitants were based on the use of Old World staples such as apples, wheat, and oats, as well as meat from sheep, pigs, and cows. This suggests that the Belleville inhabitants did not make use of many foods available to them in the New World such as maize, fresh water fish, or sugar cane.

The trichogram analysis was effective in detecting different growth phases in the hair samples and was essential for specifying the presence or absence of physiological stress. Seven of the 10 individuals used in this study showed high percentage of hairs in the catagen/telogen (resting phase). The trichogram analysis also assisted in the evaluation of the presence of a growth cycle error in the $\delta^{13}C$ and $\delta^{15}N$ values in two individuals (B262 and B343). The use of isotopic data for sequentially segmented hair

identified the timing of the metabolic reaction of an individual with pathology or trauma and this was signified by variation (>1‰) in $\delta^{15}N$ values over time associated with a lack of change in $\delta^{13}C$ values. Three individuals (B364, B339, B262) showed large variability in $\delta^{15}N$ values accompanied by little variation in $\delta^{13}C$ values, which suggests metabolic stress versus a change in their diet. Overall, the results found that $\delta^{15}N$ values were elevated for individuals with osteological evidence of pathology and depleted in cases of suspected pregnancy leading to the conclusion that nitrogen balance (either negative or positive) changes quickly in response to an individual's level of physiological stress. The lack of change in $\delta^{15}N$ values was also detectable in the segmented hair for three individuals (B76, B343, B442) suggesting they were in nitrogen balance prior to death.

The results are also in agreement with the findings of Katzenberg and Lovell (1999), who suggested that a change in $\delta^{15}N$ values can be seen within an individual undergoing physiological stress. The body is in effect consuming itself, such that as the body suffers from negative nitrogen balance, existing proteins are catabolized as a source of energy and the result is an elevated $\delta^{15}N$ value in the body. The implications of this are that the metabolic status of an individual poses a confounding factor to ancient dietary studies when using tissues such as hair that represents a relatively short time frame. Researchers analyzing isotopes in hair should be aware that short term fluctuations of $\delta^{15}N$ values may be the result of changes in an individual's metabolic imbalance.

One advantage of isotopic data from sequentially segmented hair keratin over tissues, such as bone and teeth, is that the fast formation of hair and direct measurement

of $\delta^{15}N$ values permits an assessment of the nitrogen balance in humans over a short period of time before death.

This study demonstrated that the analysis of $\delta^{13}C$ and $\delta^{15}N$ values in hair keratin facilitate the detection of short-term fluctuations in diet and can differentiate that from $\delta^{15}N$ values that indicate a shift in the metabolic status of an individual. The observed patterns of nitrogen fluctuations are in line with the concept that the body's nitrogen balance alters during stress and that the body will catabolize its own tissues to act as an energy reserve. This thesis further confirms the potential of human hair to track dietary habits and changes in nitrogen balance under conditions of physiological or nutritional stress.

Future Research

Further archaeological and genealogical information about the Belleville sample could provide important insights into the lives of the Belleville individuals. An osteobiography of certain individuals could be created by matching the Belleville hair samples with the coffin plates uncovered during the excavation of the St. Thomas' Anglican Church cemetery. By identifying the name, date of birth, and date of interment allows for the inclusion of genealogical and further historical information to aid in the interpretation of health status of the Belleville individuals. Specific knowledge of the dates of interment of these individuals may allow for the investigation of the effects of temporal changes in this population, and may permit tracing information on cause of death. Knowing cause of death for Belleville individuals would greatly aid in correlating

the isotopic data with specific physiological events such as pregnancy or trauma and could help differentiate individuals who died suddenly in accidents versus those suffering from trauma, infection, or chronic illness. Future research will include isotopic analysis of $\delta^{13}C$ and $\delta^{15}N$ values in hair samples from modern pregnant women and cadavers (whose cause of death is known) to provide control samples to use as comparative data for the isotopic results from this study.

Another area of research could use this isotopic technique in conjunction with the trichogram analysis for nutritional and medical studies of modern humans; isotopic measure of hair keratin may be a useful tool for exploring nitrogen flux and balance under various conditions such as pregnancy, malnourishment, and eating disorders. The results from this study have the potential to be applied to modern individuals with an eating disorder or malnourishment. Bulimia nervosa and anorexia nervosa affects 1-5% of high school and university age women in North America and 18% of elderly individuals (>65 years of age) are malnourished (Ahmed and Haboubi, 2010; Hatch et al., 2006; Neuberger et al., 2013). The measurement of stable isotope ratios in hair has the ability to provide a unique method to aid in diagnosing individuals with anorexia nervosa, bulimia nervosa, or malnutrition in the elderly, by determining a more precise timing of changing dietary habits. The social benefits of this research for modern individuals is that the stable isotopes of hair has the potential to provide a method to objectively aid in the diagnosis of individuals with an eating disorder or malnourishment by determining a shift in dietary intake.

Ultimately, implications of this research suggest that human hair is an important biological archive that preserves an individual's dietary and metabolic life history. Hair keratin contributes to the reconstruction of the biology and quality of life including health status, type and availability of food resources, and physical stress of past humans, providing us with a unique glimpse into life in 19th century Upper Canada. Finally, the assessment of $\delta^{13}C$ and $\delta^{15}N$ values in human hair has the potential to augment interpretations of health and trauma during the final months of life and can be used to develop a clearer understanding of the interrelationship of diet, health, and physiology.

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Appendix A: Inventory of Belleville Hair Specimens

Burial:	Description of Hair Specimen	Length of Hair
B 12	2 medium clumps of medium length hair with soil debris	3 cm
B 24	Large clump of medium to long length hair (reddish), with soil debris	>7 cm
B 35	3 left ribs with a few chest hairs	1-2 cm
B 40	Small clumps of very short hair with soil debris	<1 cm
B 45	Small clump of short to medium length hair	3 cm
B 60	Large clump of medium length hair with soil debris	3-4 cm
B 63	Very large clump of very long hair	> 7 cm
B 68	Medium clump of short hair with skull fragments	2.5 cm
B 69	Small clump of short to medium length hair with plant and soil debris	1.5 cm
B 71	Medium clump of short to medium length hair soil debris	2-3 cm
B 75	Few short hairs with soil debris	1 cm
B 76	Very large clump of medium to long length hair with soil debris	>7 cm
B 85	Fair number of short hairs from occipital, with soil debris	0.50-1.5 cm
B 92A	Very large clump of very long hair with skull fragments	>7 cm
B 100	Small clump of short hair from cranium	1 cm
B 101	Few short hairs with soil debris	0.50-2 cm
B 103	2 small clumps of medium length hair with plant debris (left occipital and parietal)	3 cm
B 108	Small clump of short to medium length hair with sand	2.5 cm
B 111	Small clump of short hair from left parietal with soil debris	> 1 cm
B 119	Large clump of medium length hair with skull fragments and soil debris	4 cm
B 124	Few short hairs with soil debris	0.50-1 cm
B 128	Medium clump of short to medium length hair with soil debris	1.5 cm
B 140	Very small clump of short hair with soil debris	>1 cm
B 141	2 small clumps of short to medium length hair	4 cm
B 151	Large clump of medium length hair with soil debris, (located under back of head)	>7 cm
B 152	Small clump of medium hair, hair net or wig?	2 cm
B 168A	Medium clump of medium length hair with soil debris	> 3 cm

Burial:	Description of Hair Specimen	Length of Hair
B 169	Large clump of medium length hair with soil debris	4 cm
B 211a	Small clump of short hair from frontal (superior, mid sagittal)	2.5 cm
B 243	Small clump of short hair (left mandibular ramus), beard hair	0.5 cm
B 249	Small clump of short hair from left posterior parietal and temporal	1 cm
B 251	Clump of short to medium length hair from right occipital	>7cm
B 251	Very large clump of very long hair including vault	> 7 cm
B 260	Medium clump of long to medium length hair with soil and wood debris	>7 cm
B 262	Clump of long hair with soil debris	>7 cm
B 276	Small clump of short hair with soil debris	2 cm
B 298	Clump of medium length hair from left posterior parietal	4-5 cm
B 298	Clump of medium length hair with soil debris	3-4 cm
B 304	Small clump of short hair from frontal and parietals	2 cm
B 310	Medium clump of short hair with soil debris	2 -3 cm
B 339	Large clump of medium to long length hair (reddish), with soil debris	>7
B 342	2 clumps of medium length hair with soil debris	3-4 cm
B 363	Small clump of short to medium length hair, blonde	1-2 cm
B 364	Medium clump of medium length hair with soil debris	>6 cm
B 383	2 small clumps of medium length hair with plant and soil debris	2-3 cm
B 386	Small clump of short hair	1.5 cm
B 404	Small clump of short hair	> 1 cm
B 406	Few very short hairs from bregma	0.25-0.50 cm
B 413	Fair number of short hairs from right anterior parietal	1-2 cm
B 423	Medium clump of short to medium length hair (left frontal)	> 1cm
B 424	Medium clump of short to medium length hair	1-2 cm
B 442	Clump of medium length hair with soil debris	>7 cm
B 465	Very small clump of short hair with soil debris	0.50 cm
B 468	Clump of medium length hair	2 cm
B 423	Tiny clump of short hair with plant debris (left anterior frontal)	< 3 cm
B 486	3-4 small clumps of short hair with soil debris	3 cm
B 487	Small clump of medium length hair with sand	4 cm
B 490	Small clump of short hair to medium length hair	3 cm
B 500c	Few very short hairs	> 0.5 cm

Burial:	Description of Hair Specimen	Length of Hair
B 513	Small clump of short hair	1 cm
B 530	Tiny clump of medium hair (blonde)	1.5 cm
B 531	Medium clump of medium length hair with soil debris (blonde)	3 cm
B 549	2 small clumps of short hair	1 cm
B 343 (mislabelled as 339)	Clump of medium length hair with soil debris, reddish	>7 cm

Appendix B: Compiled Raw Isotopic Data

Identifier 1	13C corr	C wt%		15N corr	N wt%		c/n	atomic C/N
B151a-A1	-18.8	41.64		11.3	14.3		2.90	3.39
B151a-A10	-18.0	40.64		11.4	14.9		2.73	3.19
B151a-A11	-18.0	42.71		11.0	13.9		3.07	3.58
B151a-A12	-18.1	43.39		11.1	14.8		2.93	3.42
B151a-A7	-17.9	43.00		11.2	14.8		2.90	3.38
B151b-C1	-18.5	42.20		11.1	14.6		2.88	3.36
B151b-C10	-18.2	42.05		11.1	14.6		2.89	3.37
B151b-C11	-18.3	42.10		10.9	14.5		2.90	3.38
B151b-C12	-18.1	40.99		11.0	14.6		2.80	3.27
B151b-C2	-18.6	41.85		11.0	14.6		2.86	3.34
B151b-C3	-18.4	41.71		11.1	14.4		2.89	3.37
B151b-C4	-18.3	41.94		11.0	14.8		2.83	3.30
B151b-C5	-18.3	41.56		11.1	14.8		2.81	3.27
B151b-C6	-18.4	40.92		11.1	15.0		2.73	3.18
B151b-C7	-18.4	42.00		11.0	14.9		2.81	3.28
B151b-C8	-18.4	41.76		11.0	15.0		2.79	3.25
B151b-C9	-18.3	42.02		11.1	14.7		2.86	3.33
B262 A1	-20.5	43.52		12.0	14.8		2.95	3.44
B262 A2	-20.5	42.85		10.2	14.5		2.95	3.44
B262 A3	-20.5	43.18		10.1	14.6		2.95	3.44
B262 A4	-20.3	43.89		10.1	14.9		2.94	3.43
B262 A5	-20.5	42.89		9.7	14.5		2.96	3.45
B262 A6	-20.5	44.14		9.6	14.9		2.97	3.46
B262 C1	-20.4	41.67		9.8	13.2		3.15	3.67
B262 C2	-20.5	43.80		10.0	13.9		3.15	3.67
B262 C3	-20.4	43.89		9.9	14.2		3.08	3.60
B262 C4	-20.4	43.58		9.8	14.1		3.09	3.60
B262 C5	-20.4	46.08		9.7	15.1		3.06	3.57
B262 C6	-20.2	43.96		8.8	13.0		3.38	3.94
B364 A1	-20.8	40.30		12.0	14.6		2.76	3.21
B364 A2	-20.6	41.69		10.7	13.9		3.01	3.51
B364 A3	-20.5	42.69		11.0	14.2		3.00	3.50

Identifier 1	13C corr	C wt%		15N corr	N wt%		c/n	atomic C/N
B364 A4	-20.6	38.53		11.9	14.8		2.61	3.04
B364 A5	-20.4	40.66		12.0	14.6		2.78	3.24
B364 A6	-20.5	40.17		10.7	13.7		2.94	3.42
B364 C1	-20.7	40.82		11.3	14.9		2.75	3.20
B364 C2	-20.7	40.84		10.8	13.7		2.98	3.47
B364 C3	-20.5	40.60		11.0	14.7		2.76	3.21
B364 C4	-20.6	40.55		11.9	14.6		2.77	3.23
B364 C5	-20.3	40.67		11.9	14.4		2.82	3.29
B76 A1	-20.0	36.11		11.1	13.5		2.68	3.13
B76 A2	-20.1	41.73		11.0	13.5		3.09	3.60
B76 A3	-20.1	41.85		11.1	13.6		3.09	3.60
B76 A4	-20.2	41.99		11.1	13.5		3.10	3.62
B76 A5	-20.2	41.73		11.2	13.6		3.08	3.59
B76 A6	-20.3	42.04		11.2	13.7		3.07	3.58
B76 A7	-20.2	41.74		11.1	13.7		3.04	3.55
B76 A8	-20.2	41.34		11.1	13.5		3.06	3.57
B76 A9	-20.2	41.70		11.1	13.6		3.07	3.58
B76 A10	-20.1	41.48		11.1	13.5		3.08	3.59
B76 A11	-20.3	41.81		11.1	13.6		3.08	3.59
B76 A12	-20.5	41.14		11.1	13.3		3.09	3.60
B76 C1	-20.2	41.50		11.2	13.4		3.09	3.60
B76 C2	-20.2	41.83		11.3	13.6		3.08	3.59
B76 C3	-20.2	41.84		11.4	13.7		3.04	3.55
B76 C4	-20.3	41.67		11.2	13.5		3.08	3.59
B76 C5	-20.3	41.84		11.3	13.7		3.05	3.56
B76 C6	-20.3	41.75		11.3	13.5		3.09	3.61
B76 C7	-20.1	41.11		11.2	13.5		3.04	3.54
B76 C8	-20.2	41.09		11.2	13.5		3.03	3.54
B343 A1	-19.7	37.85		10.4	13.4		2.83	3.30
B343 A2	-19.6	41.06		10.6	13.5		3.04	3.54
B343 A3	-19.6	41.48		10.7	13.6		3.05	3.56
B343 A4	-19.6	41.21		10.7	13.8		2.99	3.49
B343 A5	-19.6	41.08		10.7	13.7		3.00	3.50
B343 A6	-19.5	41.17		10.6	13.5		3.05	3.56
B343 A7	-19.6	41.26		10.8	13.6		3.03	3.54
B343 A8	-19.7	34.96		10.8	11.5		3.04	3.55

Identifier 1	13C corr	C wt%		15N corr	N wt%		c/n	atomic C/N
B343 A9	-19.7	41.28		10.9	13.7		3.02	3.52
B343 A10	-19.7	41.68		11.0	13.6		3.06	3.57
B343 A11	-19.8	41.21		10.9	13.5		3.05	3.56
B343 A12	-19.7	41.26		11.7	13.6		3.03	3.54
B343 C2	-19.6	41.25		10.4	13.6		3.04	3.54
B343 C3	-19.5	41.28		10.6	13.7		3.02	3.52
B343 C4	-19.5	37.95		10.6	12.7		2.99	3.48
B343 C5	-19.6	41.72		10.6	13.8		3.03	3.54
B343 C6	-19.6	41.45		10.6	13.8		3.00	3.50
B343 C7	-19.6	41.42		10.8	13.7		3.02	3.52
B343 C8	-19.6	41.35		10.8	13.8		3.00	3.50
B343 C9	-19.8	41.47		12.0	14.6		2.83	3.30
B339 A1	-19.5	43.02		11.5	14.3		3.02	3.52
B339 A2	-19.5	43.06		11.4	14.2		3.03	3.54
B339 A3	-19.5	43.77		10.7	14.2		3.09	3.61
B339 A4	-19.5	43.12		10.5	14.5		2.98	3.48
B339 A5	-19.5	43.17		10.7	14.5		2.99	3.48
B339 A6	-19.4	43.15		10.6	14.5		2.97	3.47
B339 A7	-19.6	43.54		10.9	14.3		3.05	3.56
B339 A8	-19.6	43.26		10.6	14.4		3.01	3.51
B339 A9	-19.6	43.28		10.8	14.2		3.05	3.55
B339 A10	-19.7	43.42		11.0	14.2		3.07	3.58
B339 C2	-19.6	34.10		11.7	11.5		2.97	3.47
B339 C3	-19.5	43.31		11.6	14.6		2.96	3.46
B339 C4	-19.7	43.03		10.4	14.3		3.00	3.50
B339 C5	-19.7	43.15		10.7	14.3		3.01	3.51
B339 C6	-19.4	43.14		10.6	14.6		2.96	3.46
B339 C7	-19.5	43.20		10.7	14.6		2.96	3.45
B339 C8	-19.8	43.22		10.7	14.0		3.08	3.59
B339 C9	-19.7	43.46		10.8	14.4		3.02	3.52
B339 C10	-20.1	43.26		10.9	14.1		3.08	3.59

Appendix C: Pathological vs. Non-Pathological Raw Isotope Values

Pathological			Non-Pathological		
Identifier	**Carbon**	**Nitrogen**	**Identifier**	**Carbon**	**Nitrogen**
B151-A1	-18.5	11.1	B343-A1	-19.6	10.3
B151-A2	-18.6	11.1	B343-A2	-19.6	10.6
B151-A3	-18.4	10.9	B343-A3	-19.6	10.7
B151-C4	-18.3	11.0	B343-A4	-19.6	10.7
B151-A5	-18.3	11.0	B343-A5	-19.6	10.7
B151-A6	-18.4	11.0	B343-A7	-19.6	10.8
B151-A8	-18.4	11.1	B343-A8	-19.7	10.8
B151-A9	-18.3	11.1	B343-A9	-19.7	10.9
B151-A10	-18.2	11.0	B343-A10	-19.7	11.0
B151-A11	-18.3	11.0	B343-A11	-19.8	10.9
B151-A12	-18.1	11.1	B343-A12	-19.7	11.7
B151-C1	-18.4	10.8	B343-C2	-19.6	10.4
B151-C2	-18.7	10.7	B343-C3	-19.5	10.6
B151-C3	-18.4	10.8	B343-C4	-19.5	10.6
B151-C4	-18.4	10.9	B343-C5	-19.6	10.6
B151-C5	-18.5	11.1	B343-C6	-19.6	10.6
B151-C6	-18.5	10.9	B343-C7	-19.6	10.8
B151-C7	-18.2	11.4	B343-C8	-19.6	10.8
B151-C8	-18.5	11.2	B343-C9	-19.8	12.0
B151-C9	-18.3	11.4	B76-A1	-20.0	11.1
B151-C10	-18.4	11.1	B76-A2	-20.1	11.1
B151-C11	-18.3	11.1	B76-A3	-20.1	11.1
B151-C12	-18.3	10.9	B76-A4	-20.1	11.1
B364-A1	-20.8	12.0	B76-A5	-20.2	11.2
B364-A2	-20.6	10.7	B76-A6	-20.3	11.3
B364-A3	-20.5	11.1	B76-A7	-20.2	11.1
B364-A4	-20.6	11.9	B76-A8	-20.2	11.1
B364-A5	-20.4	12.0	B76-A9	-20.2	11.1
B364-A6	-20.5	10.7	B76-A10	-20.1	11.1
B364-C1	-20.7	11.3	B76-A11	-20.3	11.1
B364-C2	-20.7	10.9	B76-A12	-20.5	11.2
B364-C3	-20.5	11.0	B76-C1	-20.2	11.2
B364-C4	-20.6	11.9	B76-C2	-20.2	11.3

Pathological			Non-Pathological		
Identifier	**Carbon**	**Nitrogen**	**Identifier**	**Carbon**	**Nitrogen**
B364-C5	-20.3	11.9	B76-C3	-20.2	11.3
B262-A1	-20.5	11.9	B76-C4	-20.3	11.2
B262-A2	-20.5	10.2	B76-C5	-20.3	11.3
B262-A3	-20.5	10.1	B76-C6	-20.3	11.3
B262-A4	-20.3	10.0	B76-C7	-20.1	11.2
B262-A5	-20.5	9.7	B76-C8	-20.2	11.2
B262-A6	-20.5	9.6	B92A A1	-20.0	10.0
B262-C1	-20.4	9.8	B92A A2	-19.9	10.0
B262-C2	-20.5	10.0	B92A A3	-19.9	9.9
B262-C3	-20.4	9.9	B92A A4	-19.8	9.8
B262-C4	-20.4	9.8	B92A A5	-20.3	9.8
B262-C5	-20.4	9.7	B92A A6	-20.1	9.9
B262-C6	-20.2	8.8	B92A A7	-20.1	9.4
B339-A1	-19.5	11.5	B92A A8	-19.7	8.6
B339-A2	-19.5	11.4	B92A A9	-19.8	8.4
B339-A3	-19.5	10.7	B92A A10	-19.6	8.5
B339-A4	-19.5	10.5	B92A A11	-19.6	8.4
B339-A5	-19.5	10.7	B92A A12	-19.5	8.4
B339-A6	-19.4	10.6	B92A A13	-19.45	8.3
B339-A7	-19.6	10.9	B92A A14	-19.4	8.6
B339-A8	-19.6	10.6	B92A A15	-19.5	8.5
B339-A9	-19.6	10.8	B92A A16	-19.4	8.4
B339-A10	-19.7	10.9	B92A A17	-19.5	8.7
B339-C2	-19.6	11.7	B92A A18	-19.7	9.8
B339-C3	-19.5	11.6	B92A C1	-20.0	9.8
B339-C4	-19.7	10.4	B92A C2	-20.0	9.9
B339-C5	-19.7	10.7	B92A C3	-19.9	9.9
B339-C6	-19.4	10.6	B92A C4	-20.0	9.5
B339-C7	-19.5	10.7	B92A C5	-20.1	9.6
B339-C8	-19.8	10.7	B92A C6	-20.1	9.6
B339-C9	-19.7	10.8	B92A C7	-19.9	9.3
B339-C10	-20.1	10.9	B92A C8	-19.7	8.5
B260 A1	-20.4	11.5	B92A C9	-19.8	8.4
B260 A2	-20.4	11.3	B92A C10	-19.7	8.5
B260 A3	-20.6	11.4	B92A C11	-19.6	8.5

Pathological			Non-Pathological		
Identifier	**Carbon**	**Nitrogen**	**Identifier**	**Carbon**	**Nitrogen**
B260 A4	-20.3	10.2	B92A C12	-19.4	8.4
B260 A5	-20.4	10.5	B92A C13	-19.4	8.5
B260 A6	-20.7	10.5	B92A C14	-19.4	8.6
B260 C1	-20.2	11.3	B92A C15	-19.5	8.7
B260 C2	-20.5	11.2	B92A C16	-19.5	8.6
B260 C3	-20.5	11.2	B92A C17	-19.8	8.7
B260 C4	-20.3	10.1	B92A C18	-19.9	9.9
B260 C5	-20.6	10.4	B442 A1	-19.5	11.4
B260 C6	-20.7	10.4	B442 A2	-19.6	11.2
Mean	**-19.6**	**10.9**	B442 A3	-19.6	11.1
Std Dev	**0.9**	**0.6**	B442 A4	-19.4	11.3
			B442 A5	-19.5	11.3
			B442 C1	-19.7	11.6
			B442 C2	-19.6	11.3
			B442 C3	-19.6	11.2
			B442 C4	-19.4	11.1
			B442 C5	-19.6	11.2
			B251 A1	-19.7	13.0
			B251 A2	-19.1	13.0
			B251 A3	-19.9	12.2
			B251 A4	-19.4	12.4
			B251 A5	-19.8	12.2
			B251 A6	-19.7	11.2
			B251 A7	-19.9	11.3
			B251 A8	-20.0	11.5
			B251 A9	-19.8	11.3
			B251 A10	-20.3	11.5
			B251 A11	-20.1	9.6
			B251 A12	-20.1	9.7
			B251 A13	-19.5	9.2
			B251 A14	-19.4	9.3
			B251 A15	-19.4	9.6
			B251 A16	-19.5	9.7
			B251 A17	-19.4	9.8
			B251 A18	-19.8	9.8

	Non-Pathological	
Identifier	**Carbon**	**Nitrogen**
B251 A19	-20.0	9.3
B251 A20	-20.1	11.9
B251 A21	-18.9	11.5
B251 A22	-20.3	11.6
B251 A23	-20.4	11.8
B251 A24	-19.8	11.4
B251 A25	-20.1	11.6
B251 A26	-19.4	11.9
B251 A27	-19.8	11.1
B251 A28	-20.1	11.4
B251 A29	-20.1	11.9
B251 A30	-19.8	11.5
B251 C1	-20.1	12.3
B251 C2	-19.3	12.0
B251 C3	-19.9	11.2
B251 C4	-19.2	11.9
B251 C5	-19.9	11.3
B251 C6	-19.8	11.1
B251 C7	-19.5	10.9
B251 C8	-20.1	10.7
B251 C9	-20.1	11.1
B251 C10	-19.6	10.5
B251 C11	-20.2	9.8
B251 C12	-19.8	9.5
B251 C13	-19.4	9.6
B251 C14	-19.3	9.9
B251 C15	-19.4	9.7
B251 C16	-19.3	9.7
B251 C17	-19.5	9.9
B251 C18	-19.4	9.7
B251 C19	-19.6	9.6
B251 C20	-19.9	11.1
B251 C21	-19.1	11.1
B251 C22	-19.7	11.1
B251 C23	-19.6	11.1

	Non-Pathological		
	Identifier	**Carbon**	**Nitrogen**
	B251 C24	-19.6	10.9
	B251 C25	-19.5	11.0
	B251 C26	-19.3	10.7
	B251 C27	-19.1	10.7
	B251 C28	-19.1	10.8
	B251 C29	-19.2	10.6
	B251 C30	-19.2	10.6
	Mean	**-19.8**	**10.5**
	Std. Dev	**0.3**	**0.7**

Appendix D: Comparing Paired Isotopic Values between Anagen and Catagen/Telogen Hair Phases

Nitrogen

Identifier	$\delta^{15}N$ (‰) Anagen	$\delta^{15}N$ (‰) Catagen/Telogen	$\delta^{15}N$ (‰) Anagen - Catagen/Telogen
B151-1	11.1	10.8	0.345
B151-2	11.1	10.7	0.399
B151-3	10.9	10.8	0.125
B151-4	11.0	10.9	0.137
B151-5	11.0	11.1	-0.079
B151-6	11.1	10.9	0.160
B151-7	11.0	11.4	-0.397
B151-8	11.1	11.2	-0.124
B151-9	11.1	11.4	-0.313
B151-10	11.0	11.1	-0.095
B151-11	11.0	11.1	-0.076
B151-12	11.1	10.9	0.154
B262-1	12.0	9.8	2.126
B262-2	10.2	10.0	0.242
B262-3	10.1	9.9	0.284
B262-4	10.1	9.8	0.263
B262-5	9.7	9.7	0.041
B262-6	9.6	8.8	0.816
B364-1	12.0	11.3	0.662
B364-2	10.7	10.9	-0.181
B364-3	11.0	11.0	0.010
B364-4	11.9	11.9	0.009
B364-5	12.0	11.9	0.025
B364-6	10.7		
B76-1	11.1	11.2	-0.150
B76-2	11.0	11.3	-0.255
B76-3	11.1	11.4	-0.269
B76-4	11.1	11.2	-0.152
B76-5	11.2	11.3	-0.174
B76-6	11.2	11.3	-0.160
B76-7	11.1	11.2	-0.121
B76-8	11.1	11.2	-0.041

Identifier	$\delta^{15}N$ (‰) Anagen	$\delta^{15}N$ (‰) Catagen/Telogen	$\delta^{15}N$ (‰) Anagen - Catagen/Telogen
B76-9	11.1		
B76-10	11.1		
B76-11	11.1		
B76-12	11.1		
B343-1	10.4		
B343-2	10.6	10.4	0.164
B343-3	10.7	10.6	0.116
B343-4	10.7	10.6	0.163
B343-5	10.7	10.6	0.131
B343-6	10.7	10.6	0.024
B343-7	10.8	10.8	-0.033
B343-8	10.8	10.8	-0.007
B343-9	10.9	12.0	-1.046
B343-10	11.0		
B343-11	10.9		
B343-12	11.7		
B339-1	11.5	11.7	-0.150
B339-2	11.4	11.6	-0.243
B339-3	10.7	10.4	0.285
B339-4	10.5	10.7	-0.233
B339-5	10.7	10.6	0.109
B339-6	10.6	10.7	-0.103
B339-7	10.9	10.7	0.136
B339-8	10.6	10.8	-0.172
B339-9	10.8	10.9	-0.162
B339-10	11.0		
B260 A1	11.5	11.3	0.108
B260 A2	11.3	11.3	0.084
B260 A3	11.4	11.2	0.243
B260 A4	10.2	10.1	0.069
B260 A5	10.5	10.4	0.066
B260 A6	10.5	10.4	0.101
B92A A1	10.0	10.0	-0.015
B92A A2	10.0	9.9	0.130
B92A A3	9.9	9.9	-0.038
B92A A4	9.8	9.5	0.341

Identifier	$\delta^{15}N$ (‰) Anagen	$\delta^{15}N$ (‰) Catagen/Telogen	$\delta^{15}N$ (‰) Anagen - Catagen/Telogen
B92A A5	9.8	9.6	0.144
B92A A6	9.9	9.6	0.345
B92A A7	9.5	9.3	0.203
B92A A8	8.6	8.5	0.112
B92A A9	8.4	8.4	-0.016
B92A A10	8.5	8.5	0.000
B92A A11	8.4	8.5	-0.098
B92A A12	8.4	8.4	-0.076
B92A A13	8.4	8.5	-0.120
B92A A14	8.6	8.6	-0.012
B92A A15	8.5	8.7	-0.133
B92A A16	8.4	8.6	-0.186
B92A A17	8.7	8.7	-0.011
B92A A18	9.8	9.9	-0.081
B442 A1	11.4	11.6	-0.132
B442 A2	11.2	11.3	-0.036
B442 A3	11.1	11.2	-0.002
B442 A4	11.3	11.1	0.134
B442 A5	11.3	11.2	0.124
B251 A1	13.0	12.3	0.653
B251 A2	13.0	12.0	1.008
B251 A3	12.2	11.2	0.986
B251 A4	12.4	11.9	0.487
B251 A5	12.2	11.3	0.924
B251 A6	11.2	11.1	0.074
B251 A7	11.3	10.9	0.374
B251 A8	11.5	10.7	0.813
B251 A9	11.3	11.1	0.224
B251 A10	11.5	10.5	0.944
B251 A11	9.6	9.8	-0.130
B251 A12	9.7	9.5	0.142
B251 A13	9.2	9.6	-0.375
B251 A14	9.3	9.9	-0.551
B251 A15	9.6	9.6	-0.019
B251 A16	9.7	9.7	0.005
B251 A17	9.8	9.9	-0.050

Identifier	$\delta^{15}N$ (‰) Anagen	$\delta^{15}N$ (‰) Catagen/Telogen	$\delta^{15}N$ (‰) Anagen - Catagen/Telogen
B251 A18	9.8	9.7	0.101
B251 A19	9.4	9.6	-0.275
B251 A20	11.9	11.0	0.852
B251 A21	11.5	11.1	0.448
B251 A22	11.6	11.1	0.510
B251 A23	11.8	11.1	0.676
B251 A24	11.4	10.9	0.517
B251 A25	11.6	11.0	0.649
B251 A26	11.9	10.7	1.164
B251 A27	11.1	10.7	0.471
B251 A28	11.4	10.8	0.599
B251 A29	11.9	10.6	1.279
B251 A30	11.5	10.6	0.870
		Mean:	**0.149**
		Std Dev:	**0.371**

Carbon

Identifier	$\delta^{13}C$ (‰) Anagen	$\delta^{13}C$ (‰) Catagen/Telogen	$\delta^{13}C$ (‰) Anagen-Catagen/Telogen
B151-1	-18.5	-18.4	-0.134
B151-2	-18.6	-18.7	0.057
B151-3	-18.4	-18.4	0.025
B151-4	-18.3	-18.4	0.105
B151-5	-18.3	-18.5	0.288
B151-6	-18.4	-18.5	0.050
B151-7	-18.5	-18.2	-0.251
B151-8	-18.4	-18.5	0.070
B151-9	-18.3	-18.3	0.071
B151-10	-18.2	-18.4	0.212
B151-11	-18.3	-18.3	0.005
B151-12	-18.1	-18.3	0.236
B262-1	-20.5	-20.4	-0.054
B262-2	-20.5	-20.5	-0.041
B262-3	-20.5	-20.4	-0.120
B262-4	-20.3	-20.4	0.100

Identifier	$\delta^{13}C$ (‰) Anagen	$\delta^{13}C$ (‰) Catagen/Telogen	$\delta^{13}C$ (‰) Anagen-Catagen/Telogen
B262-5	-20.5	-20.4	-0.054
B262-6	-20.5	-20.2	-0.272
B364-1	-20.8	-20.7	-0.150
B364-2	-20.6	-20.7	0.052
B364-3	-20.5	-20.5	-0.008
B364-4	-20.6	-20.6	0.067
B364-5	-20.4	-20.3	-0.064
B364-6	-20.5		
B76-1	-20.0	-20.2	0.150
B76-2	-20.1	-20.2	0.106
B76-3	-20.1	-20.2	0.097
B76-4	-20.2	-20.3	0.126
B76-5	-20.2	-20.3	0.149
B76-6	-20.3	-20.3	-0.001
B76-7	-20.2	-20.1	-0.023
B76-8	-20.2	-20.2	0.044
B76-9	-20.2		
B76-10	-20.1		
B76-11	-20.3		
B76-12	-20.5		
B343-1	-19.7		
B343-2	-19.6	-19.6	-0.042
B343-3	-19.6	-19.5	-0.108
B343-4	-19.6	-19.5	-0.157
B343-5	-19.6	-19.6	-0.074
B343-6	-19.5	-19.6	0.029
B343-7	-19.6	-19.6	-0.036
B343-8	-19.7	-19.6	-0.025
B343-9	-19.7	-19.8	0.105
B343-10	-19.7		
B343-11	-19.8		
B343-12	-19.7		
B339-1	-19.5	-19.6	0.060
B339-2	-19.5	-19.5	-0.017
B339-3	-19.5	-19.7	0.156
B339-4	-19.5	-19.7	0.168

Identifier	$\delta^{13}C$ (‰) Anagen	$\delta^{13}C$ (‰) Catagen/Telogen	$\delta^{13}C$ (‰) Anagen-Catagen/Telogen
B339-5	-19.5	-19.4	-0.076
B339-6	-19.4	-19.5	0.086
B339-7	-19.6	-19.8	0.212
B339-8	-19.6	-19.7	0.131
B339-9	-19.6	-20.1	0.478
B339-10	-19.7		
B260 A1	-20.4	-20.2	-0.243
B260 A2	-20.4	-20.5	0.084
B260 A3	-20.6	-20.5	-0.127
B260 A4	-20.3	-20.3	0.006
B260 A5	-20.4	-20.6	0.121
B260 A6	-20.7	-20.7	-0.040
B92A A1	-20.0	-20.0	-0.006
B92A A2	-19.9	-20.0	0.156
B92A A3	-19.9	-19.9	0.022
B92A A4	-19.8	-20.0	0.152
B92A A5	-20.3	-20.1	-0.227
B92A A6	-20.1	-20.1	0.046
B92A A7	-20.1	-19.9	-0.166
B92A A8	-19.7	-19.7	0.002
B92A A9	-19.8	-19.9	0.024
B92A A10	-19.6	-19.7	0.090
B92A A11	-19.6	-19.6	-0.012
B92A A12	-19.5	-19.4	-0.065
B92A A13	-19.5	-19.4	-0.022
B92A A14	-19.4	-19.4	0.012
B92A A15	-19.5	-19.5	0.010
B92A A16	-19.4	-19.5	0.053
B92A A17	-19.5	-19.8	0.253
B92A A18	-19.7	-19.9	0.212
B442 A1	-19.5	-19.7	0.118
B442 A2	-19.6	-19.6	0.011
B442 A3	-19.6	-19.6	-0.044
B442 A4	-19.4	-19.4	-0.005
B442 A5	-19.5	-19.6	0.060
B251 A1	-19.7	-20.1	0.404

Identifier	$\delta^{13}C$ (‰) Anagen	$\delta^{13}C$ (‰) Catagen/Telogen	$\delta^{13}C$ (‰) Anagen-Catagen/Telogen
B251 A2	-19.1	-19.3	0.183
B251 A3	-19.9	-19.9	-0.007
B251 A4	-19.4	-19.2	-0.144
B251 A5	-19.8	-20.0	0.187
B251 A6	-19.7	-19.8	0.092
B251 A7	-19.9	-19.5	-0.314
B251 A8	-20.0	-20.1	0.138
B251 A9	-19.8	-20.1	0.285
B251 A10	-20.3	-19.6	-0.674
B251 A11	-20.1	-20.2	0.081
B251 A12	-20.1	-19.8	-0.286
B251 A13	-19.5	-19.4	-0.077
B251 A14	-19.4	-19.3	-0.034
B251 A15	-19.4	-19.4	0.018
B251 A16	-19.5	-19.3	-0.199
B251 A17	-19.4	-19.5	0.163
B251 A18	-19.8	-19.4	-0.410
B251 A19	-20.0	-19.7	-0.311
B251 A20	-20.1	-19.9	-0.183
B251 A21	-18.9	-19.0	0.109
B251 A22	-20.3	-19.7	-0.590
B251 A23	-20.4	-19.6	-0.730
B251 A24	-19.8	-19.6	-0.238
B251 A25	-20.1	-19.5	-0.600
B251 A26	-19.4	-19.3	-0.073
B251 A27	-19.8	-19.1	-0.770
B251 A28	-20.1	-19.1	-1.028
B251 A29	-20.1	-19.2	-0.940
B251 A30	-19.8	-19.2	-0.607
			-0.041
		Std Dev:	**0.334**