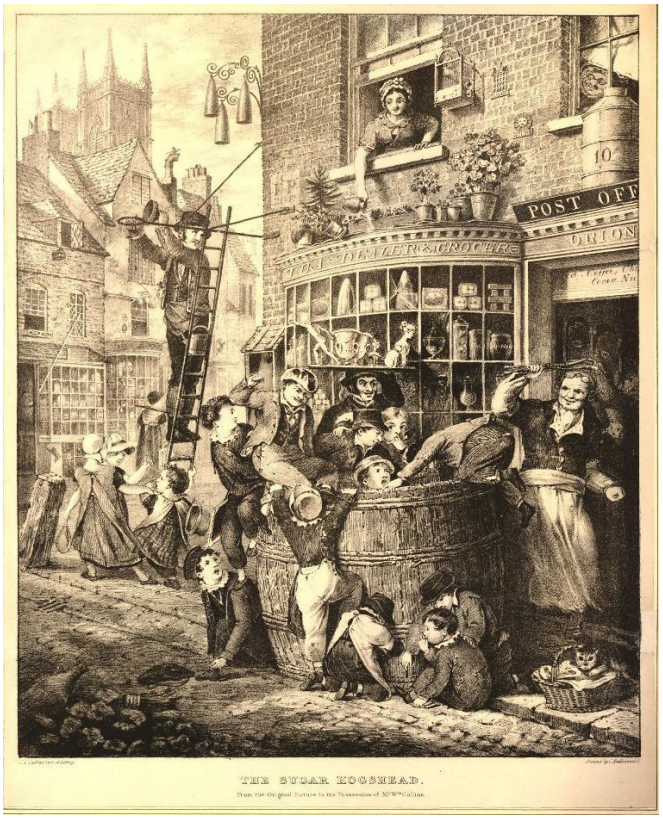
A multi-isotope, multi-tissue study of diets in industrialised societies of 17th to 19th century England

Blessing Chidimuro

PhD



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ABSTRACT

Palaeodietary research using isotope analysis has recently expanded and this research takes a novel approach by employing carbon isotope analysis of bone carbonate in conjunction with bone collagen for the first time, to explore 17th to 19th century diets in English populations. Central to the research, is the potential consumption of C_4 crops (cane sugar/maize) which increased during this period. Additionally, it pays special attention to regional, local, age, sex, race, and socioeconomic variations in the diet in selected northern manufacturing towns and London populations in England. Finally, this research explores the potential for using isotope analysis of human dental calculus carbonate as a new proxy for cane sugar consumption investigations.

Collagen isotope analysis was performed on 244 human and 168 faunal remains from (i) post-medieval sites in Halifax, Leeds, London, Manchester, Rotherham, Tunstall and York (ii) medieval sites in Southwell, Leicester, and Chester as well as (iii) modern individuals from the Forensic Anthropology Center, Tennessee, USA. In addition, FTIR-ATR was utilised to assess diagenesis and both FTIR-ATR and bone carbonate analyses were performed on 273 human bones, 27 faunal bones, and 57 dental calculus samples.Finally, enamel and calculus carbonate analyses were performed on 57 samples.

Overall, variations in the diet, especially consumption of C_4 resources and animal protein were observed between and within different populations by geographical location, age, and socioeconomic status. The combination of bone carbonate and collagen isotope analysis using linear and multivariate modelling enabled the identification of C_4 resource consumption. Londoners consumed more animal and marine protein as well as C_4 resources potentially cane sugar. Additionally, the middle- and upper-class populations from both London and northern populations had greater access to these foods compared to those of lower status. Results from a pilot study indicated that although dental calculus has the potential to be used in palaeodietary studies, it cannot yet be used to ascertain C_4 cane sugar consumption.

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Cover page: The Sugar Hogshead Painting by ET Parris in 1846 illustrating children in London playing in and around a sugar barrel outside a shop potentially to see if there is any sugar left, exemplifying sugar's growing importance in everyday diet. ©The Trustees of the British Museum

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Acknowledgments

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For my husband and son,
Collins and David

Declaration

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Statement of Copyright

Chapter 1: Introduction

This thesis examines dietary changes across eleven human and nine faunal post-medieval (17th to 19th centuries) skeletal collections in England with a focus on regional, local, age, sex, race, and socioeconomic variations in diet. This has been accomplished using stable isotopes of carbon ($\delta^{13}C_{collagen}$, $\delta^{13}C_{carbonate}$) and nitrogen ($\delta^{15}N_{collagen}$) in contrast to the previous studies that have mainly utilised only bone collagen ($\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$). This thesis is the first study to use bone carbonate isotope analysis to study the diet of English populations and interestingly, to explore the potential consumption of C_4 crops (cane sugar/maize) during a pivotal moment in food globalisation (Oddy 1990; Oddy and Burnett 1992; Burnett 2005). The use of bone carbonate isotope analysis on English archaeological populations has previously been hampered by the controversy surrounding the effects of diagenesis on the isotopic ratios (Garvie-Lok et al. 2004; Shin and Hedges 2012; Snoeck and Pellegrini 2015), and arguably, the use of carbonates to identify the potential consumption of C_4 crops is not a significant issue until the post-medieval period due to dietary history of England. For this thesis, however, in consideration of carbonate's high susceptibility to diagenesis, preservation is investigated using Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance (FTIR-ATR) before analysis, to exclude significantly altered samples from bone carbonate palaeodiet reconstruction (Hollund et al. 2013; Snoeck and Pellegrini 2015; Kontopoulos et al. 2018).

1.1 Diet and the post-medieval period in England

Studying diet will provide a key insight into the lives of people in post-medieval England. Diet in archaeology is studied in order to interpret various aspects of ancient lives. It does not only fulfil the role of nutrition that is necessary for people to survive but provides insights into how humans interacted with their food (Super 2002; Twiss 2019). The ways in which food is prepared and consumed is determined by the way people live, their occupation, faith, traditional beliefs,politics, the technologies with which they access it, and more. Consequently, this then reflects these individuals' gender roles, age differences, ethnic identities, international relations, religion,and socioeconomic status (Twiss 2019). The post medieval period is a time of food globalisation (Oddy 1990; Oddy and Burnett 1992; Burnett 2005). Increased agricultural output, improved livestock breeds and adoption of new agricultural machinery by farmers as well as improved trade links during the 17th to 19th century Industrial Revolution, led to the industrialisation of food production, a dramatic change in diets, and deviation from local seasonal produce (Oddy 1990; Oddy and Burnett 1992; Burnett 2005; Thirsk 2007). This transition from a regionally specific to a nationalised diet transformed the way people procured their food and lived (Oddy 1990; Oddy and Burnett 1992; Burnett 2005). As with many historic cultures, there is extensive information

available from written sources on these changes in the post-medieval English diet (reviewed in Chapter 3). However, most of the sources reveal the dietary resources that are "typical" to the post-medieval English population as a whole and rarely extend to the specific diets of individuals, therefore, more studies using other sources are required to reveal more information.

Archaeological research on populations from England has in the past, focused less on the post-medieval period, in favour of earlier time periods. However, post-medieval skeletal remainsare increasingly available, particularly from cemeteries excavated during urban development. These remains have the added advantage of generally good preservation, including soft tissues insome cases, and can represent named individuals where coffin plates survive e.g., Brown and Alexander (2016). Additionally, the revolutionary changes of the post-medievalperiod paved the way for the development of modern England, and therefore it is essential to investigate the implications of such changes for lifestyle and diet from every available facet of evidence. In addition, although there is a vast availability of historical documents for thisperiod, it should be kept in mind that they may present information that reflects the authors' points of view and biases and may simply be incomplete. For instance, a study of the 19th century Great Famine period in rural County Roscommon, Ireland observed that despite all the contemporary documents that were available, there was still very little information known about the rural poor of that period (Orser 1997) and this lack of understanding of particularly the poorer sections of society holds true throughout the period. Therefore, in the case of post-medieval diet, the detailed records of dietary consumption in post-medieval England do not preclude archaeological research but provide a rich complimentary resource for comparison with archaeological evidence such as isotopic data which facilitate more 'direct' dietary interpretations that take every level of society into account at the level of the individual. This in turn provides a significant source to answer broader questions such as socioeconomic, location, ethnic, gender, and age issues within these populations. Overall, by examining isotopic results alongside historical documents, interpretations of post-medieval life can be achieved in a wider context. Furthermore, Powers et al (2013) noted that the archaeological material from the post-medieval period for which the historic records are available can particularly be useful as a proving groundfor creating models that will be of great potential to utilise as interpretive tools for earlier non documented populations.

1.2 Post-medieval diet and isotopes

Since the pioneering work on stable isotope analysis four decades ago, stable isotope analysis on human skeletal remains recovered from archaeological sites has been significant in palaeodietary studies due to its ability to provide dietary patterns of individuals. The principles of using stable isotope analysis of carbon and nitrogen to explore dietary practices of ancient populations are well established (Schwarcz and Schoeninger 2012; reviewed in Chapter 2). The tissue used for this thesis, bone, is one of the tissues frequently used by archaeological researchers for palaeodietary studies as it is a hard tissue that is generally preserved in archaeological contexts. Bone tissues incorporate stable isotopes of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) from foods consumed by individuals during their lifetime and are retained after death (Ambrose and Norr 1993; Wild et al. 2000; Hedges et al. 2007; Lee-Thorp 2008). In recent years there has been a great increase in the studies of post-medieval diet in English populations, however, these are mostly from London (Trickett 2006; Nitsch et al. 2010; Nitsch et al. 2011; Beaumont 2013; Beaumont et al. 2013a; Beaumont et al. 2013b; Brown and Alexander 2016; Bleasdale et al. 2019), with few elsewhere, Birmingham (Richards 2006), Coventry (Trickett 2006) and Plymouth and Gosport (Roberts et al. 2012). These studies are consequences of the expanding developmental proposals in the South of England which in turn are resulting in an increase in the number of excavations in that area (Powers et al. 2013). For this reason, the initial part of this research redresses the southern focus, by contributing new data from selected sites in the North of England, particularly the 18th to 19th century Manchester urban community.

Previous studies have also all concentrated on the use of stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in bone collagen because of its stability relative to bone carbonate ($\delta^{13}C$) (Schoeninger and DeNiro 1982; Schwarcz and Schoeninger 2012). However, bone collagen provides information on the main protein sources of the diet, with a small proportion of non-essential amino acids synthesised from other dietary sources (Fernandes et al. 2012). The use of bone carbonate, on the other hand, has the potential to augment bone collagen stable isotopic analysis as it offers information on the whole diet (carbohydrates, lipids, and proteins) (Krueger and Sullivan 1984; Ambrose and Norr 1993). Unlike bone collagen then, bone carbonate has the power to identify the input of C_3/C_4 carbohydrates (and fats) to the diet (Kellner and Schoeninger 2007; Fernandes et al. 2012). This is of relevance for the post-medieval period in England where C_4 resources in the form of cane sugar and maize, found their way into the English diet (Mintz 1986; Ó'Gráda 1989; Brassley 2000; Thirsk 2007). Colonial exploitation and the slave trade enabled England to obtain its cane sugar from sugarcane grown and harvested by Africans who had been sold to white traders as slaves (Walvin 2017; Stack et al. 2018). Maize on the other hand was imported from America and supplied to Irish immigrants who came to work in Britain

during the Great Irish Famine (Dudley-Edwards and Williams 1956; Ó'Gráda 1989). Most of the post-medieval dietary studies have alluded to the consumption of these C_4 resources, e.g. Trickett (2006), Nitsch et al. (2010), Nitsch et al. (2011), and Bleasdale et al. (2019), however, these sources of food are less visible in collagen because their contribution to the protein part of the diet is minimal. This project uses a novel approach, bone carbonate ($\delta^{13}C_{carbonate)}$ analysis, to investigate the rise of C_4 consumption in England across eleven human and nine faunal post-medieval skeletal collections in England. These human populations comprise a range of social status and cemetery affiliation (religious denomination) to provide a wider cross-section of society than has been explored previously, particularly in the North (Table 1.1; Figure 1.1)

Table 1-1: List of the human sites sampled in this thesis, with associated dates, denomination and economic status.

Site name	*Location*	*Status*	*Period*	*Denomination*
Fewston	Harrogate	Mixed*	18th-19thc.	Church of England
Rotherham Minster	Rotherham	Low Status	1780s-1854	Church of England
Hazel Grove	Stockport	Low Status	1794-1910	Non-conformist Wesleyan
Victoria Gate	Leeds	Low Status	1796-1850s	Non-conformist Methodist
St George Crypt	Leeds	Middle/Upper Class	1840-1911	Anglican Church
Square Chapel	Halifax	Middle Class	1772-1857	Non-conformist Congregationalist
Cross Street	Manchester	Middle/Upper Class	18th-19thc.	Non-conformist Unitarian
Royal London Hospital	London	Low Status	1825-1841	-
St Brides Lower	London	Low Status	1770-1849	Church of England
Queen's Chapel	London	Mixed	1510-1854	Congregationalist
St Barnabas/St Mary Abbots, Kensington	London	Upper Class	1760-1853	Church of England

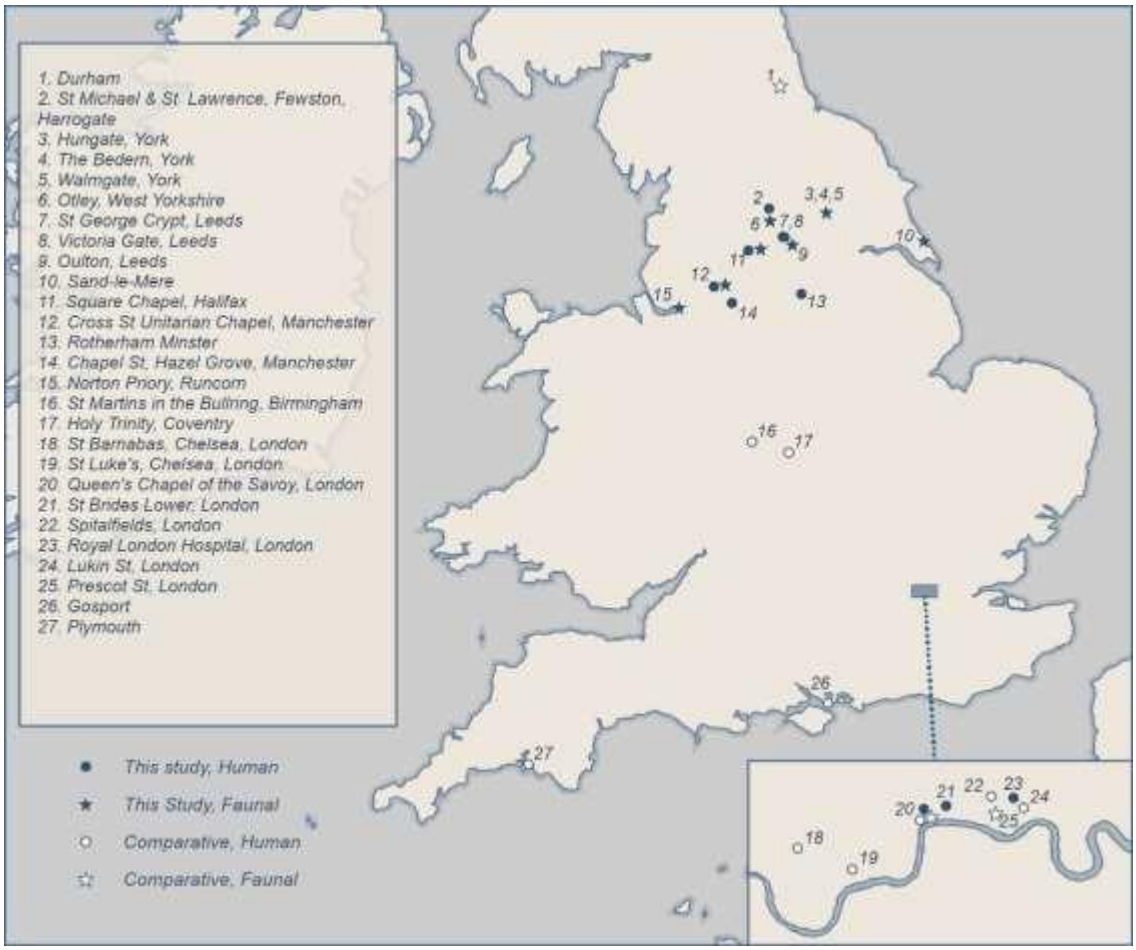


Figure 1-1: Map of post-medieval England showing the locations of the sites in this study and other post medieval sites that have been studied. The city of London is shaded, and sites are given from 18 to 25. Note that there are more post-medieval individuals that have been studied in the South of England compared to the rest of the country before this study.

Additionally, this research explores the potential for using isotope analysis of human dental calculus carbonate as a new proxy for cane sugar consumption investigations in post-medieval individuals. This tissue has been investigated for use in dietary studies previously (Scott and Poulson 2012; Poulson et al. 2013; Salazar-Garcia et al. 2014; Eerkens et al. 2014; Price et al. 2018) but only one of the studies has specifically targeted the inorganic fraction in a limited way (Price et al. 2018). This thesis presents an improved methodology as well as the use of modern human samples from the Forensic Anthropology Center, USA, taking a distinctive and novel approach to explore whether dental calculus carbonate may offer a marker for C_4 sugar consumption.

1.3 Research Aims, Questions, and Objectives

The overall aim of this research is to undertake a comprehensive analysis of diet in post-medieval England (c.17th-19th centuries) extending the previously published datasets through analysis of additional sites and using a broader range of isotopic evidence. Three research questions will be explored namely:

1. Can bone carbonates offer useful complementary evidence for diet alongside more traditional collagen approaches in post-medieval England?
2. Are there any variations in diet between and within different populations in 17th and 19th century England, e.g., by geographical location (London vs northern manufacturing towns), age (adult/non-adult), socioeconomic status, ancestry or sex?
3. Does dental calculus bicarbonate have the potential to be utilised as the most sensitive indicator target of the consumption of cane sugar and a viable substitute for bone carbonates to explore C_4 resource consumption in post-medieval England?

Objectives

1. To carry out measurements of carbon and nitrogen in bone collagen from individuals from two different socioeconomic groups from Manchester, a middle/upper-class Cross Street population and a lower-class Hazel Grove population.
2. To analyse a number of post-medieval animal remains from Manchester (Cross Street), Cheshire (Norton Priory) and Yorkshire (Oulton, Leeds; Otley, Leeds; Square Chapel, Halifax; Sand-le-Mere, Tunstall, Hungate, York, The Bedern, York, Walmgate, York) to provide an animal baseline to aid in the interpretation of human diet.
3. To evaluate the preservation of bone carbonate using Fourier Transform Infrared spectroscopy (FTIR-ATR) prior to bone carbonate stable isotope analysis.
4. To carry out measurements of carbon in bone carbonate from individuals from eleven different post-medieval populations across England
5. To develop a suitable criterion to analyse diet using $\delta^{13}C$ in paired bone, calculus, and teeth mineral samples.

1.4 Thesis Layout

The remainder of this thesis is separated into 8 chapters with the bibliography provided at the end of the thesis. Chapters 2 to 5 provide the general background of the thesis. The discussion of the results of this thesis is presented as three research articles (not submitted at the time of thesis

submission), dealing with specific case studies/research questions, intended for publication in peer-reviewed journals (Chapter 6, Chapter 7, and Chapter 8).

Chapter 2 introduces the theory and concepts behind the use of stable isotope analysis to reconstruct diet. It also provides a description of the development and structure of the bone tissues utilised in this study. Previous research into stable carbon isotopes of bone carbonate and the controversies surrounding its use are also discussed in this chapter.

Chapter 3 gives an overview of the beginning of the Industrial Revolution in England and its effects on the lives of individuals in England. The chapter also provides the historical and archaeological evidence for diet in England thereby providing the context for the interpretation of isotope results.

Chapter 4 introduces the historical and archaeological contexts of the sites studied in this thesis. This chapter also serves as Supporting information for the Chapter 7 paper.

Chapter 5 provides an outline of the sampling methodology, sample preparation method, and the analytical procedures followed.

Chapter 6 explores diet in post-medieval Manchester using stable isotope analysis of human and faunal bone collagen samples. It gives the first insight into the diet of two post-medieval populations in the Greater Manchester area in England - part of the country where isotope analysis on post-medieval individuals has not been performed before. The data are considered in the context of the constraints that governed consumption by focusing on age, sex, race, and social status.

Chapter 7 examines diets in industrialised London and Northern Manufacturing towns using bone collagen and bone carbonate analysis. This will be the first-time bone carbonate isotope analysis has been used to assess diet in England. This chapter also presents the results of FTIR-ATR to assess diagenesis prior to bone carbonate isotope analysis. Furthermore, an analysis of diet on post-medieval faunal remains across nine sites is undertaken to provide a baseline for the human diet.

Chapter 8 explores the potential of utilising dental calculus as a new proxy for C_4 consumption which increased in post-medieval England. The rare availability of modern human samples from the USA, a country whose population consumes large amounts of sugary food enables the study to determine if dental calculus is a viable tissue for sugar consumption isotopic studies. Finally, an analysis and interpretation of dental calculus FTIR-ATR results are presented.

Chapter 9: This chapter brings together all the objectives and findings drawn from the research project, concludes the research, and finishes with a discussion of its significance for future archaeological research.

Chapter 2: Stable isotopic investigation of diet and bone chemistry

This chapter introduces the basic concepts behind the use of the stable isotopes of carbon (from bone collagen and carbonates) and nitrogen (from bone collagen) as dietary biomarkers in human tissues when undertaking palaeodietary studies. It also describes the composition, isotope dietary routing, structures and diagenetic alteration of bone, the main tissue used in this research.

2.1 Bone

In order to understand the isotopic signals in animal and human tissues, it is important to first understand bone tissue, its chemistry as well as its degradation in archaeological settings. In addition, the isotopic routing for each bone tissue will be explored.

Bone is living tissue that constantly undergoes remodelling (a continuous process of simultaneous bone resorption and bone formation) throughout life (Figure 2.1). Bone formation involves the laying down of new bone tissue to replace old tissue and resorption is the breaking down of old bone tissue by a group of osteoclasts cells which in turn remove old bone tissue. The bone remodelling cycle is reviewed in detail elsewhere (Nair et al. 2013; Ralston 2017).

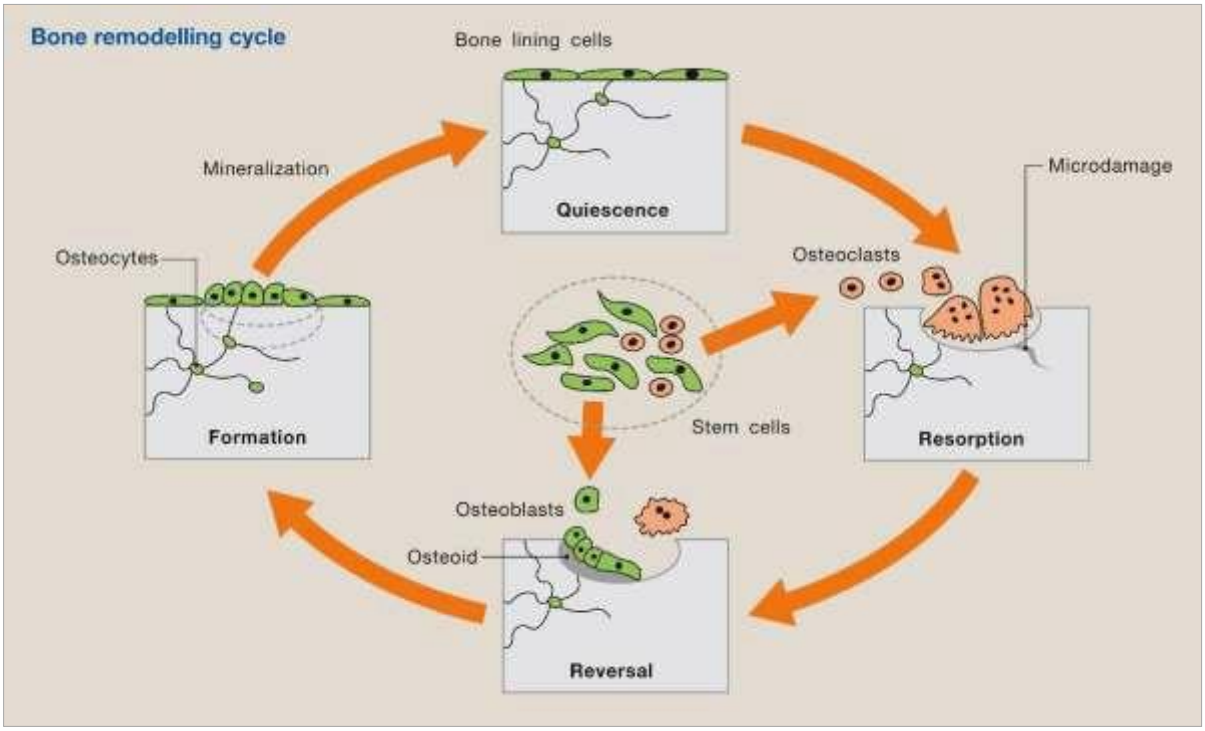


Figure 2-1: Bone remodelling cycle (Ralston 2017).

The rate of remodelling is not constant and varies throughout an organism's lifespan with bone turnover changing with the organism's bone type, age, and sex (Geyh 2001; Hedges et al. 2007). Previous studies revealed that bone collagen turnover in juveniles is faster than in adults, that of adult females is faster than that of adult males and the femoral bone reflects an individual's diet over a much longer period of time than 10 years (Cox and Sealy 1997; Hedges et al. 2007). Ribs onthe other hand have faster turnover rates and may represent diet from a more recent period priorto death - 5 to 10 years (Cox and Sealy 1997; Hedges et al. 2007; Meier-Augenstein 2017). This should be borne in mind, for those situations when mixed samples are recovered from archaeological sites.

Bone has a significant biomechanical performance due to its intricate and unique hierarchical structure (Figure 2.2).

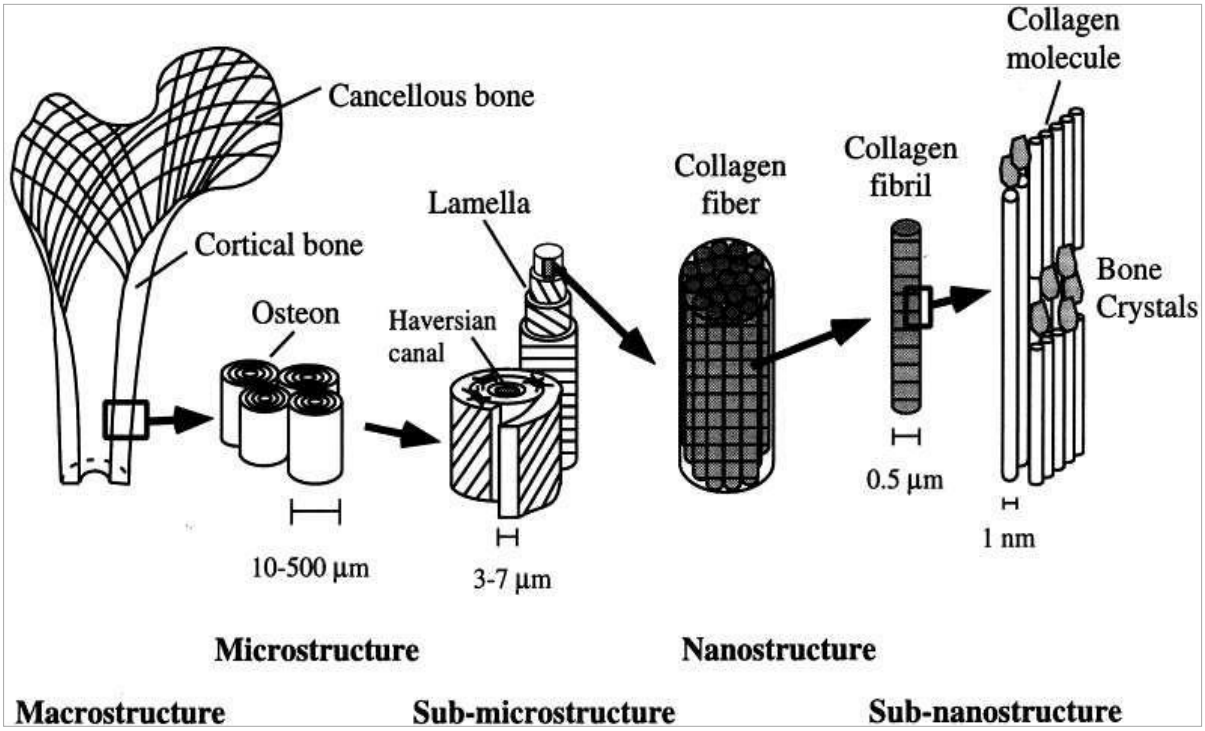


Figure 2-2 The hierarchical structure of bone at its various length scales (Rho et al. 1998).

The hierarchical structure of the bone is complex with dimensions spanning from the macro to the nanoscale (Rho et al. 1998; Liu et al. 2016). The macrostructure and the microstructure of the bone are reviewed in detail in the literature elsewhere (Rho et al. 1998; Kini and Nandeesh 2012; Liu et al. 2016), but the following section will focus on the nanostructure since the components from this are the most relevant for this study. At this scale, a multiphase nanocomposite consisting of an organic matrix (collagen protein), an inorganic matrix (apatite mineral), and water is observed.

2.1.1 Water

Water makes up approximately 25% of the total weight of bone. About 85% of water is found in the organic matrix around the collagen fibres and ground substance with the other 15% located in the intracortical canals and cavities as pore water which serves to transport bone cells and nutrients (Nordin and Frankel 2001, 27).

2.1.2 Bone collagen

The organic matrix makes up about 20% of the dry weight of a bone. Approximately 90% (by weight) of this matrix is made up of long fibrils of type 1 collagen, ~5% is a series of non-collagenous proteins such as osteocalcin and the remaining fraction is made up of lipids, water, and carbohydrates (Schwarcz and Schoeninger 1991; Nielsen-Marsh et al. 2000; Liu et al. 2016). Type 1 collagen is a fibrous protein comprising three stretched helical chains of similar length (two α-1 chains and one α-2 chain). Each chain contains 1050 amino acids that have merged into triple-helical structures called tropocollagen characterised by an abundance of three amino acids: glycine, proline, and hydroxyproline. These amino acids make up the characteristic repeating motif, with the glycine residue present at every third amino acid (Xxx-Yyy-Gly). The positions Xxx and Yyy can be any amino acid but are often proline and hydroxyproline (Dumitru et al. 2018). The tropocollagen in turn combine to form collagen fibrils that are bundled into fibres (Figure 2.3). Collagen is the most abundant protein in bone (Liu et al.2016; Nijhuis et al. 2019). The presence of mineral bioapatite embedded within the protein contributes to the bone strength and elasticity of the connective tissue of the body. Collagen is also found in skin, tendons, and a variety of other soft tissues (Antoine et al. 1992; Kini and Nandeesh 2012). Experiments revealed that the structure and composition of collagen do not vary significantly between different vertebrate species (Armstrong et al. 1983; Ambrose 1993).

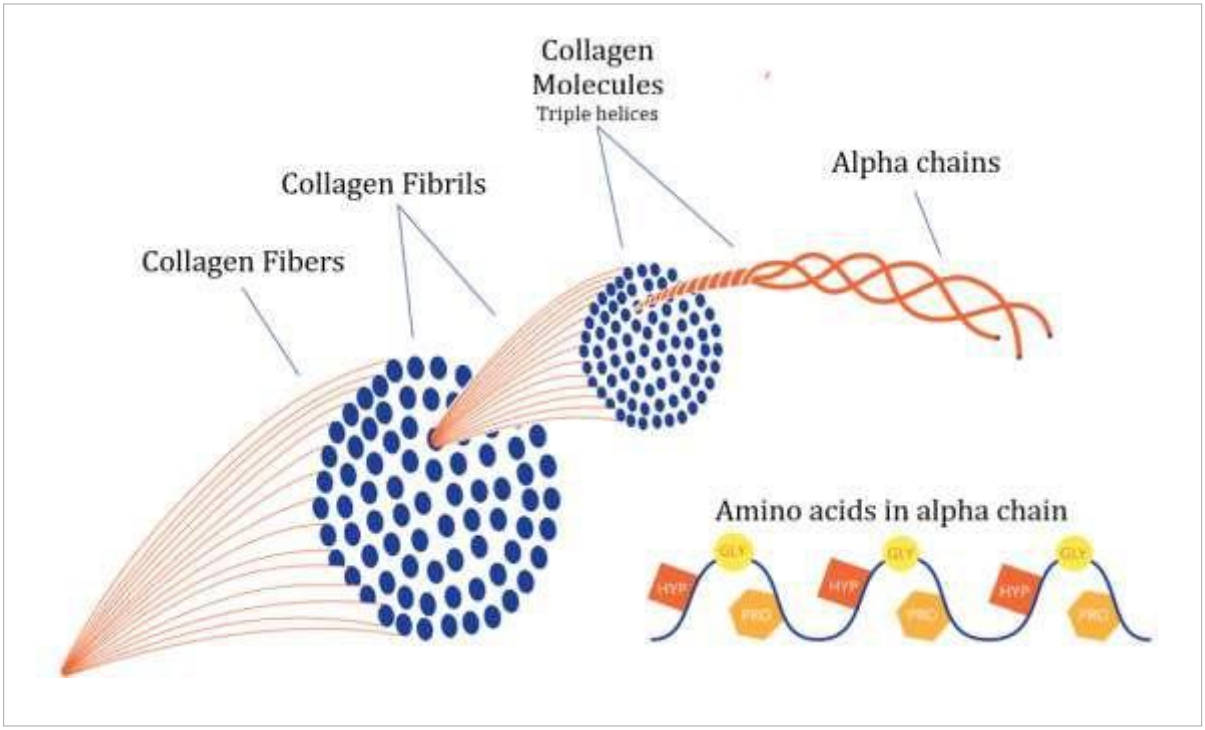


Figure 2-3: A diagram showing how collagen fibrils form into collagen (Nijhuis et al. 2019).

2.1.3 Bone mineral

The inorganic mineral component of the bone makes up approximately 70% of dry weight of the bone and is predominantly crystalline although it may exist in amorphous forms. The inorganic matrix of a bone is composed of carbonate apatite (dahllite) which is essentially a carbonated form of the geological hydroxyapatite (HA) with a general formula $Ca_{10}(PO_4)_6(OH)_2$ but containing up to 7 wt.% (percentage by weight) carbonate. Bone apatite has various crystallographic sites that allow chemical variations to occur that will fulfil the overall charge balance in the mineral (Wopenka and Pasteris 2005). The basic structure of bone apatite $Ca_{10}(PO_4)_6(OH)_2$ can be altered when CO_3^{2-} ions occupy the phosphate site (Type B carbonate substitution-major) and/or hydroxyl (OH^-) (Type A carbonate substitution-minor) sites. In addition, some additional carbonate (CO_3) or bicarbonate (HCO_3^-) can be adsorbed onto crystal surfaces. It is generally accepted that Type B substitution at the phosphate sites is the dominant form of substitution in biological apatites while Type A carbonate substitution at hydroxyl sites is very low in comparison (Wopenka and Pasteris 2005;Loreille et al. 2007; Monge et al. 2014; Von Euw et al. 2019).

The bone mineral phase consists of plate-shaped crystals of carbonated hydroxyapatite with a hexagonal crystal structure (Figure 2.4). The bone mineral crystals are mostly arranged with their broad surface facing collagen fibril surface (Lees et al. 1994), parallel with their c-axes and aligned

together with the axis of the adjacent mineralized collagen fibrils (Su et al. 2003).

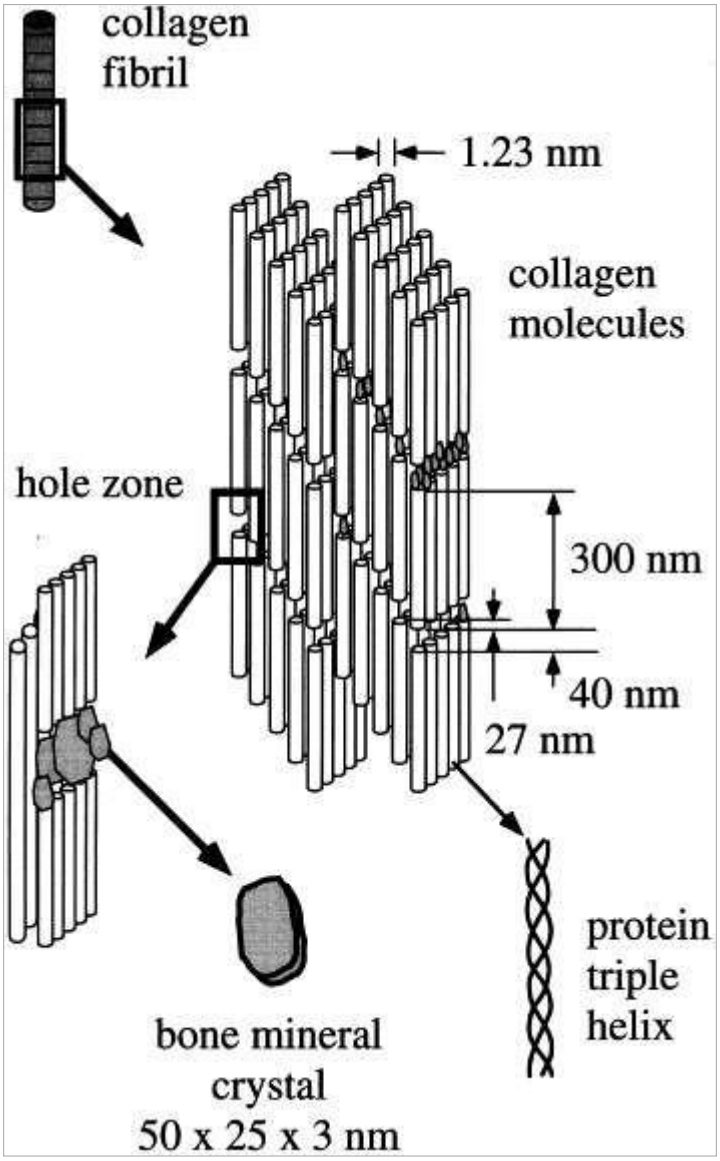


Figure 2-4: A schematic diagram illustrating the arrangement of bone mineral crystals (Rho et al. 1998).

Relatively little is known about bone mineral and its intimate association with collagen. Nudelman et al. (2010) investigated how apatite crystals form inside a fibril and suggested that there is a specific polymer that attracts calcium phosphate clusters forming complexes that transform into stable mineral droplets. When these mineral droplets come into contact with the collagen fibril they combine and fuse inside the fibril, filling all the available space. It then solidifies into a distorted fluid-like phase which is then directed by the collagen to transform into crystals, resulting in the infiltration of the fibrils with amorphous calcium phosphate (Nudelman et al. 2010).

2.2 Historical background of stable isotope analysis

The use of isotopes in archaeological studies is based on the principle that organisms incorporate chemical elements into their body tissues with isotopic compositions that resemble those of the food and water ingested during their lifetimes - "You are what you eat and drink" (Vogel and van der Merwe 1977; van der Merwe and Vogel 1978; Tykot et al. 1996). Seminal investigations into the utilisation of stable isotope analysis of human bone collagen to investigate diet were performed in the 1970s (Vogel and van der Merwe 1977; van der Merwe and Vogel 1978). These studies demonstrated that stable isotope values of human bone collagen were dramatically changed by the introduction of maize agriculture in North America, leading to the revision of the then understanding of the date of introduction of maize into the region. The authors discovered that the bone collagen $\delta^{13}C$ values of North American individuals only showed an isotope shift consistent with consumption of C_4 maize around 1150 BP (Figure 2.5).

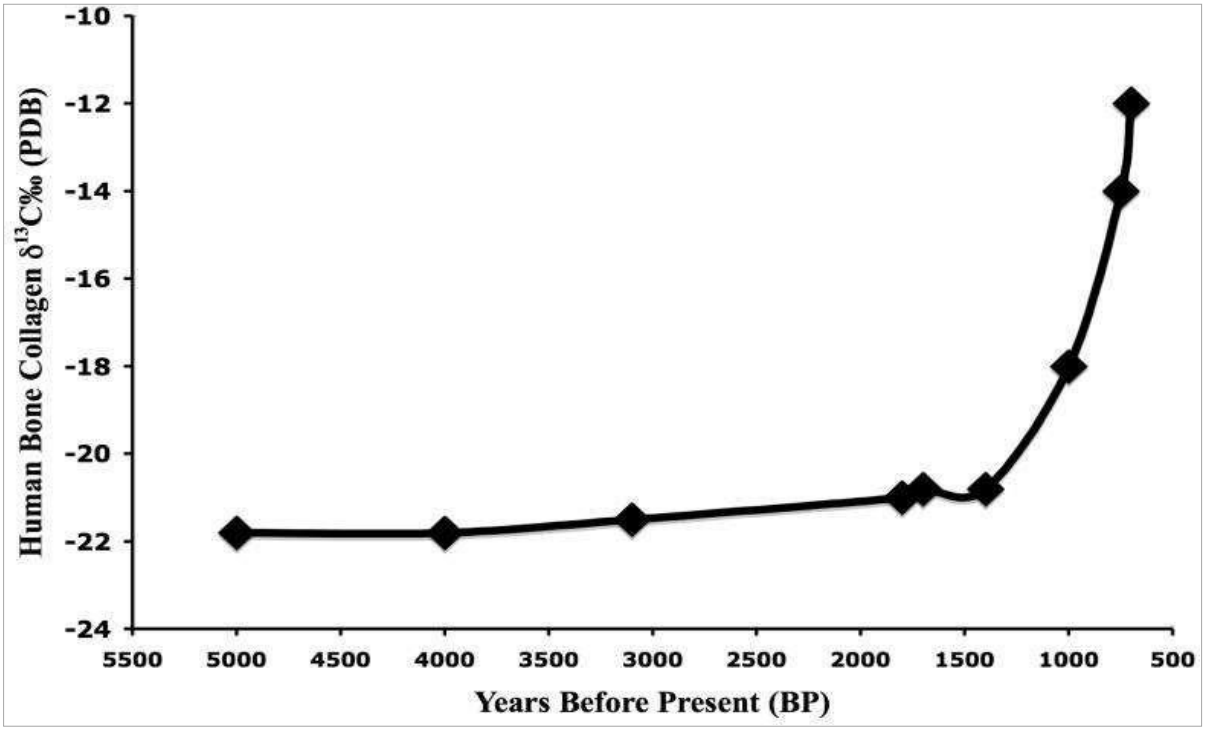


Figure 2-5: Stable carbon isotope ratios in bone collagen demonstrating the sharp rise in maize agriculture after 1150 BP (redrawn by Schoeninger (2009) from data obtained from van der Merwe and Vogel 1978; Vogel and van der Merwe 1977 studies).

This in turn demonstrated that isotope analysis could complement traditional archaeological evidence of dietary interpretations. DeNiro and Epstein (1978a) were the first to demonstrate the use of isotopic analysis of bone carbonate in a study of two sympatric hyrax species in the Serengeti Plain, Tanzania. The carbon isotope ratios of the animals in this study indicated that their diet was largely composed of C_4 plants. After this, Sullivan and Krueger (1981) published a

paper that introduced the use of the mineral phase of calcified tissues for dietary isotope studies. They showed that stable carbon isotope values extracted from a purified mineral phase were offset, but comparable in reliability to the values extracted from the purified organic phase. Following this, Tauber's (1981) study of Mesolithic fishers, Neolithic farmers, and historic fishers in Denmark became the first to quantify marine resource consumption using ^{13}C. Soon after, Schoeninger et al. (1983) noted that nitrogen isotope ratios could serve the same purpose as they varied between different food sources (differences caused by trophic level effects), especially marine and terrestrial. From this point onwards, stable isotope analysis of human bones in palaeodietary studies further developed, and rapid advances in the field have been witnessed over the years. The advanced techniques have resulted in researchers having a better understanding of palaeodietary patterns from the Palaeolithic to the historic periods (Richards et al. 2005; Lightfootand Stevens 2012; Goude et al. 2015; Robson et al. 2016; Bleasdale et al. 2019). Furthermore, they have also proven vital in characterisation of animal husbandry practices (Finucane et al. 2006; Towers et al. 2011) as well as human breastfeeding and weaning practices (King et al. 2017; Craig-Atkins et al. 2018). Moreover, isotopic studies have provided a means of exploring social identity (Leach et al. 2010; Brock 2019) as well as population mobility and migration (Shaw et al. 2016; Hemer and Evans 2018).

2.3 Basic concepts in stable isotope analysis

Isotopes are atoms of the same element that have the same number of protons and electrons but vary in the number of neutrons. The difference in the number of neutrons between the various isotopes of an element results in different masses of the various isotopes. Isotopes are divided into two kinds, stable and unstable isotopes. Stable isotopes do not decay, thus remain in the environment in constant concentrations while unstable isotopes undergo radioactive decay and lose energy over time. For example, carbon has three naturally occurring isotopes, carbon-14 (^{14}C), carbon-13 (^{13}C) and carbon-12 (^{12}C), but only ^{13}C and ^{12}C are stable forms of carbon whereas carbon-14 ^{14}C is the unstable form (Pollard and Wilson 2001; Gross 2006; Hoefs 2008). The current research is a dietary reconstruction based on stable isotopes of carbon (^{13}C and ^{12}C) and nitrogen (nitrogen-15 (^{15}N) and nitrogen-14 (^{14}N)) to reconstruct diet. Each element has a higher proportion of the lighter stable isotope relative to the heavier isotope , consequently, for these two elements, ^{12}C and ^{14}N are the most common isotopes in nature (Fry 2006; Hoefs 2015 ; see Table 2.1).

Table 2-1: Stable isotopes examined in this project and their isotopic abundances (adapted from Fry 2006, 9).

Element	*Stable isotope*	*Abundance in nature (%)*
Carbon	^{12}C	98.93
	^{13}C	1.07
Nitrogen	^{14}N	99.64
	^{15}N	0.36

Mass differences in isotopes result in different reaction rates which leads to isotopic fractionation (Schoeller 1999). This is the relative partitioning of the heavier and lighter isotopes of an element during naturally occurring processes, arising from the subtle variations in the relative atomic mass. There are two kinds of isotopic fractionation processes, equilibrium, and kinetic isotope effects, with the latter being the most pertinent to the current thesis. In general, all isotopic effects involving organic matter are kinetic and occur commonly in biological processes. In this process, molecules that have light isotopes, hence weaker bonds and therefore lower dissociation energy, react more readily than those containing heavy isotopes which generally have stronger bonds and therefore higher dissociation energy. Thus, the lighter isotope reacts more readily and becomes more concentrated in the products. Reaction products become enriched in the lighter isotope relative to the residual reactants (Hoefs 2015). An example of this effect used in palaeodietary studies is the carbon fixation via photosynthesis whereby organic matter gets depleted in ^{13}C relative to the atmosphere during photosynthesis (Hoefs 2015).

In palaeodietary studies, changes in the ratio of the heavier to the lighter isotope are expressed in delta notation (δ) in parts per thousand (per mil or ‰) relative to an internationally agreed sample material (McKinney et al. 1950; Craig 1957).

$$\delta\ (‰) = (R_{sample}/R_{standard} - 1) \times 1000$$

Where R is the ratio of the heavy to light isotope in the sample or standard. Thus, for carbon and nitrogen, the formulas are as follows respectively:

$$\delta^{13}C\ (‰) = (^{13}C/^{12}C_{sample}/^{13}C/^{12}C_{standard} - 1) \times 1000$$

$$\delta^{15}N\ (‰) = (^{15}N/^{14}N_{sample}/^{15}N/^{14}N_{standard} - 1) \times 1000$$

The international standard for carbon ($\delta^{13}C$) was a sample of $CaCO_3$ obtained from a calcareous Cretaceous fossil of *Belemnitella Americana* from the Pee Dee formation of South Carolina (Pee Dee Belemnite, PDB) (Smith and Epstein 1971; Craig 1957). The original source has been exhausted, but other reference standards were calibrated to that original sample and carbon isotope values are still reported relative to PDB but using the term VPDB to show that the data are normalised to the values of the standard (Werner and Brand 2001). A more negative $\delta^{13}C$ indicates enrichment in ^{12}C (depletion of the heavier isotope relative to the standard) whereas a more positive $\delta^{13}C$ means enrichment in ^{13}C (enrichment of the heavier isotope relative to the standard). Most natural materials contain less ^{13}C than the standard, resulting in negative $\delta^{13}C$ values (Craig 1957). Atmospheric nitrogen (AIR) is used as the international standard for nitrogen (Coplen 2011).

2.4 Carbon isotopes

Carbon is found in four primary reservoirs in the terrestrial environment: atmosphere, oceans, land, and fossil fuels (Faure 1977; Houghton 2003). The following sections discuss how the analysis of the stable isotopes of carbon can be utilised in identifying dietary variation.

2.4.1 Carbon isotope ratios in plants

The base of most terrestrial food webs is formed by plants fixing carbon dioxide (CO_2) from the atmosphere. Photosynthesis is the process used by plants, algae, and cyanobacteria in both terrestrial and marine environments to convert light energy into chemical energy (Sealy 2001). Plants employ different photosynthetic pathways to metabolise atmospheric carbon and these pathways play a pivotal role in the observed plant carbon isotope ratio ($\delta^{13}C$) variation (Bender 1968; Smith and Epstein 1971). The photosynthetic process tends to utilise a greater proportion of the lighter carbon isotope, ^{12}C than the heavier ^{13}C, resulting in all plants containing a lower proportion of ^{13}C than the surrounding atmosphere. Three photosynthetic pathways occur among higher plants namely, crassulacean acid metabolism (CAM); the Calvin-Benson cycle (C_3 pathway); and the Hatch-Slack cycle (C_4 pathway) (Ehleringer and Monson 1993). Plants using CAM photosynthesis such as pineapples were imported in the 17th century but were very expensive and in the 18th century, were grown in heated greenhouses (BBC News, 2016; Lausen-Higgins et al. 2010). However, they were always rare and could not have formed a significant dietary component, therefore they will not be considered in the analysis of results in this study. However, C_3 and C_4 plants were available, with consumption of the latter, in the form of cane sugar, increasing in England from the 17th century onwards, due to mass import and lower prices

resulting from the development of the Caribbean plantations (Walvin 2017). Therefore, for the purposes of this study, this section will focus only on the C_3 and C_4 photosynthetic pathways.

The photosynthetic pathways of C_3 and C_4 plants (named that way because the number of carbon atoms in the product of the first step of photosynthesis contains three and four carbon atoms, respectively) use different carboxylation enzymes to fix atmospheric carbon dioxide after it has dissolved through the stomata of the plant and enters the mesophyll cells (the outer layer of photosynthetic cells, see Figure 2.6).

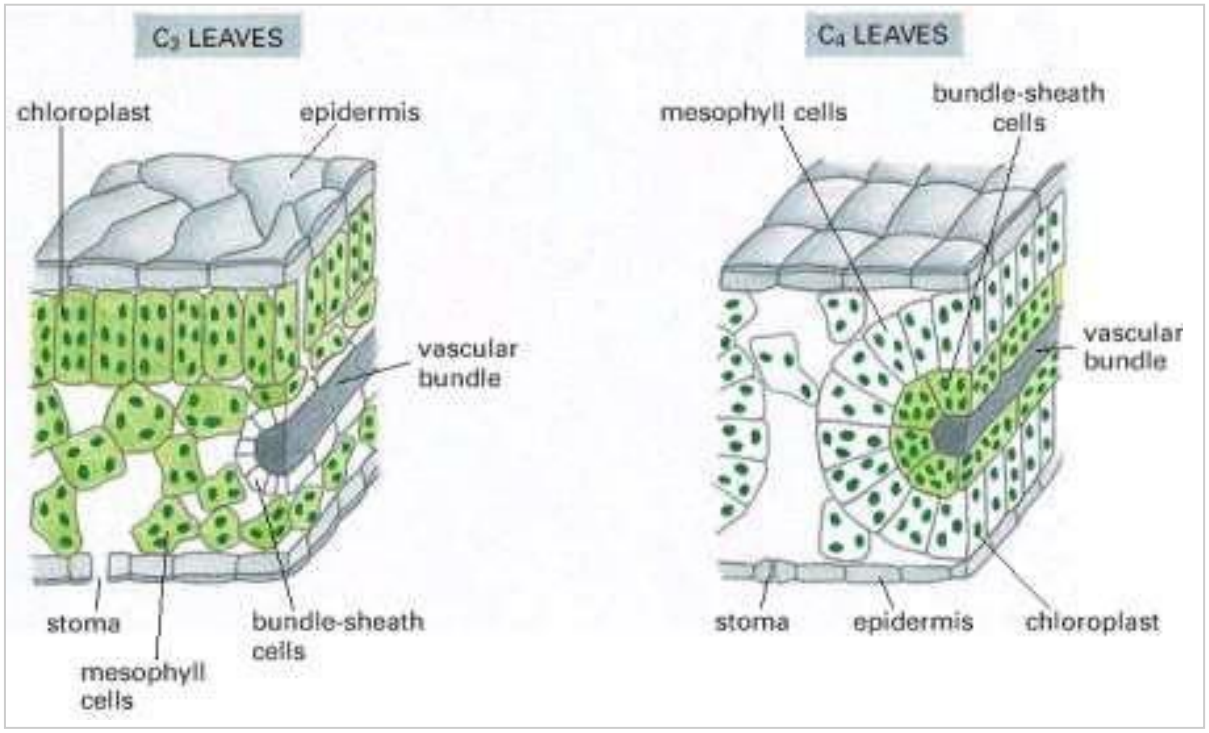


Figure 2-6: Comparison of the anatomies of C_3 and C_4 plant leaves. For C_3 plants carbon-fixation takes place in the mesophyll cells. The green coloured cells are the ones which harbour carbon-fixing chloroplasts. In contrast, for C_4 plants carbon-fixation does not take place in the mesophyll cells. Instead, the mesophyll cells facilitate CO_2 transfer to create a CO_2-rich environment in the bundle-sheath cells, where carbon-fixation exclusively takes place in C_4 plants. The vascular bundles are responsible for transporting the carbohydrates made in the leaves to other parts of the plant (Alberts et al. 2002).

2.4.1.1 C_3 (Calvin-Benson) photosynthetic pathway

The first phase in the Calvin-Benson cycle is the fixation of carbon dioxide by the action of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Usually, atmospheric carbon dioxide diffuses into the stoma of the chloroplast in mesophyll cells where it is combined with ribulose-1,5-bisphosphate (RuBP) by the enzyme Rubisco, to produce two molecules each with 3 carbon atoms (3-phosphoglycerate). The second phase is the reduction phase whereby the products of the photosynthetic light reactions (ATP, NADPH) are utilised to further reduce phosphoglycerate (PGA) into a series of intermediate products in the photosynthetic carbon reduction cycle (PCR), synthesising reduced sugars for further plant metabolism and

regeneration of RuBP to allow the PCR cycle to continue. During the final phase - regeneration of CO_2 acceptor (RuBP)- the enzyme Rubisco also catalyses the oxygenation of RuBP during which process RuBP combines with oxygen to produce one PGA and one phosphoglycolate. Further metabolism of phosphoglycolate results in the release of CO_2. The oxygenation of RuBP and eventual release of CO_2 is termed photorespiration, a respiratory process in many higher plants by which they take up oxygen in the presence of light and give out some carbon dioxide, contrary to the general pattern of photosynthesis. The plants that use this standard mechanism of carbon fixation are called C_3 plants because of the three-carbon compound (3-PGA) the reaction produces. Almost 85% of plant species on the planet earth are C_3 plants and these include plants such as rice, wheat, soybeans, all trees, and grasses suited to temperate climates (O'Leary 1988; Ehleringer and Monson 1993; Hoefs 2015).

2.4.1.2 C_4 (Hatch-Slack) photosynthetic pathway

C_4 plants use the phosphoenolpyruvate (PEP) as a carbon fixation substrate instead of RuBP. Within the mesophyll cells, is the enzyme phosphoenolpyruvate (PEP) carboxylase that catalyses the initial photosynthetic reaction. This reaction - fixation of CO_2 or carboxylation - involves carbon dioxide from the atmosphere entering through stomata into the mesophyll cells and combining with phosphoenolpyruvate (3-carbon compound) to produce oxaloacetate (OAA), a four-carbon acid; hence the name C_4 photosynthesis. Because it is not a stable compound, OAA is readily reduced into a new four-carbon molecule called malate which then diffuses into the bundle-sheath cells (See Figure 2.6). Malate undergoes decarboxylation (via malic enzyme or PEP carboxykinase) in the bundle-sheath cells where it dissociates to pyruvate and CO_2. The CO_2 that is formed enters the Calvin cycle where sugar is formed and Pyruvate returns to the mesophyll cell where it is phosphorylated to PEP, the CO_2 acceptor in the C_4 cycle, and the cycle begins again. The C_4 cycle is a special adaptation feature present in tropical or semi-tropical, high light intensity, high temperature, drought conditions. Examples of plants that utilise the cycle are maize, sugarcane, millet and sorghum. The C_4 pathway is, however, not an alternative pathway for carbon fixation but it is an additional process with the CO_2 formed in the bundle-sheath cells ultimately being fixed by the Calvin-Benson cycle (Iglesias et al. 1986; Hatch and Burnell 1990; Ehleringer and Monson 1993).

The range of $\delta^{13}C$ values in plants utilising the C_3 and C_4 photosynthetic pathway is well defined (Cerling et al. 1997; Cerling 1999; see Figure 2.7).

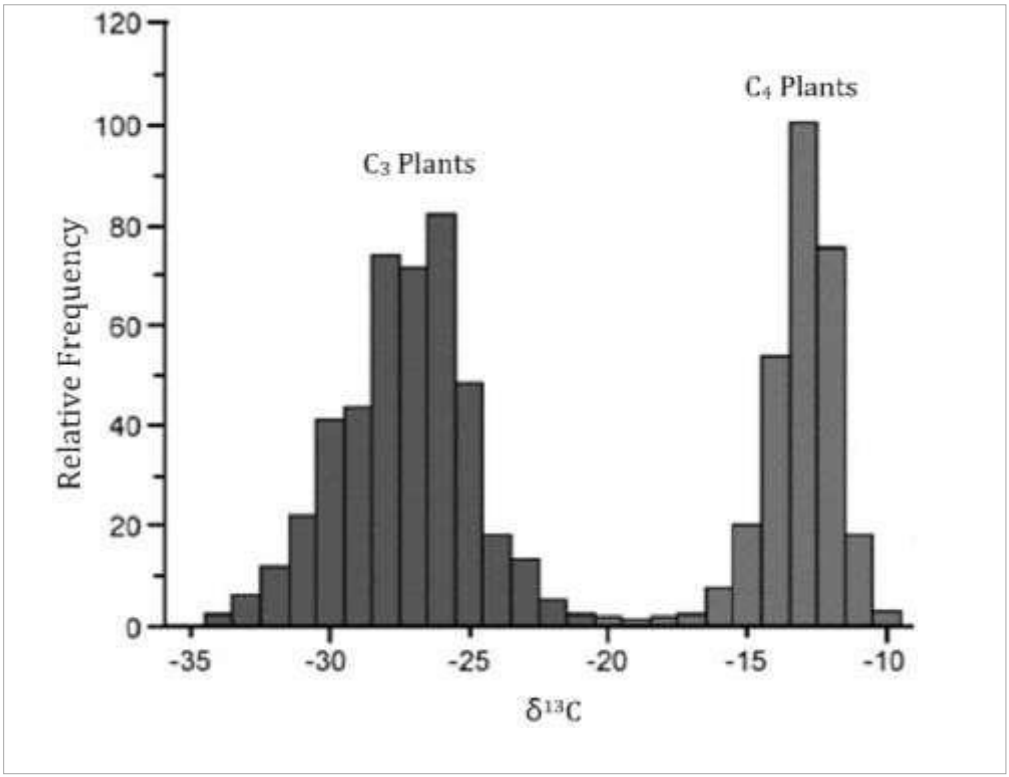


Figure 2-7: Histogram showing the distribution of $\delta^{13}C$ values of individual grasses (Cerling 1999).

Plant collagen $\delta^{13}C$ values obtained from studies of C_3 grasses such as oats, wheat, and barley are -27.0‰, -26.9‰, and -27.8‰ respectively while those from C_4 grasses such as millet, sorghum, and sugarcane are -13.3‰ -12.2‰, -13.7‰ respectively (Bender 1968, 470). This is because the ribulose bisphosphate carboxylase/oxygenase ("Rubisco") enzyme (*in the C_3 pathway*) discriminates against the heavier isotope more strongly than phosphoenolpyruvate (PEP) carboxylase (*in the C_4 pathway*). As a result, when compared to reference studies, C_3 plants are relatively depleted in the ^{13}C isotope compared to C_4 plants. (O'Leary 1981, 554; Hoefs 2015, 67–68). This C_3 or C_4 isotopic signature in plants is retained along the food chain, enabling the investigation of the relative contribution of C_3 and C_4 resources to the diet of consumers. However, there are other environmental variables that can alter $\delta^{13}C$ values in plants during photosynthesis. An in-depth description of these factors is provided elsewhere (Tieszen 1991; Heaton 1999; Dawson et al. 2003) but a brief summary of some of them are provided below.

2.4.1.3 Plant organ variations

There have been a number of studies that have shown that the plant isotopic signatures derived from photosynthesis can be altered further by post-photosynthetic fractionation processes which in turn cause distinct signatures in different plant organs (Badeck et al. 2005; Eglin et al. 2009; Zhao et al. 2017). Leaves are said to be generally depleted in ^{13}C compared to all the other plant organs (Ghashghaie et al. 2003). More recently, a study showed that the average $\delta^{13}C$ value in

mature seeds was the highest among soybean plant tissues followed by the whole shoot, then the uppermost mature trifoliolate, and finally the center leaflets (Kaler et al. 2018) . Additionally, Heaton (1999) showed that different parts of the same plants such as seeds and stems, exhibit $\delta^{13}C$ value variations of up to 1-2‰. Therefore, it is always important for investigators to specify the plant tissue from which $\delta^{13}C$ values were obtained. This is potentially important for dietary studies as plant parts vary in their digestibility. Differential digestion of particular parts by consumers may therefore result in a shift in consumer tissue $\delta^{13}C$ values (Tieszen 1991; McCutchan et al. 2003).

2.4.1.4 Climate and Environment

Climate, particularly temperature and humidity have been known to have a significant influence on the isotope ratios of plant tissues (Van Klinken et al. 2002). Arid conditions can cause partial stomatal closure in plants for the conservation of water, which also results in a limited circulation of CO_2 through the stomata. Consequently, this leads to a more complete consumption of CO_2 in sugar generation and therefore limited carbon isotope fractionation and relative ^{13}C enrichment in plant tissues (Farquhar et al. 1989; Schwarcz and Schoeninger 2012). The influence of climate on $\delta^{13}C$ values in plants and therefore those of the plant consumers have been illustrated by Van Klinken et al. (1994) and Van Klinken et al. (2002). The authors demonstrated that regional variations in climate accounted for the variations found in the $\delta^{13}C$ of Holocene charcoal, wood, and bone samples across Europe and suggested that correcting for climate differences could improve dietary comparisons of bone collagen $\delta^{13}C$ values. Additionally, it has been shown that plants from high altitudes are more enriched in ^{13}C than those in lower altitudes (Körner et al. 1991). Both the North American and English samples used in this study are from temperate climates, therefore, no corrections for climate variations are required in this study.

In dense forest canopies, local variations in carbon isotopes occur and it has been shown that $\delta^{13}C$ values tend to increase with height such that plants growing under dense tree canopy cover can have $\delta^{13}C$ values ~5‰ less than atmospheric levels. This is referred to as the canopy effect. Plants preferentially incorporate the lighter isotope ^{12}C, but at the forest floor, litter decomposition by bacteria re-releases ^{13}C depleted carbon dioxide leading to a $\delta^{13}C$ -depleted local environment and therefore plants in that environment will absorb ^{13}C-depleted air (van der Merwe and Medina 1991; Cerling et al. 2004). Some studies have found that plants growing under dense, forested, canopied areas in Africa can be depleted in $\delta^{13}C$ by up to 2-5‰ compared to those in open areas (Ambrose 1993). The canopy effect, therefore, could potentially have an impact on herbivore values from densely forested areas in England. In addition, the microenvironment such as drainage and soil type has also been shown to cause a variation of around 2‰ in $\delta^{13}C$ values of plants. Soils low innutrients can result in a 2‰ depletion in plant $\delta^{13}C$ whereas salinity can cause

higher $\delta^{13}C$ values (O'Leary 1981; Farquhar et al. 1982; Tieszen 1991; Heaton 1999). This will be taken into consideration when interpreting results.

2.4.2 Carbon isotopes in consumers

2.4.2.1 Herbivores and carnivores

Carbon is integrated into animals through the consumption of plants and/or meat resources depending upon each species. DeNiro and Epstein (1978b) conducted controlled feeding experiments with insects and animals fed on diets of known isotopic composition and demonstrated a direct relationship between the stable carbon isotope composition of animal tissues and the $\delta^{13}C$ values of the diet. However, there is usually a poorly defined systematic difference between the isotopic composition of the diet and consumer tissues (Ambrose and Norr 1993). As a result, Ambrose (1993, 60) suggested that for accurate dietary reconstruction, it is essential to analyse purified tissue and to have an understanding of fractionation of that tissue as well as that of isotopic compositions of different classes of resources. Herbivore diets consist only of plants whereas those of carnivores consist of meat of other animals. Thus, the micronutrient of herbivores would consist of a great deal of carbohydrates with minor contributions of lipids and proteins from the plants consumed. On the other hand, a carnivore diet would consist of mostly proteins and lipids but with almost no carbohydrates (Krueger and Sullivan 1984, 212–13). Observations conducted on herbivores revealed that $\delta^{13}C$ values in collagen are enriched by +5‰ relative to the diet whereas that of carbonate is enriched by +12‰ over the assumed diet (Vogel and Van Der Merwe 1977; Vogel 1978; Krueger and Sullivan 1984; Lee-Thorp 1989). Thus, the herbivore carbonate and collagen $\delta^{13}C$ difference is ~7‰. It has also been established that carnivore carbonate $\delta^{13}C$ is enriched by 8–9‰ relative to the diet (Krueger and Sullivan 1984; Lee-Thorp 1989), therefore if the carnivore collagen $\delta^{13}C$ is also enriched by 5‰, then the carnivore carbonate and collagen difference should be 3–4‰. It has been suggested that the differences in herbivore and carnivore $\delta^{13}C_{carbonate\text{-}collagen}$ values are because carnivores consume a greater proportion of dietary lipids which are depleted in ^{13}C relative to other dietary macronutrients. Therefore, since the carnivore diet is richer in lipids than that of herbivores, then carnivores should have lower $\delta^{13}C_{carbonate}$ values, and thus smaller $\delta^{13}C_{carbonate\text{-}collagen}$ values (Krueger and Sullivan 1984; Lee-Thorp et al. 1989; O'Connell and Hedges 2017).

2.4.2.2 Aquatic ecosystems

The photosynthetic processes by primary producers in the marine environment such as algae and phytoplankton make use of carbon derived from dissolved inorganic carbon, of which a major constituent is hydrogen bicarbonate ions (HCO_3^-) with a $\delta^{13}C$ value of 0‰, around 7‰ more

positive than modern atmospheric carbon (Craig 1954, 117; Schwarcz and Schoeninger 2012, 730). When compared to uptake of carbon by plants in terrestrial environments, the uptake of dissolved inorganic carbon by algae and plankton during photosynthesis in seawater, involves a larger kinetic fractionation which leads to algal $\delta^{13}C$ values of about -19 to -24‰ (Peterson and Fry 1987, 301). Due to the difference in carbon sources between marine and terrestrial plants, the $^{13}C/^{12}C$ ratios of the carbon they utilise is different. These differences, however, cause $\delta^{13}C$ offsets that are maintained in the marine and terrestrial food webs. Consequently, the $\delta^{13}C$ values of marine mammals tend to be ~7‰ higher than that of terrestrial animals. Therefore, in addition to being valuable in distinguishing between C_3 and C_4 plant intake, $\delta^{13}C$ can be useful in differentiating marine and terrestrial protein diets. Marine mammal flesh $\delta^{13}C$ values have been found to range between -17‰ to -18‰ with a human diet entirely on marine foods expected to be between -12‰ and -13‰ (Chisholm et al. 1982; Mays 1997). This, however, can be confusing in populations consuming C_4 plants as the $\delta^{13}C$ ranges will overlap, in which case a combination of carbon and nitrogen isotopes is employed to distinguish between the dietary sources in question (Chisholm et al. 1982; Schoeninger and DeNiro 1984; van Klinken 1999).

It has been demonstrated that the $\delta^{13}C$ values in freshwater ecosystems are highly variable (Katzenberg 2008). This is because the different sources of dissolved CO_2 in freshwater available to aquatic plants affect the isotopic composition of carbon. The sources of carbon in freshwater include dissolved CO_2 from the atmosphere, CO_2 in the water, mineral springs, dissolved organic carbon from carbonate and bicarbonate rock weathering, respired organic matter as well as faecal pellets from various aquatic fauna (Peterson and Fry 1987; Zohary et al. 1994; Katzenbergand Weber 1999). Compared to marine resources, freshwater shows more negative $\delta^{13}C$ values (Robson et al. 2016). However, it has been shown that fish from pelagic environments (offshore) may exhibit more negative $\delta^{13}C$ values than those in littoral environments (nearshore) (France 1995b; Briones et al. 1998). Pelagic algae are subject to turbulent conditions and an unstable boundary layer allowing greater diffusion of CO_2 and hence greater levels of ^{13}C depletion, while benthic algae are located in more stable conditions with more stable boundary conditions which prevent CO_2 diffusion and lead to greater assimilation of ^{13}C (France 1995a). This habitat differentiation was also reported in Katzenberg and Weber (1999) whereby littoral species exhibited more enriched $\delta^{13}C$ values (-12.9‰) than pelagic species (-24.6‰). Moreover, fish that move seasonally between marine and freshwater environments can exhibit $\delta^{13}C$ values intermediate between marine and freshwater resources (Schoeninger and DeNiro 1984; Guiry 2019). This freshwater $\delta^{13}C$ value variability may result in difficulties in the interpretation of diet in populations in this study.

2.4.3 Challenges for interpreting $\delta^{13}C$ values in this study

Overall, the expected diet of individuals from the 18th and 19th century England in this study is omnivorous with some of the individuals having higher $\delta^{13}C$ values from either aquatic resources or C_4 diets in the form of cane sugar or maize (see Chapter 3). It has been determined that individuals relying on a primarily C_3 diet should show a mean $\delta^{13}C_{collagen}$ value of ~-21.5‰ and a mean $\delta^{13}C_{carbonate}$ value of ~-14.5‰ whereas those on a C_4 diet, a mean $\delta^{13}C_{collagen}$ value of ~-7.5‰ and mean $\delta^{13}C_{carbonate}$ value of ~-0.5‰ (Ambrose 1993; Mays 1997; Tykot 2004, 435). However, as stated above, the interpretation of diet using carbon isotopes can be confusing in populations consuming both C_4 and marine foods as the $\delta^{13}C$ ranges overlap (Chisholm et al. 1982; Schoeninger and DeNiro 1984; van Klinken 1999). Nevertheless, the use of nitrogen ($\delta^{15}N$), as well as carbon in both bone collagen ($\delta^{13}C_{coll}$) and bone carbonate ($\delta^{13}C_{carb}$), has been shown to have the capability to differentiate between marine and C_4 terrestrial resources e.g. Reitsema et al. (2010) and Yoder (2012), therefore, all three isotopes were utilised in this study.

Another challenge this study faces is the effect of industrialisation on carbon isotope ratios. In the absence of industrial activity, the $\delta^{13}C$ value of atmospheric carbon dioxide is -7.8‰, however, the burning of fossil fuels has since reduced $\delta^{13}C$ in atmospheric CO_2 over the last 200 years by approximately 1.5‰ (Keeling et al. 1979; O'Leary 1981; Marino and McElroy 1991; Graven et al. 2017). This change is commonly referred to as the "Suess effect". The Suess effect is particularly important in dietary reconstruction for the modern individuals included in this study. A time-dependent correction per year is applied to each modern sample in this study according to Graven et al. (2017). The isotopic data in Graven et al. (2017) covers the period 1850 – 2015. The variations associated with fossil fuel burning in industrial areas before 1850, which is after the beginning of the Industrial Revolution in the late 18th century have therefore not been determined. Although there is an awareness of the impact that the Suess effect could potentially have on post-medieval individuals in this study, the correction cannot be applied due to the lack of any data on ^{13}C data prior to 1850.

2.5 Nitrogen isotopes

The processes that determine the fractionation of nitrogen isotopes are not as well understood as those of carbon. The nitrogen cycle involves many external and internal fluxes and the influxes and effluxes of nitrogen in the ecosystem influence soil and plant baseline $\delta^{15}N$ values (Hogberg 1998; Amundson et al. 2003; Pardo and Nadelhoffer 2010). These effects on soil and plant isotopic compositions then extend up the trophic levels to animals and humans, therefore, in order to understand animal and human diet using $\delta^{15}N$ values in this study, factors that affect nitrogen isotopes in soils and plants must be considered first.

The earth's most abundant reservoir for nitrogen (N) is the atmosphere (about 78% by volume). Despite its abundance in the atmosphere, primary producers such as plants cannot utilise nitrogen in its gaseous form. Consequently, for organisms to be able to utilise nitrogen for growth and in some cases to derive energy, atmospheric N_2 undergoes different transformations which include nitrogen fixation, assimilation, ammonification, nitrification, and denitrification in the ecosystem (Long et al. 2013; Galloway 2014; Sharp 2017; see Table 2.6). This is commonly known as the Nitrogen Cycle (Figure 2.8).

Table 2-2: Processes involved in the Nitrogen Cycle and their definitions compiled from Long et al. 2013;Galloway 2014a; Sharp 2017.

Process	*Definition*
Nitrogen fixation	It is the conversion of atmospheric N_2 into nitrogen compounds such as ammonia (NH_3) and ammonium (NH_4^+) using the enzyme nitrogenase in an anaerobic environment
Assimilation	It is the uptake of NH_3 and NH_4^+ by a living organism to become part of its biomass in the form of organic nitrogen compounds.
Nitrification	It is the aerobic oxidation of ammonia (NH_3) to nitrite (NO_2^-) and then to nitrate (NO_3^-) by ammonia oxidizers and nitrifying organisms (e.g., chemotrophic bacteria). This process generates energy.
Denitrification	It is the conversion by anaerobic bacteria, fungi, and aerobic bacteria of nitrate (NO_3^-) and nitrite (NO_2^-) into gaseous N_2O (nitrous oxide) and ultimately N_2.
Ammonification or mineralisation	It is the degradation of tissues in the form of organic nitrogen (e.g., amino acids, DNA, etc) to release inorganic nitrogen as ammonia back to the ecosystem through the action of microorganisms such as fungi and prokaryotes. The ammonia produced here then becomes available for use by plants and other microorganisms for growth.

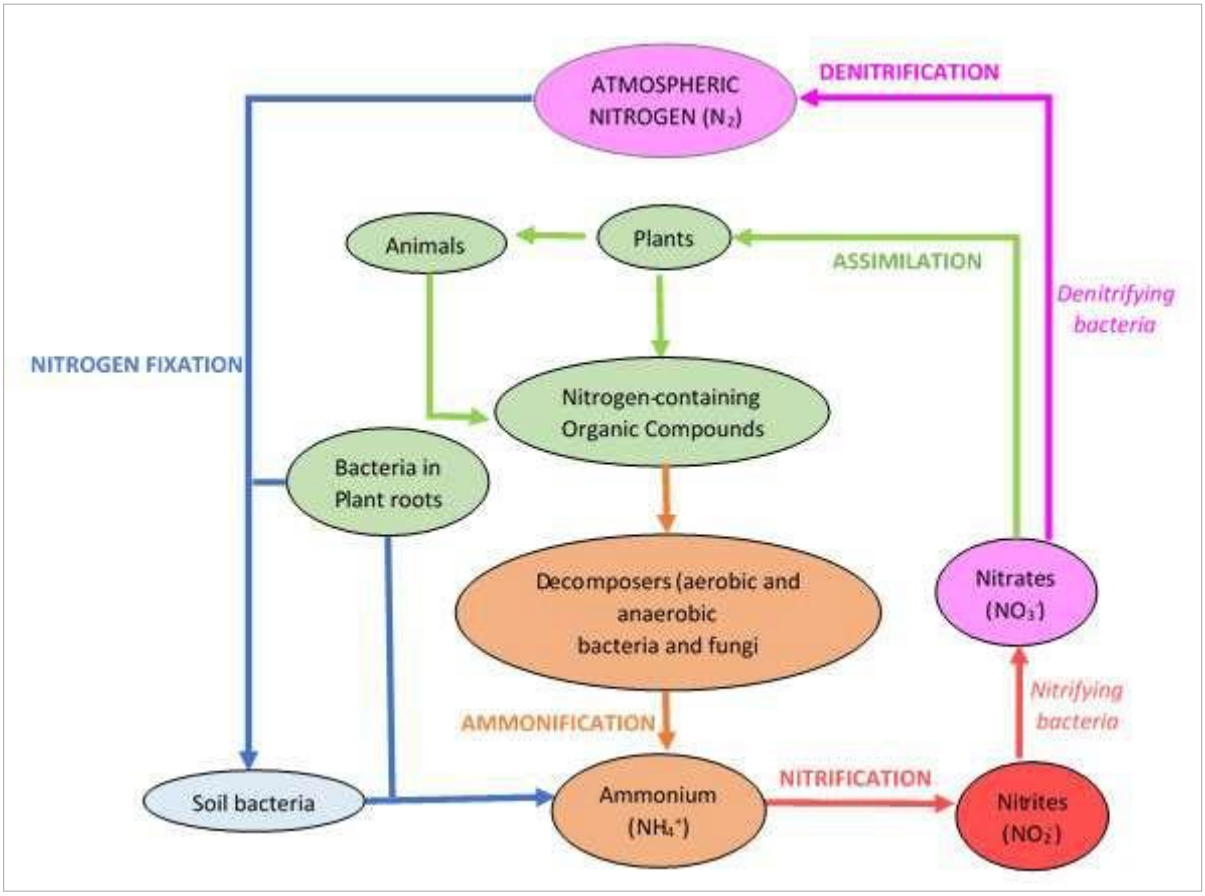


Figure 2-8: Schematic representation of the Nitrogen Cycle (Modified from the Nitrogen Cycle diagram in the Encyclopædia Britannica, Inc.).

2.5.1 Nitrogen isotope ratios in plants

Unlike carbon isotopes that have known fractionation processes that occur during photosynthesis and can be traced in food webs, there is no singular fractionation process available for nitrogen isotopes. The various biogeochemical factors that influence the variation in plant nitrogen isotopic compositions in plant-soil systems include the following : (i) whether the nitrogen was obtained through uptake mediated by symbiotic microbes (e.g. in legumes) or direct uptake of soil; (ii) the type of nitrogen obtained (e.g. N_2, NO_3^-, NH_4^+); (iii) where it was assimilated (in roots or shoot) and (iv) the part of the plant to which nitrogen is allocated (Szpak 2014). For instance, leguminous plants (soybeans, clover, chickpeas, etc.), which are nitrogen-fixing plants, gain nitrogen through symbiotic bacteria found on their roots. The microorganisms fix nitrogen directly from the atmosphere which, as the standard, has a $\delta^{15}N$ of 0‰ and is, therefore, not enriched in nitrogen. As a result, N_2 fixing plants exhibit values close to atmospheric levels at 0‰. On the other hand, non-N_2 fixing plants (most plants) receive nitrogen from inorganic compounds (e.g. NH_4^+, NO_3^-) in the soil or surrounding waters in the case of marine plants, and fractionation results in higher $\delta^{15}N$ values than the atmospheric resulting in them typically exhibiting $\delta^{15}N$ values of close to 3‰ (Rennie et al. 1976; Wada and Hattori 1976; Schwarcz and Schoeninger

1991; Jaffe 1992; Hoefs 2015). In addition, it has also been shown that plant species existing alongside each other can have different $\delta^{15}N$ values that can reach as much as 10‰. Moreover, $\delta^{15}N$ in plant organs can vary usually between 2-3‰ within the same plant, with differences sometimes as much as 7‰ in desert plants (Handley et al. 1999; Evans 2001; Yoneyama et al. 2003).

Other factors such as anthropogenic activities, temperature, salinity, and proximity to the coast have also been shown to strongly affect the $\delta^{15}N$ of soil and by extension, plants (Heaton 1987; Ambrose 1991; Amundson et al. 2003; Bogaard et al. 2007). Plants treated with artificial fertilisers such as ammonium nitrate have low $\delta^{15}N$ values ranging from -4‰ to +4‰ reflecting atmospheric source of the nitrogen used in manufacturing processes (Kendall 1998). However, in the past, agriculturists used animal manure to improve overall soil fertility. Soil that has been treated with animal manure has high $\delta^{15}N$ values due to loss of ^{14}N in volatile gaseous ammonia which leaves the resulting ammonium enriched in ^{15}N. The ^{15}N enriched ammonium is subsequently converted to nitrate with high $\delta^{15}N$ values, which in turn, is assimilated by plants (Heaton 1986; Bogaard et al. 2007; Fraser et al. 2011). Processes occurring in animal waste have resulted in organic fertilisers having a $\delta^{15}N$ range of +6‰ to 30‰ (Kendall 1998). In addition, local climatic conditions cause differences in $\delta^{15}N$ values with colder and wetter ecosystems tending to conserve and recycle nitrogen between organic nitrogen pools without significant fractionation of the lighter ^{14}N isotope which results in lower $\delta^{15}N$ values whereas hot and arid ecosystems tend to lose ^{14}N more since nitrogen is easily converted to forms that can be lost through the gaseous phase such as ammonia leading to higher $\delta^{15}N$ values in the ecosystem (Handley et al. 1999; Amundson et al. 2003). Britton et al. (2008) demonstrated that modern plant species growing in coastal ecosystems were significantly enriched in ^{15}N compared to other terrestrial plants. A similar trend was also recognised in plant tissues as well as coastal/saline soil (Heaton 1987). Virginia and Delwiche (1982) had previously proposed that high $\delta^{15}N$ values in coastal areas were probably a result of ocean derived nitrate from sea spray. Heaton (1987) suggested that the high values were likely due to the enrichment of the nutrient nitrogen in the soil rather than to the metabolic responses of plants themselves. In addition, the author proposed that increased local rainfall could reduce the effects of sea spray and salinity on the soils by diluting salt and oceanic derived nitrate thereby lowering $\delta^{15}N$ values.

2.5.2 Nitrogen isotope ratios in consumers

It has been demonstrated that nitrogen isotopes are useful in determining plant contribution to food chains. When a consumer eats plants or animals that have consumed these plants, the process of fractionation results in more enriched nitrogen levels with each step up the food chain. As is the case with $\delta^{13}C$ measurements, the $\delta^{15}N$ measurements in animals rely on the distribution

and incorporation of food into the tissues of consumers. However, it has been determined that different tissues in the same body do not necessarily have the same $\delta^{15}N$ values. For instance, bile and urinary urea tend to have $\delta^{15}N$ values that are 2‰ to 4‰ more negative than that of diet, and blood and muscle have values that are 1‰ to 3‰ heavier than diet. Bone and skin tend to have higher $\delta^{15}N$ values than most tissues (Ambrose 1991; Kelly 2000). The differences in tissue $\delta^{15}N$ values will not be a major problem for this study as $\delta^{15}N$ values were only obtained from one tissue (bone collagen) for all the samples in this study.

2.5.2.1 Herbivores and carnivores

There are rarely any plants available to measure stable isotope ratios in palaeodietary studies and where fragments are available, the integrity of $\delta^{15}N$ measurements is usually questioned (Fogel and Tuross 1999). However, the principle applied in interpreting diet is that animals conform to the phenomenon "You are what you eat" which proposes that the $\delta^{15}N$ values of animals are related to their diet (Deniro and Epstein 1981; Tykot 2004). Several studies have found that animals have a step-wise ^{15}N-enrichment of ~3–5‰ with trophic level (Minagawa and Wada 1984; Schoeninger and DeNiro 1984; Peterson and Fry 1987; Schwarcz and Schoeninger 1991). It is normally assumed that local herbivores will have $\delta^{15}N$ values that reflect the local plants they are consuming. The $\delta^{15}N$ values in flesh and bone collagen (body protein) of herbivores have been found to be ~3‰ to 5‰ higher than the plants they consume. Animals which consume other animals are expected to have higher $\delta^{15}N$ values than the animals they eat, therefore, the $\delta^{15}N$ values of carnivores are higher than those of herbivores in terrestrial environments (~7‰ over herbivores) (Schoeninger and DeNiro 1984; Schoeninger 1985; Richards and Trinkaus 2009; O'Connell et al. 2012). It has been suggested that omnivores with mixed plant and animal diet occupy the highest trophic level (Koch et al. 1994).

Nevertheless, it should be noted that quantifying the diet-body nitrogen spacing has proven difficult, for instance, large scale ecological studies suggest enrichments of approximately 3‰ to 4‰ while small scale feeding experiments show values between 1.5‰ and 6‰ (Caut et al. 2009; O'Connell et al. 2012). Underestimation of the diet-collagen offset can result in an overestimation of higher trophic foods such as fish, meat, and milk (O'Connell et al. 2012). Additionally, interpretation of human $\delta^{15}N$ values is limited because there is no definitive value for the ^{15}N enrichment in humans (Hedges and Reynard 2007). This value has previously been assumed to be 3-5‰ in the archaeological literature but has recently been estimated to be approximately 6‰ (O'Connell et al. 2012). Moreover, the trophic level effect varies due to a range of environmental conditions (aridity, temperature, altitude), as well as physiological factors such as starvation, the physiology of digestion, and diet composition (McCue and Pollock 2008; O'Connell et al. 2012). All these factors show that more information is still needed to understand trophic

level ^{15}N enrichments. The factors that directly affect the analysis of this study individuals will however be reviewed in a later section (Section 2.5.3).

2.5.2.2 Aquatic ecosystems

Vertebrates in marine ecosystems exhibit $\delta^{15}N$ values 6-8% higher than animals at similar trophic levels in most terrestrial environments (Schoeninger and DeNiro 1984). This has been attributed to the high $\delta^{15}N$ of particulate organic matter and nitrate in seawater (Guo et al. 2004). In addition, the longer trophic systems in the marine ecosystem have been attributed to the amplification of $\delta^{15}N$ values (Peterson and Fry 1987; Fry 2006). Freshwater fish also have high $\delta^{15}N$ values but have lower $\delta^{13}C$ values than marine fish, therefore distinguishing between freshwater and marine diets is best accomplished by applying a combination of $\delta^{15}N$ and $\delta^{13}C$ values (Fuller et al. 2012). The consumption of freshwater fish in this study will not be inferred exclusively from the $\delta^{15}N$ and $\delta^{13}C$ values, but site contextual knowledge will also be taken into consideration – for example, whether fish is known to have been a major resource at the site or if the site was close to freshwater fish resources such as rivers.

2.5.3 Challenges on $\delta^{15}N$ values in this study

This general increase in $\delta^{15}N$ values of animals at successive trophic levels seems to be well-founded and will be used as such in this study. However, for archaeological dietary interpretations, it is accepted that there are other factors that influence $\delta^{15}N$ values and can affect how data is interpreted. There is therefore the need to evaluate these factors in relation to individuals in this study in order to perform reliable estimations. It must be noted that mechanisms behind nitrogen isotope variations are still being debated, therefore only the potential challenges pertinent to the interpretation of diet using $\delta^{15}N$ values in this study are reviewed below.

2.5.3.1 Consumption of dairy products

The post-medieval populations in this study are known to have been consuming dairy products and eggs in addition to their meat protein. In an Oxford, England $\delta^{15}N$ study, O'Connell and Hedges (1999) found clear differences between individuals with vegan (strictly no animal products consumption) and those of either vegetarian - with secondary animal product (e.g. dairy) consumption but no marine intake – or omnivorous (meat, marine and secondary animal products) diets, however, their data did not reveal any appreciable difference between the vegetarian and omnivorous group. With the knowledge that some secondary animal products such as dairy and eggs cannot be isotopically distinguished from meat, these are taken together as terrestrial mammal protein in this study.

2.5.3.2 Metabolic effects

Osteological analysis was carried out for all individuals in this study, and some had a range of health conditions that can cause nutritional stress in the body. Nutritional stress in the body can in turn lead to an elevation of $\delta^{15}N$ values (Fuller et al. 2005). When protein is used to build body tissues, isotope fractionation occurs, resulting in body tissues being enriched in ^{15}N and body waste enriched in ^{14}N relative to diet (Hedges and Van Klinken 2002; Koch 2007; Martínez del Rio et al. 2009). It is known that when an individual does not ingest sufficient nitrogen to meet metabolic requirements, the individual catabolises its own tissues to meet the demands of protein synthesis. Furthermore, when an individual is on a low protein diet, all of the amino acids consumed are utilised to build body tissues whereas, for an individual on a protein-rich based diet, not all of the dietary amino nitrogen is required for metabolic processes. As a result, for the low protein diet more ^{15}N is incorporated in the body tissues than excreted resulting in elevated $\delta^{15}N$ in the body tissues (Hedges and Van Klinken 2002; Koch 2007; Martínez del Rio et al. 2009). This was first observed in wild bird controlled feeding studies (Hobson and Clark 1992; Hobson et al. 1993) that reported elevated levels of $\delta^{15}N$ in the body tissues of birds undergoing nutritional stress and fasting. Hobson et al. (1993) attributed this to protein synthesis from products of existing body proteins. This pattern has also been observed in humans (Katzenberg and Lovell 1999; Fuller et al. 2005; Mekota et al. 2006; Huelsemann et al. 2009). Katzenberg and Lovell (1999) compared $\delta^{15}N$ values of individuals of known medical history and found elevated $\delta^{15}N$ values in the diseased (osteomyelitis) bone segment of an individual who had died of AIDS relative to the healthy segments of the bone. Furthermore, isotopic studies of pregnant women's hair revealed that $\delta^{15}N$ values increased during periods of nutritional stress caused by morning sickness (Fuller et al. 2005) In addition, elevated $\delta^{15}N$ values were also observed in individuals diagnosed with eating disorders (Hatch et al. 2006; Mekota et al. 2006; Mekota et al. 2009). However, it is important to point out that most of these studies were conducted on sequentially forming tissues such as hair that represent a short period of time. For this effect to be visible in bone collagen an individual must have been ill for a very long time. In addition, in cases where this has been studied in bone, the studies have explored $\delta^{15}N$ values of woven pathogenic bone (Olsen et al. 2014a; Olsen et al. 2014b). Therefore, on balance, this should not be a major issue for this study. However, since osteological analysis was undertaken for most individuals in this study and it identified some individuals who had pathological conditions, the effect of specific diseases on individuals' $\delta^{15}N$ values will be explored where relevant.

The samples in this study include both adults and nonadults, therefore the effect of age on $\delta^{15}N$ values is relevant and must be accounted for. Previous studies have reported age-dependent $\delta^{15}N$ values between children and adults e.g. Richards et al. (2002), Fuller et al. (2006a) and Hannah

et al. (2018). Richards et al. (2002) hypothesised that the variation in $\delta^{15}N$ values in adults and nonadults was due to slightly different diets between the two groups, possibly due to nonadults' diets being more plant-based. In relation to the infants in this study, the suckling effect on $\delta^{15}N$ values will be explored. The premise is that suckling infants' consumption of milk raises the trophic level above that of the mother and when they gradually incorporate other foods into their diet, their $\delta^{15}N$ values decline until they are similar to those of the adults (Fogel et al. 1989; Fuller et al. 2006a; Fuller et al. 2006b). Milk has been reported to be 2‰ to 3‰ more positive than the mother's diet (Figure 2.9) (Reynard and Tuross 2015; King et al. 2017). Therefore, assuming that both the mother and infant are consuming an isotopically constant diet, the infant's bone collagen should be 2‰ to 3‰ more enriched than the mother's (King et al. 2017).

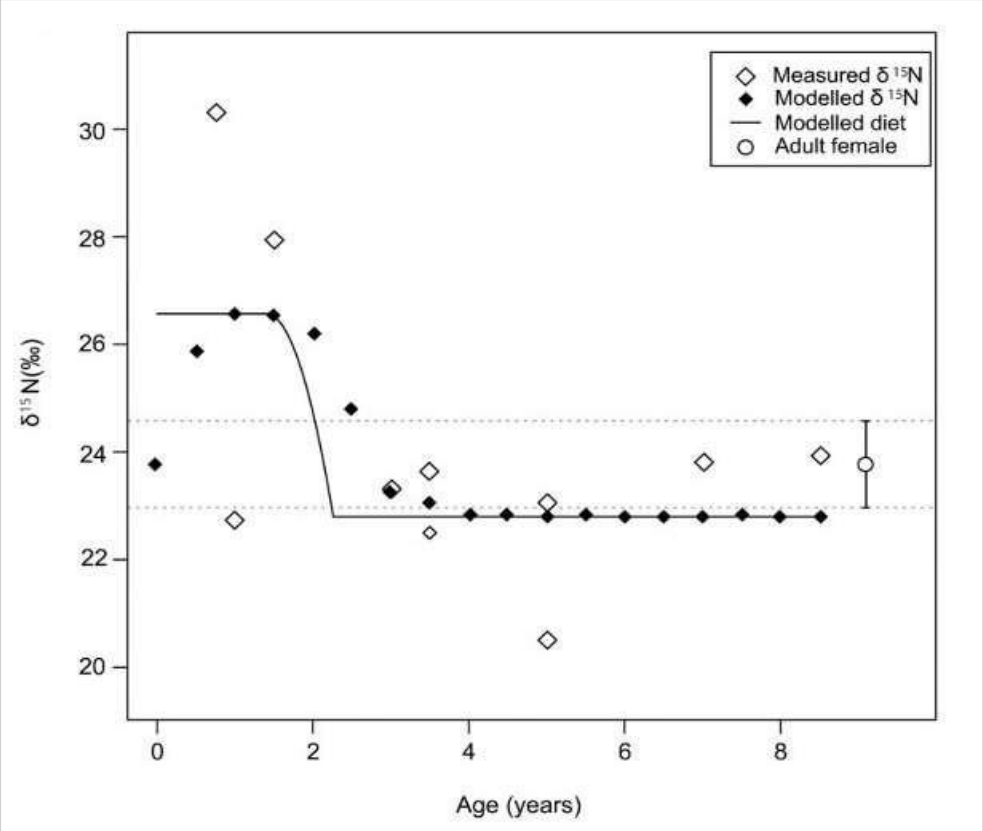


Figure 2-9: Changes in nitrogen isotope values by subadult age (Late Periods) (King et al. 2017).

2.5.3.3 Abiotic effects

Agriculture efficiency was significantly enhanced just before the Industrial Revolution as a result of the increased use of manure and the growth of stock density (Allen 2003; Drouard and Oddy 2013). As explained in Section 2.5.1, the use of manure affects $\delta^{15}N$ levels in plants. In the 18th and19th centuries, intensive manuring was enabled by the application of sewage sludge, especially in London (Drummond and Wilbraham 1939; Beaumont et al. 2013b), therefore it is expected that this might have an effect on the $\delta^{15}N$ values in the faunal and human individuals in this study.

Furthermore, a link between high stocking density or grazing rate with enrichment in $\delta^{15}N$ values has been demonstrated in several archaeological studies (Schwertl et al. 2005; Britton et al. 2008; Fraser et al. 2011; Müldner et al. 2014), and will need to be considered in interpreting $\delta^{15}N$ values in this study.

2.6 Routing: collagen and carbonate

2.6.1 Collagen routing

Experimental feeding studies have shown that carbon atoms in bone collagen are largely sourced from dietary protein (Ambrose and Norr 1993; Jim et al. 2006; Kellner and Schoeninger 2007; Fernandes et al. 2012; Froehle et al. 2012). Dietary protein is made up of essential and nonessential amino acids (EAAs and NEAAs respectively) and the bone collagen $\delta^{13}C$ values in consumer tissues represent the weighted average of the isotopic compositions of all component EAAs and NEAAs. Early workers originally believed that the majority of carbon in bone collagen deriving from NEAAs (~80%) was synthesised from carbohydrates, lipids, and protein (Schwarcz 1991; Ambrose and Norr 1993), but controlled feeding experiments have shown that collagen is predominantly synthesised from dietary protein (Ambrose and Norr 1993; Tieszen and Fagre 1993; Jim et al. 2004). It was determined that EAAs and NEAAs whose sole precursor molecules must be derived from dietary protein, contribute ~18.5 - 21.7% of collagen carbon atoms. This amount, therefore, represented the minimum amount of routing from dietary protein to bone collagen (Ambrose 1993; Schwarcz 2002; Jim et al. 2004; Jim et al. 2006). The body cannot generate EAAs therefore the consumer has to ingest them in sufficient quantities whereas, NEAAs can be synthesised *in vivo* or directly assimilated from dietary protein. Depending on the amount of protein in the diet, NEAAs can be synthesised using carbon from dietary carbohydrates, lipids, and proteins or directly routed into consumer tissues (Jim et al. 2004; Martínez del Rio et al. 2009). Carbon is therefore routed in from dietary protein to consumer tissue protein in high protein diets whereas carbohydrates and lipids make a greater contribution to the isotopic composition of consumer collagen on low protein diets (Ambrose and Norr 1993; Tieszen and Fagre 1993; Howland et al. 2003; Jim et al. 2004).

The $\delta^{15}N$ values in animal tissues are determined by those absorbed from the food minus those excreted in the form of nitrogenous waste during metabolism. The primary cause for elevated $\delta^{15}N$ values in animals relative to their diet has been attributed to the loss of urine which is ^{15}N depleted (Sharp 2017). It has been determined that the primary sources of nitrogen for most animals are amino acids derived from digested proteins. When amino acids enter the bloodstream, they are absorbed by the cells mainly in the muscles and liver and converted to ammonia which in turn is converted to urea. It has been proposed that during metabolism, amino

acids containing ^{14}N dissociate more easily than those containing ^{15}N resulting in ^{14}N being excreted as urea at a higher rate than ^{15}N, leaving the residual amine (NH_2) pool in the plasma enriched in ^{15}N. It is this process that is believed to drive fractionation of nitrogen isotopes (Steele and Daniel 1978; Yoneyama et al. 1983; Minagawa and Wada 1984; Ambrose and DeNiro 1986; Schoeninger 1995). However, it must be noted that diet-tissue nitrogen isotope discrimination is variable as in some cases it has been shown that there can be preferential uptake of ^{15}N relative to diet. For instance, Sponheimer et al.'s (2003) study of llamas revealed that urinary nitrogen was depleted in ^{15}N, and faeces were enriched in ^{15}N relative to diet resulting in no significant difference between dietary and total excreted $\delta^{15}N$ values.

The proportion of dietary protein in different sources has implications on palaeodietary research when using isotope analysis of bone collagen alone. For instance, animal sources are about 90% protein by weight whereas plant sources are 10 -20% protein by weight, therefore it is expected that consumption of plant foods may be underrepresented in the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen compared to animal/fish products (Ambrose and Norr 1993; Schoeninger 1995; Schwarcz 2002).

2.6.2 Carbonate routing

In contrast to dietary routing in bone collagen, current understanding based on controlled feeding experiments is that bone carbonate $\delta^{13}C$ values reflect the organism's whole diet (carbohydrates, lipids, and proteins) because bone carbonate forms in equilibrium with blood carbonate that is itself a product of energy metabolism. Therefore, unlike bone collagen $\delta^{13}C$ values which provide mainly the protein portion of the diet, bone carbonate $\delta^{13}C$ values increase the visibility of carbohydrates (e.g., cane sugar or maize) in the diet as will be reflected in this study. The principle is that in the metabolic state, virtually all carbon atoms (>99%) that have been ingested by the animal consumer leave the body as respired carbon dioxide (CO_2). This CO_2 comprises virtually all the carbon from different macronutrients as represented in the diet. Blood bicarbonate equilibrates with respired CO_2 which in turn equilibrates with bone carbonate during bone formation (Krueger and Sullivan 1984; Lee-Thorp et al. 1989; Schwarcz 1991; Ambrose and Norr 1993; Tieszen and Fagre 1993; Kellner and Schoeninger 2007).

2.7 Bone degradation and diagenesis

Understanding the diagenetic processes in archaeological skeletal material is essential when deciphering the extent to which the original chemical information stored in biomolecules has been retained or altered. Such knowledge is fundamental in demonstrating the requirement that authentic biogenic isotopic signatures are still preserved in the skeletal remains that will be used in this study to interpret diets.

After burial, archaeological bone undergoes alterations due to biological and physicochemical processes that degrade archaeological information (Hollund et al. 2013). These alterations can occur due to interaction with the environment (Monge et al. 2014). It is important to note that bone diagenetic processes are generally site-specific and not linearly related with burial time but rather controlled by different external factors such as pH, hydrology, temperature, microbial attack, and humidity (Nielsen-Marsh and Hedges 2000b; Tütken and Vennemann 2011). Major advances have been made over the years to understand the processes affecting bones or other hard tissues in the burial environment e.g. Collins et al. (1995) Hedges and Milliard (1995), Nielsen- Marsh et al. (2000), Nielsen-Marsh and Hedges (2000b) and Collins et al. (2002). Considering bone as a composite of collagen and bioapatite, different pathways of bone deterioration that include collagen and mineral degradation are reviewed. However, it should be noted that the extent to which any of these pathways occurs in isolation is still not very clear.

2.7.1 Collagen degradation

The degradation of the collagen as a result of the microbial attack and/or hydrolysis is one of the earliest and most fundamental alteration steps during bone diagenesis (Collins et al. 2002). It has been suggested that attack by microbes such as bacteria and fungi is probably the most critical factor affecting bone deterioration (Trueman and Martill 2002). However, the mechanisms by which microbial activity occurs are relatively unclear. For instance, it is not clear whether microbial attack depends on changes occurring to the bone prior to chemical alterations or it is the first process to occur.

It has been argued that for collagen to be attacked by enzymes, some inorganic matrix must have been removed. It was suggested that the large size of microbes and collagenases does not allow them to fit into the collagen-apatite porosity, therefore the collagen triple helix must be exposed by mineral removal first (Turner-Walker 1993; Child 1995a; Child 1995b; Nielsen-Marsh and Hedges 2000b; Hedges 2002). Microbes in the soil gain access into the skeletal material through the Haversian and Volkmann canals (natural hollow spaces in the bone) and produce acid

metabolites which then attack the osteon tissue resulting in the dissolution of the mineral fraction (Hackett 1981; Balzer et al. 1997). Dissolution of the mineral phase in turn exposes the collagen to extracellular microbial enzymes attack (Collins et al. 2002). The most important bone microstructure alteration consists of conspicuous zones of destruction known as microscopic focal destruction (MFD) which are probably caused by microorganisms (Hackett 1981; Dixon et al. 2008). Grupe and Piepenbrink (1989) suggested that MFD can change trace element composition in bone, damage bone microstructure completely, and lead to the loss of collagen. Bone alteration as a result of microbial attack is demonstrated either by the existence of tunnels or exogenous biomass in the bone, both of which result from solubilisation and leaching of bone mineral (Child 1995b; Jans et al. 2004).

Chemical degradation of collagen, a protein held together by peptide bonds (Collins et al. 1995; Collins et al. 2002), is, however, influenced by several factors. Although collagen is insoluble in normal environmental conditions, its degradation results in soluble peptide fragments that can be lost from the bone (Nielsen-Marsh et al. 2000). Diagenesis involves the hydrolysis of peptide bonds brought about by acidic conditions within a bone, resulting in fragmentation of the protein into smaller peptide molecules (Child 1995b; Collins et al. 1995; Nielsen-Marsh et al. 2000). The breaking down of protein into smaller peptide molecules causes the protein to lose its cohesion and organisation as a result of the loss of intact fully bonded molecules. The broken-down peptide molecules may be leached out of the bone or remain within it (Hare 1980; Tuross et al. 1980; Collins et al. 1995). The rate at which collagen is lost depends on time since burial, environmentalpH, and temperature. Extremes of pH accelerate hydrolysis and cause collagen swelling - a process that is used to explain fracturing observed in bones deposited in aqueous environments (Pfretzschner 2004). High temperatures accelerate the rate of collagen loss (Collins et al. 2002) and as a result, it is expected that samples deriving from tropical and arid zones produce low collagen yields (van Klinken 1999). Additionally, the remaining collagen is subject to structural changes such as cross links formation which can incorporate contaminants such as humic acids, and lipids into the protein structure (Tuross et al. 1980; Collins et al. 1995; van Klinken 1999). Contaminants such as humic acids often have $\delta^{13}C$ values that can lead to isotropic alteration e.g. Katzenberg (1989) and van Klinken and Hedges (1995). All archaeological samples in this study are, however, from England, a temperate zone, therefore the effect of high temperatures will likely not be an important factor in the bones sampled here.

2.7.2 Bone mineral diagenesis

While bone collagen degradation processes are occurring, the bone mineral will also be experiencing changes. Because the biogenic apatite is unstable, the survival of bone mineral post

burial depends on the formation of a more stable structure (recrystallisation) and loss of less stable components through dissolution (which determines the mineral alterations that occur in a bone) (Nielsen-Marsh et al. 2000; Trueman 2013). There are suggestions that the bone mineral is protected to a certain extent in cases where bone collagen survives (Sillen 1989) but Tuross et al. (1989) have suggested that the mineral protects the collagen instead. The process of bone mineral degradation is complex, but it has been established that after burial, the bone hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) lattice has the potential to accommodate substitutions at the Ca^{2+}, PO_4^{3-} and the OH^- sites. Uptake of diagenetic carbonate may occur simultaneously via the following processes (i) crystallisation of the calcite into pore spaces (ii) recrystallisation of the bioapatite matrix and (iii) exchange of CO_3^{2-} and PO_4^{3-} between the environment and bone (Krueger 1991; Wright and Schwarcz 1996; Koch et al. 1997; Nielsen-Marsh et al. 2000). The exchange of CO_3^{2-} and PO_4^{3-} between the environment and bone is understood to be a key contributing factor to the alteration of $\delta^{13}C$ and $\delta^{18}O$ values in bone carbonate (Wright and Schwarcz 1996).

One way of identifying diagenesis is the inclusion of carbonate-containing substances such as calcite ($CaCO_3$) into the empty spaces of bone such as the Haversian canals (Hassan and Ortner 1977; Hassan et al. 1977). Previous studies have detected the presence of calcite through Fourier Transform Infrared spectroscopy (FTIR) e.g., Nielsen-Marsh and Hedges (2000a) and Kontopoulos et al. (2019b). Therefore, if these carbonate-containing substances are precipitated into the bone, there is potential for bone carbonate $\delta^{13}C$ values to incorporate values of the exogenous minerals (Lee-Thorp et al. 1989; Saliège et al. 1995). FTIR will be performed to investigate the presence of calcite in these study samples.

In addition, bone mineral undergoes dissolution and recrystallisation after burial due to interaction between the bone and groundwater. The relative insolubility of bone mineral determines the survival of bone (Hedges and Millard 1995; Nielsen-Marsh and Hedges 1997) and the rate of bone mineral dissolution is affected by pH, saturation of water with respect to Ca^{2+} and PO_4^{3-} ions, and the rate of groundwater movement (Nielsen-Marsh et al. 2000). The action of groundwater on the bone in the burial environment dissolves and leaches the mineral component leading to a subsequent alteration of the thermodynamically unstable, nanometre-sized bioapatite crystals which in turn results in recrystallisation, increase in porosity, and inclusion of exogenous ions. Adsorption of exogenous ions such as carbonates (CO_3^{2-}), phosphates (PO_4^{3-}), calcium (Ca^{2+}), and strontium ($Sr2^+$) to the surface of bioapatite, or exchange with its ions can occur during this process (Nielsen-Marsh and Hedges 2000b; Nielsen-Marsh et al. 2000; Collins et al. 2002; Tütken and Vennemann 2011). If changes in crystallinity (recrystallisation) follow, these ions may be incorporated deeper into the mineral structure. Consequently, these changes in crystallinity and carbonate content will lead to inaccuracies in the interpretation of isotopic signatures as indicators of diet (Monge et al. 2014;

Wright and Schwarcz 1996; Kontopoulos et al. 2019b).

Higher crystallinity in diagenetically altered bone samples is a result of an increase in crystal size and atomic order due to Ostwald ripening, loss of smaller crystals, or both (Kontopoulos et al. 2018). Theoretical and empirical evidence suggests that once bone crystallites are removed from the organic matrix, they experience simultaneous dissolution and growth in a manner analogous to Ostwald ripening. Ostwald ripening is a thermodynamic process (flow of energy) whereby smaller crystals melt, and larger crystals grow at the expense of the smaller ones as a result of the difference in the chemical potential of the crystals. Due to the bones having higher energy small crystals and low energy larger crystals, the atoms on the small crystals readily detach from the surface, diffuse and deposit on the larger crystal because the system tends towards a lower total energy state which is more stable. This in turn shrinks the smaller crystals. Once the bone is removed from biological control of the live organism, the bone crystallites spontaneously grow, unless this process is constrained by the organic matrix. Therefore, as the organic matrix is broken down, bone crystallites increase in size thereby increasing the infrared splitting factor (IRSF), a parameter critical to checking bone diagenesis when using FTIR (Tuross et al. 1989; Trueman et al. 2004). Increased porosity in turn accelerates mineral dissolution. Alternatively, if recrystallisation is due to loss of the less crystalline, less stable material, re-adsorption of exogenous ions associated with dissolving bioapatite occurs (Nielsen-Marsh and Hedges 2000b; Nielsen-Marsh et al. 2000; Collins et al. 2002; Tütken and Vennemann 2011). In addition, another chemical change that can occur during bone diagenesis is the transformation of hydroxyapatite to brushite in acidic environments (Molleson 1990).

It is expected that because bone carbonate is analysed in this study, the effect of bone degradation from the burial environment on stable isotope composition has to be taken into consideration. In most archaeological studies, collagen has been preferred because there are available established quality indicators to recognise extracted collagen preservation levels (Ambrose 1990; van Klinken 1999; Brock et al. 2010; Brock et al. 2012; Lebon et al. 2016; Kontopoulos et al. 2019a). Even if bone mineral usually survives a lot longer than collagen (Lee-Thorp 1989), until recently it has received little attention as sample material for palaeodietary studies because carbonate undergoes exchange with external carbonate ions from the surrounding environment after burial (see Section 2.7.3) (Sullivan and Krueger 1981; Schoeninger and DeNiro 1982; Krueger and Sullivan 1984). The review of the bone degradation methods has revealed that diagenetic processes can alter the isotopic compositions of bone collagen and bone carbonate. However, sample preparation methods used in this thesis will either remove the diagenetically altered material or identify the altered samples to be discarded. More detailed information on the analytical methods is in Chapter 5.

Chapter 3: Post-medieval period in Britain and diet (17th to 19th century)

This chapter aims to describe the historical background and context upon which this thesis is based so that the analytical data obtained can be assessed with reference to the appropriate socio-economic or regional background of the 17th to 19th centuries. Firstly, the beginning of the Industrial Revolution will be introduced along with its links to the Agricultural Revolution (which allowed for the expansion of the urban workforce). The significance of improvements in manufacturing techniques and the role of industry in the movement of labour from rural to urban societies will follow. Next, the effects of the Industrial Revolution in Britain such as population growth and changes in modes of transportation will be explored. As it forms the fundamental basis of this thesis, the diet consumed during this period will be discussed in three parts, (i) regional variations (ii) socio-economic status and its implications on diet, and (iii) the emergence and nature of C_4 food resources during that period. Finally, a summary of the chapter will be presented. It should be noted that this thesis is being presented in the form of individual research papers, therefore, some of the information from this chapter features in later chapters. Additionally, it should be noted that although this thesis focuses on sites from England only, some authors in the extant literature may refer to Britain when providing information and where that occurs, the term British is used.

3.1 Historical Context

The period from the seventeenth to the nineteenth centuries in Britain witnessed unprecedented changes in almost all aspects of daily life, among them, shifts in food acquisition and consumption across both rural and urban environments linked to the Agricultural and Industrial Revolutions (Burnett 2005). However, numerous scholars have criticised the predominant image of the Industrial Revolution as a universal and continuously progressing period of transition that appears to relegate the Agricultural Revolution to an insignificant role within this period of change (Mokyr 1985; Wrigley 1990). It is difficult to attribute the economic growth that occurred over the period of the two revolutions to either of the phenomena separately or the combination of the two. It has been argued that they coincided and that they both had an impact on food transformations (Crafts 1985). In contrast, O'Brien and Quinault (1993) suggest that agricultural productivity in the seventeenth century increased rapidly enough to strengthen conditions for the continued growth of Britain's advanced manufacturing industries during the Industrial Revolution. Overton (1996) and Muldrew (2011) argue that the Agricultural Revolution started in the late seventeenth century, gradually accelerated into the mid-nineteenth century, and reinforced both urbanisation and the associated Industrial Revolution. Despite these debates,

agricultural accounts reveal huge increases in arable output as well as improvements in animal husbandry during this period (Turner et al. 2001; Clayton andRowbotham 2008). It, therefore, cannot be disputed that fundamental changes in agriculture which supplied raw materials, food and allowed for the fuelling of a growing workforce, sustainedthe advance of industrialisation and an increasingly urbanised economy.

3.1.1 The Agricultural Revolution

In pre-industrial Britain, the dependence of the society on domestic agriculture meant that people subsisted on a limited range of food material, some of which were only available seasonally (Overton 1996). There was a system in which food was largely derived from small-scale peasant farms, heavily reliant on human labour for production and processing, with peasants practicing subsistence agriculture as well as producing food for their landowners. However, with industrialisation and improved agricultural productivity, came consolidations of landholdings, new discoveries in science as well as technologies for land cultivation techniques and animal breeding, which in turn led to people becoming dependent onwage labour, with little or no access to land to produce food for themselves (O'Brien 1985; Overton 1996).

Changes in farming techniques included new turnips and clover rotation, the watering of meadows, the introduction of machinery, and the improvement of livestock breeds. Arguably, the new turnips and clover rotation was the most critical agricultural development as it improved both arable output and animal husbandry immensely (Overton 1996). In this new rotation system, the fallows were replaced with fodder crops (turnips and clover) replacing the unproductive fallow period when nothing was grown thereby increasing yields (Overton 1996, 2). Historians argue that the increase in fodder crops allowed farmers to increase their livestock, resulting in increased generation of manure which would in turn increase crop yields. The effect of manure on crops will be of interest when interpreting isotope results in this thesis. Nevertheless, increased farming productivity meant that the amount of food produced per capita was significantly enhanced (Overton 1996, 8). For instance, between 1705 and 1765, wheat exports from England increased tenfold (Overton 1996). In addition, the increase in fodder crops necessitated the prevention of animal slaughter at the onset of winter resulting in fresh meat being more widely available and therefore cheaper, throughout the year. Animals were previously killed before the winter set in as there was not enough food to feed them, thereby providing meat during the coldest months (Overton 1996; Clayton and Rowbotham 2009).

With changes occurring in farming, most people's self-sufficiency declined, and the traditional skills used previously to produce food disappeared in many parts of the country. The changes meant that agriculture was producing considerably more supplies than before but required significantly fewer workers to do so. As a result, many of the rural poor moved to the developing towns and cities for work and the number of landholders that were farming primarily for subsistence decreased (Overton 1996). The workforce engaged in agriculture in the country declined from 55% of the whole population in 1700 to nearly 36% by 1801 (Overton 1996). This in turn led to expanding numbers of urban dwellers who required an industrial provisioning system reliant upon new food technologies in order to adequately feed themselves (Oddy and Burnett 1992; Allen 1994).

3.1.2 Changes in the manufacturing industry

During the late eighteenth to the early nineteenth century, Britain became more efficient in manufacturing than elsewhere in Europe (Hudson 1992). This period saw a marked increase in the regional relocation of industry and the establishment of specialised, internally unified industrial regions connected to national and international markets (Hudson 1992). The invention of new machinerysuch as the steam engine as well as machinery for working cotton gave rise to the progression ofthe manufacturing industry which in turn had a profound effect on the British society (O'Brien and Quinault 1993). Specific industrial sectors dominated particular regions such that these regions came to be identified with specific types of manufacture. The cotton textiles became more dominant in South Lancashire and Manchester whereas the wool textiles were leading in East Anglia, the southwest, and the West Riding of Yorkshire. Small metal wares and hardware tradeswere specialised in the Midlands while coal, engineering, and shipbuilding were dominant in theNorth East (Hudson 1992; Green 2010). By 1861, approximately 15% of the workers in London were involved in the manufacturing industry, making London a key industrial centre (O'Brien and Quinault 1993). In the whole country, the increase in manufacturing accelerated the migration of labour force away from the rural areas towards the cities where there were better job opportunities in the coal mines, ironworks, and factories (O'Brien and Quinault 1993; Burnett 2005). The working day itself changed, with a shift from the working hours being governed by daylight, to being in the control of the factory clock (O'Brien and Quinault 1993). With the movement into urban cities, the general occupational patterns and the structure of the workforce changed, with children and women becoming more involved in the labour market as mechanisation meant that they were now able, by operating machines to complete tasks that were previously performed only by men. Moreover, women and children were a cheaper form oflabour in comparison to men (Pike 1966). These changes led to significant population growth in urban centres.

3.1.3 Population growth

The population of England increased noticeably in the late 18th century/early 19th century to mid-19th century, doubling from about 8.9 million people in 1801 to about 18 million by 1851 (Burnett 2005, 3). In 1801, about 3.6 million people resided in towns and 5.3 million lived in rural areas but by 1911, 28.1 million lived in towns whilst 7.9 million lived in rural areas (Law 1967). Forinstance, the population of the city of Manchester grew from about 75,000 people to about 330,000 during that period (Burnett 2005, 3). Individual city population growth for each of the populations studied for this thesis is supplied in Chapter 4. Apart from the increase of population as a result of the remarkable movement of people to the cities, other studies (McKeown and Record 1962; Razzell 1993; Burnett 2005) have suggested other contributory factors to this phenomenal urban demographic change. For instance, there is a view that the key driver was a decline in mortality (Razzel 1993) while others attribute the growth to increased birth rate (McKeown and Record 1962; Burnett 2005). It was suggested that the birth rate was increased due to a decline in marriage ages, itself a consequence of rising wages caused by economic development while the decline in mortality was attributed to the improved medical interventions as well as a decline in infectious diseases (McKeown and Record 1962; Razzell 1993; Burnett 2005). Nevertheless, with rapid population growth and urbanisation, came the problem of food supply and transport.

3.1.4 Changes in transportation

Before the Industrial Revolution, apart from London, market towns had been able to procure food easily enough from the surrounding rural areas. Before the establishment of canals in 1755 and railway systems in 1830, the produce from the countryside was transported to the great markets of London and other provinces by wagons on turnpike roads or at times on foot (Drummond and Wilbraham 1939; Engels 1845). Initially, when the canals were built, they were used only for the movement of grain rather than perishable goods. Canals were required for the movement of heavy produce created by the changes occurring in manufacture. Transportation of perishable foodstuffs such as meat and vegetables was a very slow process when compared to today. It meant that sometimes by the time the food reached the urban areas, it was of poor quality (Burnett 2005).

"A vegetable market is held in the street, baskets with vegetables and fruits, naturally all bad and hardly fit to use obstruct the sidewalk still further, and from these, as well as from the fish-dealers' stalls, arises a horrible smell." - (Engels 1993, 59)

The establishment of the new railway system in 1830 became crucial in the transportation of perishable fruit and vegetables from ever-increasingly distant sources. In London, due to its status as the nucleus of industry and manufacture, there was an enormous demand for food. As a

result, London depended on wider areas such as Surrey, and even as far as Wales, and Scotland for its vegetables, beef, and mutton respectively (O'Brien and Quinault 1993; Burnett 2005). The new railway system improved the transportation of fresh foods to the city. For instance, fruit and fresh vegetables loaded at the train stations in Kent, the Vale of Evesham and the Sandy area of Bedfordshire late in the afternoon, would be sold within hours in London (Burnett 2005, 8). For the rest of the country, it has been suggested that the railway system generated a national food market, eliminating variations in regional prices, preventing local shortages, and making locally produced foods available due to an increase in supply (Burnett 2005). Within a few years after the development of the railway system, the other urban areas were able to obtain highly perishable foods in excellent conditions in all seasons. The rail system was cheap, fast, and was generally unaffected by weather (Burnett 2005, 8). By the mid-nineteenth century cities such as Manchester, Birmingham, Leeds, and Nottingham, that in the past, had only been able to obtain foods mostly directly from the surrounding countryside were transformed and emulated London in demanding food from further afield. This will be a factor to consider when comparing London and the northern individuals' isotopic data in this thesis.

3.1.5 Other factors affecting food procurement

Other factors that might have affected the diet of individuals during this period need to be considered. On the foreign policy front, the political and social systems in Britain also had to contend with factors such as the Napoleonic Wars (1793-1815) during that period. Since the outbreak of the Napoleonic wars in 1793, there were average to poor harvests throughout the 22 years of war resulting in food shortages (Oddy 1970a; Oddy and Burnett 1992). Around this time, the British parliament preferred protecting the income of landowners and farmers, therefore, after the end of the Napoleonic Wars, the Corn Laws which imposed tariffs and other trade restrictions on grains and imported food were introduced in 1815. The Corn Laws also inflated the price of barley and wheat (and therefore bread) to protect local farmers from cheap imports of grain (Oddy and Burnett 1992; Horrell and Oxley 2012). The resulting food scarcity, as well as rapid inflation during the late eighteenth century, led to an increase in food prices at a rate above that of wage rises, forcing agricultural workers to shift their diet from relative luxuries such as meat, tea, bread, and sugar to a diet lacking both in variety and nutritional quality (Hudson 1992, 83; Burnett 2005, 22). Withthe expanding population, growth in urban or industrial regions such as London and the manufacturing northern towns increased competition, and social imitation among different classes led to differences in affordability and eating habits as well as distinctive class-driven tastes (Shammas 1984; Burnett 2005). Dietary surveys conducted in the 19th-century by Dr. Edward Smith also revealed marked regional differences in diet (Smith 1864; Barker et al. 1970).It is, therefore, the intention of this thesis to explore these differences in diet between social classes as well as between the manufacturing north and London in industrial

Britain.

3.2 Diet in 17th to 19th century Britain

In general, the 17th to 19th century diet in England consisted of locally produced C_3 plant foods such as wheat, rye, barley, and oats in the form of staples such as bread and porridge (Burnett 2005). C_4 crops in the form of cane sugar and maize were also available but in small quantities (Mintz 1986; Thirsk 2007). Although the available meat included beef, pork, poultry, and sheep, the most popular meat consumed by the British was pork and poultry (Broomfield 2007; Clayton and Rowbotham 2009; Burnett 2005). Most of the meat consumed in Britain was obtained from livestock that had been reared from the pastoral areas of the west and north of the country (Broomfield 2007; Clayton and Rowbotham 2009; Burnett 2005), however, there were some that were not from Britain. For instance, some pigs were imported as whole animals or as preserved pork mostly in the form of salt pork and bacon from the Americas, Africa, Asia, Australia, and Ireland while some cattle were from Ireland and Canada (Huttman 1978; Capie and Perren 1980; Plumb 2010; White 2011; Harris 2016). Therefore, it is possible that animals that may have consumed C_4 resources in this study may derive from overseas. In Australia, for example, C_4 shrubs are used as fodder (Hattersley 1983; Lefroy et al. 1992). Unlike livestock, the majority of fish consumed up to the end of the 18th century was locally sourced due to transportation and preservation challenges. Until the mid-19th century, transport costs and perishability restricted regular fish consumption to the British elite, those closer to rivers as well as Britain's coastal areas (Alexander 1970; Burnett 2005). With regard to vegetables, in the early 18th century, due to the continuing demand for vegetables by the poor, market gardening spread such that by the end of the 18th century, most of the large towns in Britain were supplied with locally grown fresh vegetables (Thirsk 2007). However, as indicated in sub-section 3.1.5, it should be noted that the consumption of these various foodstuffs in Britain was affected by the aforementioned rising food prices that occurred as a result of a series of bad harvests throughout the 18th century, the Napoleonic Wars, and the Corn Laws of 1815. This in turn caused the British diet to vary according to region and social status (Oddy 1970b; Oddy and Burnett 1992; Horrell and Oxley 2012).

3.2.1 Regional differences

At the beginning of the 19th century, populations living in the north of England were eating barley bread and porridge made from oatmeal and milk, however, this had changed by the mid-1830s when barley bread was substituted by white wheat bread (Mathias 1969; Burnett 2005). In London, the relative demand for wheat rose as white bread consumption became more widespread after the Napoleonic Wars (Mathias 1969). Regarding meat, it has been suggested that the Londoners consumed more meat than the rest of the country during this period (Trow-

Smith 2013). In London, meat was supplied from the wide pastoral counties of the south-west mainly for the city trade. London populations consumed livestock in the form of cattle, pigs, sheep, and poultry (Hardy 2002, 374). Cattle and mutton were brought into the city from Inverness in Scotland and the west of England (Spencer 2000; Picard 2006; Trow-Smith 2013; Metcalfe 2015) and the sheep from the chalk hills of Breckland (Trow-Smith 2013) while pigs and poultry were reared in larger quantities within the city itself (Mayhew 1851). In the northern towns, meat consumed also included pork, mutton, veal, beef, and poultry. Similar to London, poultry and pigs were locally available in great numbers (Chaloner 1959; Scola 1992). It is worth noting that the distribution of meat in the North also differed within this region. In Greater Manchester, sheep and cattle were brought in from the Welsh Borders, North Wales, Lincolnshire, and the neighbouring counties. Some of the cattle fattened for London supply from Scotland were also purchased and retained by local farmers in Manchester (Redhead et al. 1792; Marshall 1818; Scola 1992). Furthermore, following the abolishment in 1759 of the Cattle Acts of the 17th century which banned the importation of livestock from Ireland, as well as the end of the Napoleonic Wars, the import of cattle from Ireland to Greater Manchester began (Blackman 1975). By the mid-19th century, market reports showed that about 80 to 90% of cattle were obtained either directly from Ireland or from Liverpool, in which case they were still of Irish provenance (Scola 1992, 47). On the other hand, in Yorkshire, cattle were supplied from the North Riding and Lancashire while mutton was sourced from all the surrounding counties (Drummond and Wilbraham 1939; Scola 1992; Trow-Smith 2013).

It is important to highlight that while the majority of sites in this study are from London and northern manufacturing urban cities, there is one northern site (Fewston) which includes some rural labourers. The Fewston assemblage includes rich landowners, lower status labourers as well as mill workers (Alexander et al. 2017; Gowland et al. 2018). Generally, rural labourers in Britain were said to have been consuming more meat and bacon than the urban poor (Oddy and Burnett 1992; Burnett 2004). This is because they often received meat as part of their payment for their work instead of cash (Oddy and Burnett 1992; Burnett 2004). Moreover, many of them still had access to small patches of land where they could grow vegetables or keep a few animals including pigs and poultry for additional sources of food resulting in them having more than that of urban families (Oddy 1970b; Greaves2018). Bacon was the traditional meat for rural people because pigs were relatively easy to feed,and pork could easily be preserved by salting and smoking (Fraser 1981, 28). However, it has been noted that meat availability increased within the urban poor communities after 1850 and that by the 20th century the urban supply of meat had increased enough to exceed that of the rural poor (Oddy 1970b; Flandrin 1999; Teuteberg and Flandrin 1999). The proportion of high trophiclevel meat in the rural individuals' diet will be explored by use of stable isotope analysis in this thesis.

Fish were generally not consumed in rural areas unless they were situated close to the sea or local rivers. In London, fish such as cod, haddock, whelks, salmon, halibut, lobsters, and mullet were obtained from the Billingsgate fish market, established as London's principal fish market in the 16th century (Mayhew 1967; Burnett 2004; Panayi 2008). Furthermore, the London elite classes also made use of garden pond cultures to rear fish such as bream, pike, and tench for their own consumption (Dyer 1988, 27; Thirsk 2007) whilst the poor ate cheaper fish such as smoked herring and other cheap sea food such as oysters (Broomfield 2007; Clayton and Rowbotham 2009). Fish available in the North mostly for the middle/upper-class individuals included cod, lobsters, turbot, and soles from the Yorkshire coast, Lancashire coast, and the East coast. The poor could only afford mackerel from the Isle of Man in times of glut, as well as cured herring which was being produced in large quantities (10% in 1811 and 50% by 1816) by the east coast herring fleets from Scotland (Aikin 1785; Chaloner 1959; Coull 1996, 72). These are low trophic level fish/seafood sources that may stand apart from high trophic level fish such as cod in the stable isotope analysis of diet.

Additionally, dairy products such as milk, cheese, and butter formed part of the diet in post-medieval England. In the 18th century, there was generally relatively low milk consumption in the English diet (Burnett 2005). In the south of England, milk was popular among the middle/upper classes as it was very expensive, but the supply and quality were poor, with the milk often contaminated or watered down (Drummond and Wilbraham 1939; Molleson and Cox 1993; Cox 1996). London city obtained its supply of milk from the town dairies, mostly from cowsheds in the expanding urban area or surrounding areas (Oddy 1990; Scola 1992). Despite itsavailability to the upper classes, it was bought in small quantities to use mostly in cream teas forindividuals frequenting resorts and pleasure gardens within London (Atkins 1980). Since there was a limited market for milk, most of it was converted to cheese and butter (Drummond and Wilbraham 1939). However, it has been established that an increase in the number of town diaries and cowsheds around the 1820s as well as improvements in transportation mid-century,meant that by 1865, milk had replaced cheese and butter in relative importance as a product for London dairy farmers (Scola 1992). On the other hand, milk was consumed in larger quantities in the north of England due to extensive cattle farming in that part of the country, although the milk was of poor quality due to being watered down or contaminated (Drummond and Wilbraham 1939; Scola 1992). Cattle farming enabled the poor in the North to consume higher quantities than those in the South (Marshall and Marshall 1796). Cheese and butter were major dairy products compared to milk during the late 18th and early 19th centuries but by the mid-19th century, the paucity of the town dairies in the northern towns and the increase in demand for liquid milk reduced the cheese andbutter trade (Holt 1794; Scola 1992).

Higher consumption of dairy products by populations in the North compared to the South may

affect isotope ratios, however, this will be difficult to determine as studies have shown that dairy products cannot be isotopically distinguished from meat in body tissue stable isotope analysis (O'Connell and Hedges 1999). As a result, the consumption of dairy products and meat will be taken together as terrestrial mammal protein in this thesis.

Other foodstuffs consumed during this time include fruits and vegetables. However, there were certain prejudices against these in the country such that until the end of the 19th century, they rarely contributed much to the diet (Oddy 1970a; Oddy 2000). The prejudices against fruit and vegetables were due to negative publicity which began around the 16th century when it was suggested that fruit and vegetable consumption would cause diseases such as putrid fever (Oddy 1970b; O'Hare-May 1971). Despite this, the relatively smaller quantity of vegetables consumed mainly came from market gardens, and some fruits such as apples, were locally sourced while others such as oranges were imported from America. Grapes and walnuts were also imported into Britain (Oddy 1970a; Scola 1992). The development of market gardens had been enabled as a result of a combination of the introduction of a variety of vegetables in the country in the 17th century and the rapid growth of urban towns in the 18th century. These gardens are said to have started in London but as of 1742,the northern towns had not appreciably adopted the enterprise. However, by the end of the century, most towns had their own gardens which supplied the markets with fruits and vegetables. The northern towns were supplied vegetables such as cauliflower, cabbage, peas, beans, celery, and lettuce from the expanding gardening in Lancashire and Cheshire (Young 1771; Porter 1977; Thirsk 1985; Uings 2013). In London, vegetables such as cauliflower, lettuces, beans,peas, cabbages, and roots were grown in London kitchen gardens and supplied into the local markets (Thirsk 1985).

3.2.2 Social status differences

Post-medieval Britain was characterised by a high degree of socioeconomic stratification which was reflected in the dietary patterns of the rich and the poor throughout this period. Generally, the majority of the English population's diet comprised a large proportion of cereals and a smaller meat component especially for the poor (Oddy 2000). As a whole, food for the upper classes included white wheaten bread, vegetables, fruits, cane sugar, tea, cheese, eggs, meat, and fish (Oddy 2000; Clayton and Rowbotham 2009). On the other hand, the poor relied on readily available cheap filling foods such as bread and potatoes with very little meat, most of which was often allocated to men (Barker et al. 1970; Oddy 2000; Burnett 2005). However, as a consequence of the Corn Laws at the beginning of the 19th century, food prices increased, particularly affecting the new urban industrial workers (Jones et al. 2001). Tax on bread, which provided the working class with the large amount of calories required in highly physical labouring jobs, was increased, rendering bread very expensive (Oddy2000; Burnett 2005; Clayton and Rowbotham 2009). Up to

half of the earnings of the working class were required to buy bread (Burnett 2005). Furthermore, wheat supplies per capita reduced, resulting in a decline in bread consumption which meant that other foods were requiredto provide energy for the working class (Oddy 1970b; Oddy 2000, 51). This, in turn, caused potatoes, which had been previously regarded by the farmers and urban workers with extreme distaste, to become increasingly important in the diet (Drummond and Wilbraham 1939, 216; Chapman 2000). Unlike the upper classes who did not have qualms with consuming potatoes, the working classes, especially the northern industrial workers earning relatively higher wages thantheir co-workers and whose status was determined by their ability to consume wheaten bread, initially did not want to eat potatoes despite their high vitamin and nutrient content. They viewed potatoes as only fit for feeding pigs and also applied a racial association on the consumption of potatoes as they believed that it would make them uncivilised workers or, given the prejudice against Irish immigrants at the time, be associated with Irishness, pejoratively becoming "English Irish" (Thompson 1963, 315). Nevertheless, despite this distaste for potatoes by some of the working class individuals, although bread remained the main English staple food, by mid-19th century, potatoes were widely consumed throughout the country by most people owing to theirhigh starch content which was a ready calorie source in times of famine and deprivation (Thirsk 2007; Horrell and Oxley 2012). In addition to the Corn Laws, there are suggestions that an increase in the consumption of potatoes was mainly brought about by the large numbers of migrants from Ireland during the mid-19th century and also that potatoes were considered easierto grow compared to wheat (Burnett 2005; Clayton and Rowbotham 2009).

The amount of meat and fish consumed during this period was also determined by individuals' socioeconomic status, such that meat and fish formed a significant part of the middle and upper classes' diet while the working-class people ate little to no meat or just meat offcuts (Stead 1985; Thirsk 1990; Chaloner 1959; Scola 1992). Until the 17th century, people had consumed fish on Fridays and Saturdays, on which days religious tenets prohibited the consumption of other types of meat (Thirsk 2007, 265). This tradition persisted mostly among the elite classes after the 17th century underlining the consumption of fish as a status symbol even in the 18th century onwards (Stead 1985; Aston 1988, 35).

As is the case with meat and fish consumption, dairy products, fruits, and vegetables were widely available to the middle and upper classes across the country during this period. However, as previously stated in Section 3.2.1, there was a lot of distrust for eating fruits and vegetables. In spite of that, commonly consumed vegetables by the middle and upper classes included cabbage,

turnips, carrots, onions, lettuce, and cucumbers and fruits including apples, plums, cherries, and berries (Alexander 1970; Oddy 1970b; Thirsk 1985; Uings 2013). Beans were however associated with the poor (Thirsk 2007).

3.2.3 Age and sex differences

Besides socioeconomic status and regional differences, the allocation of food resources differed according to sex and age. Among the poor, necessities such as sugar, cheese, and occasional supplies of meat were mostly allocated to males who supposedly required more energy to be able to work inhighly physical jobs and as a consequence, females and children subsisted on mainly cheap rye or barley bread and/or potatoes (Burnett 2005; Horrell and Oxley 2012). On the other hand, Davidoff and Hall (2018)'s account of men and women of the English Middle Class 1780–1850 revealed that during this time, middle-class gender roles were flexible such that women were working in businesses and other occupations as much as men. Given this, one would not expect to observe gender driven differences in the middle/upper-class diet as expected for the poorer communities.

Preferential allocation of high calorie foods to men in poor families meant that children from lower classes were weaned early leading to a greater likelihood of chronic child malnutrition. They were generally given artificial feeds such as pap (a mixture of flour or breadcrumbs cooked in milk or water), panada (a bread broth, or milk flavoured with sugar, eggs, or spices), or cow's milk which were not nutritious. On the other hand, most middle or upper-class children were breastfed but there were some that weaned early because breastfeeding was considered inconvenient or unfashionable, therefore, they were also fed pap (Drummond and Wilbraham 1939; Wickes 1953; Fildes 1995; Nitsch et al. 2011). Although the upper classes could afford a variety of foods, the belief that fruit and vegetables were bad for the children was common (Powell 1939).

3.2.4 C_4 diet (Maize and Cane Sugar)

One of the main aims of this study is to investigate the consumption of C_4 maize and cane sugar, therefore their introduction to the British diet is examined in great detail here. While crops consumed in Britain were predominantly C_3 plants such as wheat, rye, barley, oats, and increasingly potatoes, a small number of C_4 foods such as maize and cane sugar found their way into the country through slavery and colonial links with the Americas, Asia and Africa (Mintz 1986; Brassley 2000; Thirsk 2007). Maize, a native plant from Mesoamerica was introduced to Europe in 1492 and later found its way into Britain around 1882 (Brassley 2000, 69). Despite this, there are suggestions that during the Great Irish Famine of 1845 to 1849,the Irish staple, potato, failed due to an infestation by *P. infestans* (a microbe species which causes potato blight)

prompting the then UK Prime Minister Robert Peel to import 'Indian meal' (maize)from America in 1845 as relief food for the poor (Dudley-Edwards and Williams 1956; Crawford, 1984; Ó'Gráda 1989). It was during this period that many Irish poor migrated to Britain to work in workhouses to escape poverty and starvation. However, the Irish poor did not readily use maize at first because it was improperly ground and stale (Crawford, 1984). In addition, they considered it as poison, therefore its consumption was limited to what the government provided people with (Crawford, 1984). Some of the maize was given to them in the form of porridge in their daily rations and a few bought it from local relief communities to cook at home if they were not staying there (Dudley-Edwards and Williams 1956; Ó'Gráda 1989). However, the introduction of relief maize lasted for only about two years after the then-new Prime Minister Charles Trevelyan closed the food depots that had been sellingmaize and stopped its importation from America (Dudley-Edwards and Williams 1956; Ó'Gráda 1989; Swift 2002). Historical sources indicate that although maize became a staple for the Irish poor, until the 20th century, maize was not widely accepted by the English regardless of class except as animal fodder (Holland 1919; Brassley 2000; Messer 2000; Martin 2012). Therefore, with the limited amount of maize consumed even by the Irish migrant population in Britain, it is possible that its signature may be difficult to find even when using bone carbonate isotope analysis.

Consumption of cane sugar, on the other hand, increased from the 17th century in Britain due to the slave trade and Britain's colonisation of the West Indies. Before this time, it was very expensive, with one pound of sugar costing one shilling - equivalent to a poor labourer's two-day wage (Shammas 1990, 81). As a result, it was mostly affordable only to the nobility and wealthy, featuring in their food, medicine, displays of rank and literary images. (Shammas 1990; Walvin 2017). In the wake of British colonisation of the West Indies in the seventeenth century, slaves were forced to work in sugar plantations (e.g., Jamestown Island in 1607, Island of St. Christopher in 1624, Barbados in 1627, Nevis in 1628, Antigua and Montserrat in 1632 and Jamaica in 1665), producing sugar that would then be supplied to consumers in Europe (Galenson 1986; Mintz 1986). In his book, Mintz (1986) revealed how sugar consumption came about in England

"The English connection between sugar production and sugar consumption was wielded in the seventeenth century, when the British acquired Barbados, Jamaica and other 'sugar islands'....."
(Mintz 1986, 38)

The growing success of England's own sugar colonies in these islands led to an increase in shipments of sugar to England. This resulted in the country controlling the sugar trade in Europe during that century. Records indicate that England imported 1200 barrels of sugar in 1660, 50000 barrels in 1700 and 11000 barrels in 1753 (Mintz 1986).

London is said to have been the commercial, financial and trading centre behind Britain's sugar colonies (Walvin 2017). Around the 1650s Londoners beganusing cane sugar as an additive to hot beverages such as tea, which by 1704, had become popularbecause of the transformation in British tea-drinking when shiploads of tea were transported to the city by the restructured United East India Company from China (Walvin 2017). By 1800, the poor had also become attached to cane sugar as a sweetener in tea. This was because, by this time, there was a lot of cheap imported tea which had been helped by the reduction of tea duties. Furthermore, London employers began giving their domestic servants sugar and tea as a replacement for the ration of beer traditionally granted to servants as part of their food allowance(Graham 2008, 72). Therefore, this thesis will explore the potential for enriched carbon-13 (^{13}C) isotope values in the London individuals including the poor as a result of hypothesised high canesugar consumption for all social classes in the city.

Despite London being home to Britain's first sugar refineries and having the majority of sugar refineries in the country, the rapid expansion of the sugar industry also saw a rapid increase in the number of refineries at many of the country's other major ports such as Bristol, Greenock and Liverpool (Schwarz 1992, 41). In the early 19th century, cane sugar duties began to be equalised throughout Britain, leading to a sugar price drop that resulted in the average per capita cane sugar intake in Britain increasing from 1.8 kg in 1700 to 8.1kg in 1800 then rising to a further 13.6kg in 1850 (Mintz 1986; Johnson et al. 2007; Walvin 2017). Additionally, the 1874 Abolition of Sugar Tax led to an even greater reduction in sugar prices, resulting in an even larger increase in consumption by all classes in the country (Mintz 1986; Langer et al. 1991; Marshall 1998; Walvin 2017). During the Napoleonic wars, the British Navy blocked cane sugar imports from the West Indies from being sent to other European countries such as Germany, France, Italy, and Austria. This in turn resulted in the shortage of cane sugar in these countries therefore they began developing techniques to extract sugar from beets. As a result, by the 19th century, these Europeans were consuming more beet sugar than cane sugar (Ballinger 1974; Mintz 1986; Johnson et al. 2007). With the British consuming more cane sugar than other western European countries, and more Irish who relied on maize coming to Britain during this period, it is therefore very important to try and investigate the consumption of these C_4 resources here through stable isotope analysis.

3.3 Summary

What is certain from this chapter is that the Industrial Revolution in Britain was characterised by significant changes in agriculture. These changes brought about population growth in urban areas, changes in manufacturing as well as changes in transportation. These in turn all had a remarkable impact on the procurement of food for different people. These impactsvaried along

social and regional lines throughout Britain. With all the information available about the socioeconomic context at the time, the addition of stable isotope analysis of human remains will assist in answering some of the main questions from this period relating to the status and regional based variations in the English diet. At the same time, by using both the historical sources and isotope analysis, it will be possible to investigate the exploitation of C_4 resources such as maize and cane sugar, animal protein, and fish by different urban populations in England. The sources of the different food resources will have an impact on the isotope results. For instance, it is expected that animals that were obtained from areas with significant manure usage will have different nitrogen isotope compositions from those that were sourced elsewhere. Furthermore, it is expected that the high dependence on C_3 cereals such as wheat, barley, and oats compared to the C_4 maize and cane sugar has to be supported by stable isotope analysis. Similarly, the higher consumption of meat (high trophic level) and fish resources by the rich and by Londoners (when compared to the northern individuals) should also be visible in stable isotope analysis results. While historical sources can provide community level information on the broad dietary changes that took place during the period in question, they are not able to provide answers on what specific individuals' diet was based on, given that individual accounts such as diaries are rare. Stable isotope studies therefore provide a way to directly investigate dietary compositions through the analysis of each individual's bones themselves, bringing an illuminating granularity that can lead to a greater understanding of social changes that were taking place in history. Still, it should be noted that isotope analysis will not be able to distinguish between consumption of C_3 resources (e.g., either between consumption of wheat and potatoes or between consumption of barley bread and white bread) or between dairy products and meat protein. As a result, these limitations will be considered when exploring both the historical information and stable isotope analysis. At the very least, the approaches complement each other in the attempt to gain a comprehensive understanding of human history.

Chapter 4: The sites & their historical and archaeological context

This chapter introduces the specific archaeological context and historical background for each site chosen for this thesis, most of which are contained in three paper chapters. This will particularly serve as a background for Chapter 7 and will be submitted as Supplementary Information for that paper. It should be highlighted that some of the things included in this chapter may therefore be repeated in the papers themselves.

In order to explore the range of isotopic variation in post-medieval populations in England, human samples for this thesis were selected from eleven different sites to provide dietary data from a variety of social groups (Table 4.2; Figure 4.1). Particular attention is paid to the Manchester sites as these are the sites that make up most of the analysed samples in this thesis. A brief background on the comparative sites used for the dental calculus case study (Chapter 8) is also provided in this chapter. Environmental influences can cause regional and temporal variations in the isotopic values of foodstuffs; therefore, it is important to interpret the human diet within the context of a faunal isotopic baseline for the potential foodstuffs available at a given location. To that end, faunal remains were also analysed from nine different sites in England to provide a baseline to aid in the interpretation of human diets across the dataset. Altogether, a total of 419 human and faunal remains were sampled for isotopic analysis from 20 sites (Tables 4.1 and 4.2). Collagen isotopic data for 70 of the 216 human samples analysed for carbonate isotope analysis were already available, having been generated in previous undergraduate and postgraduate dissertations at the University of York. Collagen data for faunal remains from Hungate, the Bedern and Walmgate were provided by Sean Doherty.

Table 4-1: Faunal remains from all sites, with associated dates, periods, and the total number of each animal species analysed in this thesis.

Location	*Site*	*Date*	*Cattle*	*Sheep*	*Pigs*	*Domestic Fowl*
Manchester	Cross Street	18th - 19th c	14	13	8	2
Runcorn, Cheshire	Norton Priory	16th - 20th c	20	23	20	12
Halifax	Square Chapel	18th - 19th c	2	3	2	0
Leeds	Otley	Unknown	3	3	2	0
Leeds	Oulton	Unknown	4	0	0	0
Tunstall	Sand-le-Mere	16th - 19th c	3	0	0	0
York	Hungate	16th - 19th c	0	21	0	0
York	The Bedern	15th - 19th c	0	11	0	0
York	Walmgate	18th - 19th c	0	3	0	0
Total analysed			46	77	32	14

*Table 4-2: Individuals sampled for bone collagen, bone carbonate, and FTIR-ATR analysis from all sites, with associated dates, economic status, and reference for Osteological analysis information. *The Fewston assemblage includes wealthy landowners, rural farmers, and industrial mill workers. Although the individuals recovered at Fewston date between 1550-1908 all 7 individuals date between the 18th and 19th centuries.*

Site name	*Collagen Analysis (n)*	*Carbonate and FTIR-ATR Analysis (n)*	*Status*	*Period*	*Osteological analysis Reference*
Fewston, Harrogate	0	7	Mixed*	18th-19thc.	(Caffell and Holst 2010; Gowland and Caffell Forthcoming)
Rotherham Minster, Rotherham	21	21	Low Status	1780s-1854	(Keefe and Holst 2011)
Hazel Grove, Manchester	34	31	Low Status	1794-1910	(Newman and Holst 2016)
Victoria Gate, Leeds	0	3	Low Status	1796-1850s	(Caffell and Holst 2014)
St George's Crypt, Leeds	9	9	Middle/Upper Class	1840-1911	(Caffell and Holst 2009)
Square Chapel, Halifax	5	32	Middle Class	1772-1857	(Keefe and Holst 2015)
Cross Street, Manchester	92	54	Middle/Upper Class	18th-19thc.	(Keefe and Holst 2017)
Royal London Hospital, London	11	11	Low Status	1825-1841	(Fowler and Powers 2012)
St Brides Lower, London	15	15	Low Status	1770-1849	(Miles and Conheeney 2005)
Queen's Chapel of the Savoy, London	0	10	Mixed	1510-1854	(Sibun and Ponce 2018)
St Barnabas/St Mary Abbots, Kensington, London	0	23	Upper Class	1760-1853	(Goldsmith 2016; Bleasdale et al. 2019)

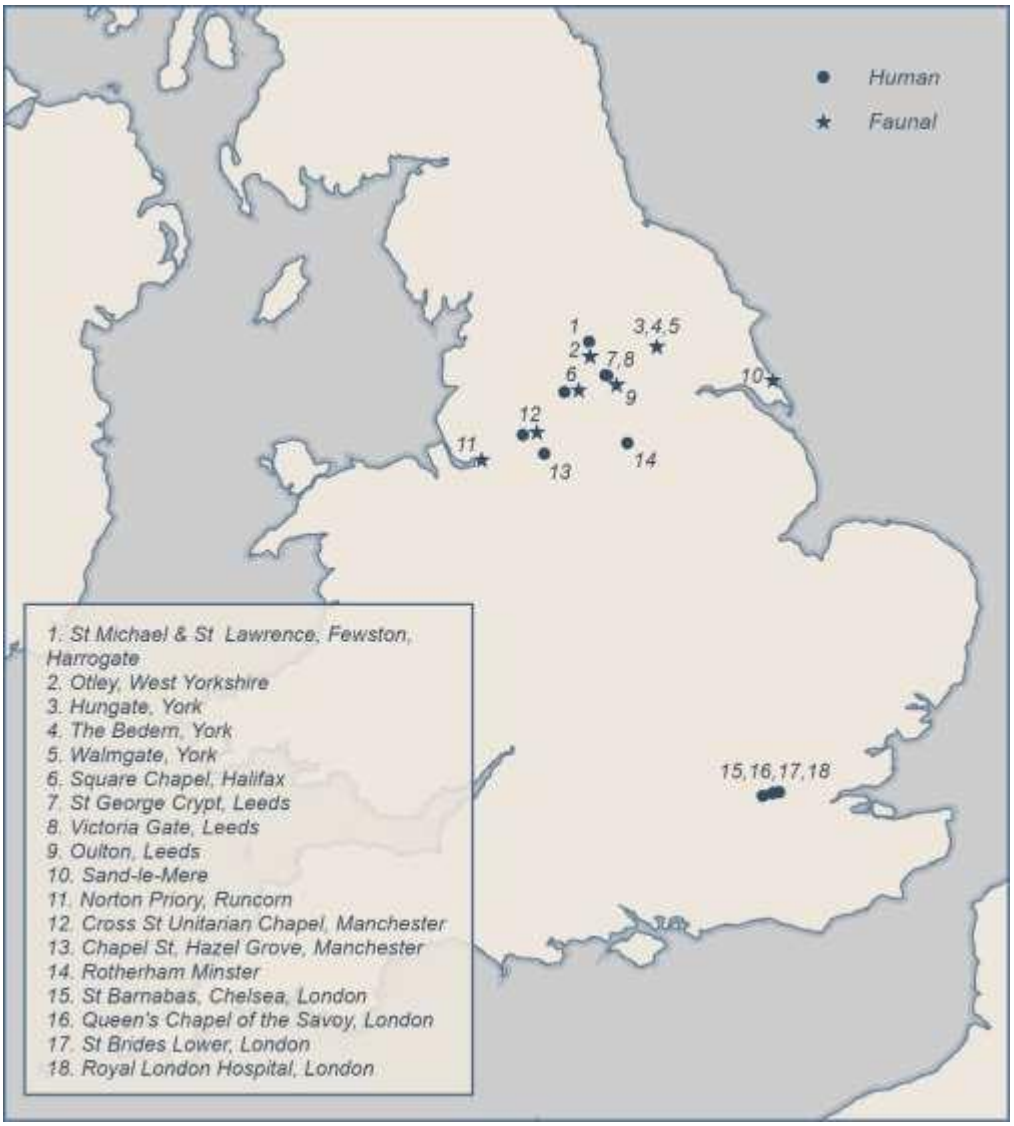


Figure 4-1: Map of post-medieval England showing the locations of the sites studied in this thesis.

4.1 Sites in Manchester

Manchester was originally a Roman fort, named *Mamucium,* which means a breast-shaped hill, and it was built in AD 79 in a place now known as Castlefield (Gregory 2007). Until the second half of the 18th century, Manchester remained a manorial township at the confluence of the Irwell and Irk rivers (Douglas et al. 2002, 237). In the second half of the 18th century, Manchester city rose rapidly from a series of small towns to a large industrially developed area (Brockbank 1929; Frangopulo 1977). The construction of the Bridgewater canal and the passenger railway to Liverpool resulted in the city attracting a large labour force from different areas. A combination of the availability of coal mines, the Bridgewater canal, and passenger railway in the area (which increased free trade and massive imports of cotton), as well as technological innovations, transformed the city into a major industrial conurbation (Douglas et al. 2002). According to the

autobiography of William Aitken, a man who resided in Audenshaw in Tameside, Greater Manchester, in the 19th century, a lot of people (men, women, and children) were employed in cotton mills, working 15-hour shifts (Bronstein 1998) while others worked in coal mines (Aitken 1869).

These industrial activities led to increased immigration into Manchester, mostly from districts surrounding the city and a small number from Scotland and Ireland resulting in population growth (Chaloner 1959). By the middle of the 19th century this industry-driven population expansion meant that about 55% of people in the Manchester-Salford area were from other parts of Britain and 1% were from abroad (Douglas et al. 2002, 237). For instance, the Irish comprised 12% of the Manchester population by 1841, a proportion further increased by 1848 due to the Irish fleeing the Famine of 1846-1848 (Lawton 1959). According to Douglas et al. (2002, 236), the British establishment viewed Manchester as an experiment through which they could learn the true nature of economic and what social and environmental problems this progress entailed, Population growth in turn increased the local demand for food with supplies originating from increasingly distant parts of Britain and other countries (Scola 1992; Douglas et al. 2002). Additionally, the late 18th century saw the city divided into two classes; the working class, which included farmers and low-skilled factory workers who did not own any means of production; and the wealthy, who owned most of the means of production (Scola 1992, 9). For this thesis, individuals from Manchester are from two sites representing different classes to enable the investigation of diets according to age, sex, and, specifically, socioeconomic status. All collagen, FTIR, and carbonate data from this region were generated as part of the laboratory work carried out for this thesis, as none existed beforehand.

4.1.1 Cross Street Unitarian Chapel

The Cross-Street Unitarian Chapel in Manchester is located in the northern half of Manchester city centre. The chapel was founded in the late 17th century and its period of use is likely to have been until 1852 (Keefe and Holst 2017). It was built by Reverend Henry Newcome in order to accommodate the increasing number of his congregation. Like many other non-conformist groups, the chapel was attended by the poorer classes in the beginning, however with time, the chapel recruited the more prosperous members from manufacturing classes who were artisans or merchants, like the first Mayor of Manchester, Sir John Potter (Baker 1884; Messinger 1985, 59). Although Sir John Potter himself was not exhumed during the redevelopment of Cross Street, individuals were excavated who had grave markers denoting the burials of members of the Potter family, with one member, skeleton 2.31 (Elizabeth Potter) included in this thesis.

The chapel was excavated by CFA Archaeology from October 2014 to November 2015, in advance of the construction of the Second City Crossing tramway along Cross Street. Among the individuals recovered at this site, were sixteen individuals who were buried with legible nameplates, five of whom were nonadults, and eleven were adults (seven males and four females). Nine of these individuals were studied in this thesis (Table 4.3).

Table 4-3: Named individuals from Cross Street sampled in this thesis and their potential dates of death.

Sample	*Named individual*	*Sex*	*Age (years)*	*Date of death*
2.03	William Crane	M	85	1 January 1808
2.21	Ann Goodier	F	46+	Unknown
2.31	Elizabeth Potter	F	46+	Unknown
2.40	Harriet Slater	F?	6	10 August 1819
2.52	Ellen Rylande	F	62	12 July 1835
4.38	Sarah Baron	F?	46+	Unknown
5.16	John Blinkhorn	M	64	17 April 1838
5.23	Mary Mason	F	64	24 August 1809
61.03	Joseph Guildford	M	26-45	1829

There were slightly more females in the young adult (18 -25 years) age group and more males in the middle adult (26-45 years) age group (Keefe and Holst 2017). Of the 241 skeletons and 17,679 fragments of disarticulated bone recovered at the site, 92 individuals representing the middle/upper class who were in a good state of preservation and were able to be aged and sexed were selected for isotope analysis in this study.

4.1.2 Chapel Street Hazel Grove

Hazel Grove site is located approximately 9 miles from Manchester city centre. The site is located at a former Wesleyan Chapel dated at c.1785 and a Sunday school in place c.1823-1912 (Jessop and Beauchamp 2015). Most people from Hazel Grove were said to have been from the low socioeconomic class. Until 1836, historical records indicate that the Hazel Grove area was known as Bullock Smithy and the renaming of the town to Hazel Grove was an attempt to overturn the negative reputation of the area (Errington 2001). The name Bullock Smithy had come about in 1560 when Richard Bullock acquired land for a smithy. The area of Hazel Grove encompassed this smithy and four medieval manors surrounding it, Bramhall, Handforth in Bosden, Torkington, and Norbury (Trowsdale 1985). Around 1750, the Bullock Smithy was referred to as a place of "wickedness" (Jutsum 1876, 17) possibly due to the cockfighting, gambling, bull-baiting, and dogfighting which were being carried out there as well as an abundance of inns and beer houses (Trowsdale 1985). There was no formal parish church in Hazel Grove prior to the 19th century, however, by the 19th century, non-conformist congregations were popular within this area, with

several Methodist churches situated in the area (Newman and Holst 2016). The 19th century saw Hazel Grove's cotton and silk industry expanding due to its location adjacent to the main trading routes south of Manchester (Trowsdale 1985).

Excavations by CFA Archaeology in advance of a housing development revealed at least 39 burials. The osteological analysis showed that the burial population comprised a slightly higher proportion of females than males, which is common in non-conformist burial grounds. However, this may have been skewed by the small sample size of the cemetery. Half of the overall population at this site was made up of non-adults, with a high proportion of infants (1 month to 11 months) and young juveniles (1 to 6 years). Of the 39 recovered at this site, 34 individuals were selected for stable isotope analysis. The studied sample includes SK 1, 10, 14 and 21 who had tentative evidence of being of African/mixed ancestry (Newman and Holst 2016).

4.2 Sites in Yorkshire

The rapid growth of industrialisation in Yorkshire occurred during the 18th century as a result of improvements in manufacturing, new inventions, together with the region being dominated by a wealth of resources in the form of waterways and various minerals such as copper, coal, iron, and lead (Marshall 1788). Another major feature in Yorkshire's industrialisation was the expansion of the textile industry which led to the urban manufacturing network of worsteds and woolens becoming centred in the region. This expanding growth of industry also saw associated rapid population growth in the region which in turn influenced food procurement patterns (Gray 2002; Mantoux 2013).

Human remains from this region were sampled from five sites: Fewston, Harrogate; Square Chapel, Halifax; St George's Crypt, Leeds; Victoria Gate, Leeds; and Rotherham Minster, Rotherham. Collagen data for Fewston, Harrogate; Square Chapel, Halifax; and Victoria Gate, Leeds were obtained from Poppy Yapp and Chloe Brown (Fewston), Sarah Delaney and Orsolya Czére (Square Chapel), and Michelle Alexander's unpublished material (Victoria Gate). However, the remaining collagen data for five of the Square Chapel, Halifax individuals, all Rotherham Minster, Rotherham and St George's Crypt, Leeds individuals as well as all FTIR and carbonate data were generated as part of the laboratory work carried out for this thesis.

4.2.1 Fewston, Harrogate

Fewston is a small village located in the Washburn Valley, 9 miles west of Harrogate, 14 miles East of Skipton, and 9 miles North of Otley. The 18th century saw many local families in this village earn the right to acquire and maintain land for stock rearing and better cultivation. Many of the

named individuals in this assemblage were farmers and textile workers of varying social status (Caffell and Holst 2010). Seven of the human individuals sampled for this thesis were part of 145 skeletons recovered during archaeological excavations in 2009 and 2010, of the churchyard of St Michael and St Lawrence, Fewston, North Yorkshire (Caffell and Holst 2010). The excavations were carried out by John Buglass Archaeological Services (JBAS) prior to the construction of a Heritage Centre at Fewston Church. Of the 145 individuals excavated, twenty-six of these were confidently identified and named; 11 from their association with grave monuments and 15 from legible and partially legible coffin plates (Buglass 2009; Caffell and Holst 2010). A notableaddition to the information available for this population are the diaries of John Dickinson (Harker 1988; Caffell and Holst 2010), a late 19th century resident of the adjacent village of Greater Timble. They were written between 1878 and 1912, furthermore, an edited version of the diaries is also available (Harker 1988). These diaries refer to some of the identified individuals within the skeletal assemblage, providing unique personal insights into everyday life in the Washburn Valley (Caffell and Holst 2010). Information from coffin plates and headstones revealed that burials were predominantly of the latter half of the 19th century (Caffell and Holst 2010). The seven individuals sampled for this thesis date to the 19th century and include three named individuals identified by coffin plates: George Lister, date of death 19th July 1882 (Skeleton 130); Christiana Patterson nee Hardwick (Skeleton 156); and Elizabeth Demaine, nee Dickinson, date of death 6th April 1888 (Skeleton 238).

4.2.2 Square Chapel Halifax

The town of Halifax is situated approximately 17 miles southwest of Leeds and 28 miles northeast of Manchester. The town began to transform in the late 18th century due to the development of woollen and textile industries in the area. There are prints available from the 18th century that reveal that Halifax was largely rural with many small enclosed fields (Hargreaves 1999, 112; Engl et al. 2013). However, by 1790, the town has been described as a place filled with smoke emitted from factories, with about 46 cotton mills (Hargreaves 1999, 70). During this period, there were several people migrating into the township from outside Halifax including many, again, from Ireland (Hargreaves 1999). The population in Halifax rose from 5,000 to 25,159 between 1743 and 1851, turning the town from a rural settlement to an urban centre (Hargreaves 2003, 73). However, it must be noted that the town did not develop as much as the nearby Manchester and Leeds urban cities because of its geography that made transportation of goods difficult, preventing the large scale shipments of the coal necessary to power the steam engines in mills, restricting the manufacturing industries from becoming wholly dominant in the town (Hargreaves 2003). During the 18th and 19th centuries, the town appears to have consisted of a

population from a variety of economic backgrounds, including those living on or below the poverty line, the working classes, artisans, and the newly established middle classes (Hargreaves 2001). Those living below the poverty line were often malnourished and lived in damp and filthy houses where there was inadequate sewerage system (Hargreaves 2003). Studies have indicated that most of the poor people in Halifax worked in the textile industry, working for most of the middle and upper classes who were manufacturers and merchants (Iwama 2003).

The Square Chapel site is located on the eastern side of the modern Halifax town. The site, previously known as Square Independent Chapel, was a Wesleyan non-conformist church. It was excavated in 2014 by the Archaeological Services WYAS and osteological analysis was carried out on 203 skeletal remains of the 1449 burials excavated (Keefe and Holst 2015; Keefe 2016). The burial register held at the National Archive in Kew shows records for Square Chapel date burials occurring between 1771 to 1837 (Keefe and Holst 2015). Although Square Chapel ceased being a chapel in 1857 gravestone inscriptions indicate that burials continued until 1870. Six individuals were identified by coffin plates and only one of them (William Fletcher, Skeleton 35) was included in this thesis. These six coffin plates indicate that the individuals died in the 19th century (Keefe and Holst 2015; Keefe 2016). Osteological analysis revealed that the burial population comprised a slightly higher proportion of females than males, which, as previously mentioned, is common in non-conformistburial grounds (Hargreaves 1999, 97–98). About 65% of the skeletal assemblage were adults with 35% being non-adults, with a high proportion of infants (1-12 months) and young juveniles (1-6years). It appears that there are likely variations in the socioeconomic status of those buried at Square Chapel, but it is impossible to differentiate this by burials themselves. The mixed socioeconomic status of these individuals may be due to the individuals being part of nonconformist churches as these churches appealed to individuals from a variety of backgrounds and professions, such as artisans, shopkeepers, manufacturers, and did not typically attract only one class (Keefe and Holst 2015). In addition, historical sources imply that living conditions were mixed, with the poorest of society enduring the worst conditions close to the factories, while those that could afford the luxury of living further away from the industrial pollution enjoyed better conditions (Webster 1998; Hargreaves 2003). Individuals sampled from this site are therefore viewed here as people who were on a mixed-status diet in England.

4.2.3 St George's Crypt, Leeds

St George's Crypt is located in the city of Leeds, West Yorkshire in Northern England, approximately 170 miles north of central London. As was the case for Manchester city, inventions of new processes in linen and wool manufacturers in Leeds developed rapidly in the late 18th century resulting in population growth from 15,000 to 309,119 between 1760 and 1881 (Jacob 2007; Caffell and Holst 2009).

In February 2009, St George's Crypt in Leeds was excavated prior to the construction of an extension to the south of St George's church in the city. This church, built between 1836 and 1839, had no graveyard (Caffell and Holst 2009). Instead, it had a crypt and burial vaults beneath the podium which were in use between 1840 and 1911 (Caffell and Holst 2009). Osteological analysis was carried out on eight articulated skeletons and disarticulated human bone and the analysis identified the individuals as 22 adults and 8 non- adults (one neonate, two infants, one young child, and four adolescents). Both sexes were represented. Since the individuals recovered at this site were found in vaults, they were probably buried within small family groups because it has been suggested that in the 19th century wealthyfamilies would reserve particular vaults for future burial of their members (Mays et al. 2009). Unfortunately, there was not enough information on the identities of the individuals buried within each vault to confirm if this was the case at this site. Although vault burials are associatedwith wealthier classes, the social status of the individuals in this study is unknown as there was no churchyard to accommodate the poor. The males were taller (175.8cm) than average for the post-medieval period, while the females were slightly below average height (158.0cm). The above- average height of the males however could suggest that these individuals were not accustomed to episodes of poor nutrition and disease during their childhood, indicating that they may have been wealthy. Furthermore, data from the grave markers in the crypt suggest that some of the individuals may have been surgeons (Caffell and Holst 2009). Therefore, these individuals will beconsidered to have been from the middle/upper class in this thesis.

4.2.4 Victoria Gate Leeds

Victoria Gate is located in the centre of Leeds City. The human remains from this site were associated with a Methodist church, having been excavated from the vicinity of Ebenezer Chapel, a Baptist church sold to the Methodists in 1797 (Beckwith 1938). The remains were excavated in 2013 and 2014 and osteological analysis was carried out by York Osteoarchaeology Ltd in 2014. They date to the late eighteenth or nineteenth centuries and were believed to represent some of the poorest in Leeds. Overall, the assemblage represented a minimum of 28 individuals: twelve adults and sixteen non-adults. Nine of the twelve adults at the site had developed metabolic diseases which included scurvy, rickets, and possibly anaemia (Caffell and Holst 2014).

4.2.5 Rotherham Minster

Rotherham is located in South Yorkshire, England, approximately 32.2 miles to the south of Leeds and 43 miles to the east of Manchester. Archaeological excavations of Rotherham Minster, previously known as All Saint's Church, were carried out between early December 2009 and late February 2010 by Archaeological Services WYAS. Osteological analysis was performed on 60

skeletons by York Osteoarchaeology Ltd (Keefe and Holst 2011). Skeletal evidence suggests that this population was probably of the lower social class who worked in coal mines, foundries, and glassworks, as well as other industries in the town. Historical records indicate that the Rotherham Minster graveyard was closed due to the expanding population as a result of the growth in the iron and steel industry between 1800 and 1854. These sources also suggest that in Rotherham, the poor were living in cramped areas with poor facilities, air and water pollution, poor hygiene, poor sewage disposal, limited access to clean water, and inadequate nutrition, and medical care (Jones 1995; Jones 1996; Munford and Munford 2000; Munford 2003). These poor living conditions resulted in two outbreaks of cholera in 1832 and 1849 (Underwood 1935; Hague 2018). Osteological analysis revealed that the quantity and severity of disease manifestations compared with those from contemporary populations were unusual thereby supporting the extent of poor living conditions in Rotherham (Keefe and Holst 2011). Nonadults made up 40% of the assemblage whereas 60% was made up of adults, a group for which there was near parity of sexes. The analysis also revealed that children often did not survive beyond the age of three years due to nutritional deficiencies. It has been suggested that the sizeable number of adolescents (13-17 years) among the nonadults most likely represents the immigration of adolescents into towns for work, where they would have been exposed to infectious diseases they had no immunity against (Keefe and Holst 2011).

4.3 Sites in London

By the early 18th century, London had become a place of Britain's central governance, political movements, a marketplace of all sorts of exotic produce, centre for traders, financiers, and specialist manufacturers (Garside 1990; Walvin 2017). With the population rising from about 575,000 people in 1700 to about 900,000 in 1801, London required broad supplies of daily essentials such as food. To satiate this demand, there was a rapid spread of market gardening which increased crop specialisation especially in areas like Kent and Surrey (Wrigley 1967). Improved agricultural practices, trade links, and the establishment of the canals in 1755 enabled the distribution of food in the city from different parts of the country. Furthermore, the River Thames offered opportunities for the movement of people and goods internationally (Oddy and Burnett 1992; Atkins 2007; Stubenrauch 2016). The development of the new railway system in the 1830s meant that food in London was imported quickly and over long distances. Moreover, the new transportation system provided populations in the city with a variety of foodstuffs in greater volume and fresher than any other cities of equivalent status (O'Brien and Quinault 1993; Tames 2003; Atkins 2007). Compared to other regions, the movement of people and goods to London was dominant throughout this period (Pooley and Turnbull 1988, 77).

Human remains from this region were sampled from four sites, Queen's Chapel of the Savoy; St Barnabas/St Mary Abbots, Kensington; Royal London Hospital; and St Bride's Lower sites. Collagen data for Queen's Chapel of the Savoy and St Barnabas/St Mary Abbots, Chelsea were obtained from Bleasdale et al. (2019). However, the remaining collagen data for Royal London Hospital andSt Bride's Lower sites and all FTIR and carbonate data in this study were generated as part of the laboratory work for this thesis.

4.3.1 Queen's Chapel of the Savoy

The Queen's Chapel of the Savoy cemetery site is located in the City of Westminster London. King Henry VII commissioned the Savoy Hospital to be built in 1502 on the former site of the Savoy Palace once owned by Henry, Duke of Lancaster, which had been destroyed in the 1381 Peasant's Revolt (Somerville 1960). The Savoy hospital, which included the Queen's Chapel and churchyard serving as a burial ground, was first used in 1523 for the poor (Thornbury 1878). Following this, the hospital was used as a military infirmary during the British Civil War (1642 - 1651), a naval hospital for injured seamen during the Dutch Wars (1652-1667) and finally in 1679, part of the hospital was converted to a prison, housing offenders, deserters and prisoners of war (Strype 1720; Firth 1902; Keevil 1957). The hospital was dissolved in 1702 after its possession by the Treasury; by 1816, all buildings except the chapel had been demolished (Somerville 1960). The burials in the churchyard began in 1552 and throughout the hospital's many changes, hospital patients, parishioners, criminals, seamen, and military personnel were buried there until 1854 when it was closed after the Burial Act of 1853 (Somerville 1960; Sibun and Ponce 2018). The graves could not be phased; however, Sibun and Ponce (2018) suggest that the surviving graves are most likely those from later years, but it is difficult to be certain without burial records. Archaeological excavations by Archaeology South East UCLwere performed in 2011 in response to the redevelopment of the chapel. In total 609 burials wererecovered and for those whose sex could be determined, 76% were male and 24% were female (Sibun and Ponce 2018). It has been suggested that the difference in the sex distribution at the site was due to the considerable use of the cemetery for military personnel burials (Sibun and Ponce 2018). Additional information regarding the site is found in Bleasdale et al. (2019).

4.3.2 St Barnabas/St Mary Abbots Kensington

St Barnabas/St Mary Abbots individuals were buried between 1831 and 1853 and had a collective lifespan of 1760 to 1853 (Goldsmith 2016). St Barnabas Church site is located on Addison Road, in the borough of Kensington in London which to this day is a very affluent area. There is very little information known about this church. The church, consecrated in June 1829, was expanded in 1860 and remodelled in 1909 to allow for more seating. The church continued to be remodelled

throughout the 19th century thereby suggesting the congregants were rich enough to afford the additions (Sheppard 1973a; Sheppard 1973b). It is therefore assumed that the individuals recovered in 1991 from a crypt at the site in response to construction work were wealthy members of the society (Goldsmith 2016; Bleasdale et al. 2019). In addition, it should be highlighted that although the individuals analysed here cannot be identified directly, the burial ground includes known named wealthy individuals, further supporting the understanding that the 23 individuals analysed for carbonate isotopes in this thesis were also wealthy. Additional information regarding the site is found in Bleasdale et al. (2019).

4.3.3 Royal London Hospital

The Royal London Hospital is located on Whitechapel Road, about a mile to the east of London city centre. The Hospital, constructed in 1752 by Boulton Mainwaring and opened in 1757, was set up to be run as a charitable institution (Clark-Kennedy 1962). Like all hospitals operating during the 18th and 19th centuries in England, the Royal London Hospital had to make burial provisions for patients who had passed away at the hospital but had no friends or relatives to collect their bodies. Some of the families and friends did not collect their relatives' remains due to the high costs of providing decent burials and since the London Hospital was one of the few hospitals that did not charge a burial fee, many of the hospital patients and their families opted for them to be buried at the hospital (Howard 1791; Fowler and Powers 2012).

Archaeological excavations undertaken by The Museum of London Archaeology (MOLA) in 2006, revealed 636 articulated contexts and 175 contexts of disarticulated bones of unclaimed patients buried between c.1825 and 1841 (Fowler and Powers 2012). The presence of decayed coffins, multiple burials within graves, and other nonstandard burials containing multiple body parts made it difficult to make an exact count of the number of individuals buried there. Furthermore, the absence of a burial register for the Hospital made it difficult to determine if the number of excavated remains was proportionate to the number of the buried population (Fowler and Powers 2012). It has been suggested that some of the disarticulated bones may have been removed as a result of surgical removal or anatomical dissection for medical training. A variety of diets were specific to each patient's circumstances and were divided into two: the common diet and the middle diet (Howard 1791, 131). The common diet consisted of 1 pint of milk pottage or water gruel for breakfast; 8 oz of meat every day for dinner; broth for supper six days a week; and 12 oz of breada day (Howard 1791, 131). The middle diet consisted of 1 pint of panada or water gruel, 4 oz of meat every day for dinner; a pint of broth or panada for supper, and 8 oz of bread (Howard 1791, 131). There were no vegetables includedin both diets (Howard 1791, 131). Some cattle and sheep/goat animal remains, displayingevidence of butchering, were recovered from some of the graves suggesting that they may have been food waste for the hospital patients. However, the

hospital kitchens supplied meat to both hospital staff and patients, and it has been suggested that some of the more expensive meat was most likely to have been served to staff (Millard 1825; Mitchell et al. 2011; Morris 2014). However, as this was a hospital patient burial ground, the eleven people sampled here are not expected to include members of the hospital staff.

4.3.4 St Bride's Lower

The site of St Bride's Lower, believed to have received burials between 1770 and 1849 (Miles and Conheeney 2005), is locatedwithin the parish of St Bride's on 75-82 Farringdon Street in the city of London. The burial cemetery is one of the three burial cemeteries of the parish linked to St Bride's Church on Fleet Street. St Brides Lower cemetery was a consequence of overcrowding of two other burial cemeteries at the same location - the original churchyard and the church crypt (Miles and Conheeney 2005). Excavations at the St Bride's Lower carried out between 1991 and 1992 by theMuseum of London Archaeology Service (MoLAS), revealed stacked human burials in wooden coffins that had since degraded. A total of 606 individuals, 47 in vaults and 497 from the open yard were excavated from the site (Miles and Conheeney 2005). Many parish burial grounds in England charged different rates per burial location and the types of burials were connected to different socio-economic backgrounds which resulted in two very contrasting skeletal assemblages from the parish of St Bride's (Miles and Conheeney 2005). While those buried in crypts were prominent members of the parish, the individuals buried at the Lower Churchyard (sampled as part of this thesis) were poorer members of the parish, servants, prisoners from Fleet prison, lodgers, and inhabitants of Bridewell workhouse. A total of 41 former prisoners were said to be buried at this site (Miles and Conheeney 2005; Kausmally 2008). There is, however, no information available that would indicate which group each of the fifteen individuals analysed for this thesis belong to.

4.4 Animal remains

Except for Cross Street Unitarian Chapel Manchester faunal remains, there were no other contemporaneous animal remains at all the sites from which human remains were sampled, therefore other animal materials utilised in this thesis were sampled from contemporaneous remains recovered from excavations near these areas (Table 4.2). All faunal collagen data from Hungate, Bedern, and Walmgate in York were generated by Sean Doherty and Chloe Brown and those from London were derived from Bleasdale et al. (2019).

4.4.1 Cross Street, Manchester

Excavations at Cross Street Unitarian Chapel uncovered a wide variety of faunal resources, but the animal remains were unstratified and could have originated from sources other than the site itself. In addition, as the city of Manchester entered into the 19th century, in the process increasing its industrial activity (Brockbank 1929), it is possible that industrial services from this time onwards may have highly disturbed the burial ground, so that any animal bone might easily have originated from a later date than the graveyard. As a result, interpretation using these remains will be considered with great caution.

4.4.2 Norton Priory, Chester

Animal samples utilised from the Norton Priory site were part of the remains recovered during excavations at Norton Priory between 1970 and 1987 by the Runcorn Development Corporation, under the direction of Patrick Greene. The excavations focussed on buildings of the Priory of St Mary (1134 - 1536) as well as some of the areas occupied post-dissolution: Period 2-The Suppression of the priory, and the Tudor and later occupation of Norton Hall, between 1536 and c1730; Period 3-the Occupation of the Georgian Mansion between c1730 and 1928 (Wright et al. 2017). However, dating of the faunal assemblage in this context proved difficult because of landscaping and the disturbance surrounding the demolition of the buildings at the site in 1928 as the remains originated from undifferentiated topsoil deposits during excavations such that they could be originating from Period 2. Therefore, considering that the samples are from both periods, the samples used for this thesis date between 1536 and 1928 (Brown and Howard-Davis 2008; Wright et al. 2017).

4.4.3 Otley, West Yorkshire

Animal remains from Otley were recovered during excavations undertaken by CFA Archaeology Ltd on behalf of Gladmans Ltd on land at 12 Bridge Street, Otley, West Yorkshire in 2014 prior to development. Various fragments of 'post-medieval' animal bones and large pieces of butchered animal bone were found in secondary fills belonging to one of the pits at the site (Hunt 2014). The assemblage is, however, yet to be studied in detail to give more accurate dating or how the bones were deposited at the site. There is not much information yet on the activities that occurred at the site. Nevertheless, Hunt (2014) states that the remains are dated to the post medieval period without giving any evidence that supports that assertion. However, without dating, it is difficult to assign an exact date for them .

4.4.4 Oulton, Leeds

Excavations on the Oulton site in Leeds were undertaken by CFA Archaeology Ltd on land to the south of Fleet Lane, Oulton, between 10 October 2014 and 4 February 2015 during a topsoil strip for a housing development. Evidence for activity dating from the 18th century to modern times was scattered across the development. Cartographic evidence and plough scarring reveal that the land was being used for agricultural purposes in recent history, with sporadic disposal of young and neonate lambs as well as calves. There is a structure at the site that appears likely to relate to an old Corn Mill that was on the site during the 18th and 19th centuries (Hunt 2015). Most of the animal bones were recovered from the northern part of the proposed development and Hunt (2015) indicated that they are likely to be from the 18th century to modern times. As is the case with Otley, this assemblage is yet to be studied in great detail (Hunt 2015).

4.4.5 Hungate, York

Excavations on the Hungate site in York were carried out by York Archaeological Trust between 2007 and 2011 as part of the development of a new Hungate neighbourhood. There are currently no publications from the Hungate excavations but brief information from unpublished work by the York Archaeological Trust has been provided for this thesis (Rainsford 2012a; Rainsford 2012b). Rainsford (2012a; b) revealed that the site was a human settlement since the Viking period. Several animal remains possibly dating from the 17th century onwards were recovered at the backyards of Victorian houses at the site (Rainsford 2012b) and the animal tissues studied in this thesis are dated between the 16th and 20th century (Rainsford 2012a).

4.4.6 Bedern, York

The Bedern site excavations were performed by York Archaeological Trust between 1973 and 1981 at a site where there was a former medieval and post-medieval College of the Vicars Choral, a religious house of priest vicars who deputised for the canons at York Minster's daily services. There is evidence of human activity pre- and post-dissolution including its use as a slum that housed Irish Immigrants in the early 19th century. A number of faunal remains were excavated at the site but for this thesis only sheep dated between the 15th and 19th century were analysed (Richards 2001; McComish 2016).

4.4.7 Walmgate, York

The excavations on Walmgate in York were carried out by York Archaeological Trust between July and October 2000 following the Time Team television broadcast at the site in September

1999. This area was a wealthy place in the early medieval period, containing six churches and a large house (O'Connor 1985; Macnab 2000). Walmgate increasingly became industrial from the 14th century through to the 17th centuries (O'Connor 1985; Macnab 2000). Animal bone assemblages were recovered at the site and the sheep remains used for this thesis date between the 18th and 19th centuries (O'Connor 1985; Macnab 2000).

4.4.8 Sand-Le-Mere Caravan Park, Tunstall

The animal remains from this site were recovered from an archaeological excavation during an evaluation undertaken between the 17 May and 24 May 2011 by CFA Archaeology Ltd on behalf of Prospect Archaeology. The evaluation was intended to assess the archaeological potential of the land to the west of an existing caravan park prior to its extension and the construction of associated infrastructure, including roads and lakes. A total of 192 fragments of animal bones were present in the assemblage. The bones were in a range of sizes from large fragments of large cattle bones down to a small fragment of goose bone. The small quantity of identifiable animal bone indicates the exploitation of cattle, sheep, and goats, as well as the consumption of domestic or wild goose. Most of the animal bones appear to derive from disturbed cattle burials which may be of medieval date. However, among the finds were post-medieval pottery from the plough soil suggesting that it is possible the site was in use until then. No dating of the animal remains has been performed yet, but the associated finds suggest the remains to be from the medieval to post-medieval period (Lightfoot 2011).

4.5 Comparative sites used for the calculus case study in Chapter 8

As part of this thesis, bone, enamel, and dental calculus from 20 modern humans from the Forensic Anthropology Center (FAC), University of Tennessee, USA and 22 archaeological individuals Southwell Cemetery, Nottinghamshire, St Peter's Cemetery, Leicester, and Nun's Field, Chester were also sampled to provide a comparative dataset for the calculus case study (Chapter 8). The modern individuals represented populations who consumed an abundance of C_4 sugar and maize while the medieval populations represented no known C_4 component in their diets.

4.5.1 The Modern Population, Forensic Anthropology Centre, USA

Permission was granted to sample and analyse twenty modern individuals who died between 1996 and 2016, from the William M. Bass Donated Skeletal Collection Forensic Anthropology Center (FAC), housed in the Department of Anthropology, University of Tennessee, USA

(Appendix E). All body donations were anonymised to protect the identities of the donors. All modern human tissue stored at the University of York must comply with the Human Tissue Act (2004) as enforced by the Human Tissue Authority (HTA), and therefore approval was sought and granted from the National Research Ethics Service (NRES) Committee Yorkshire & The Humber – Leeds East Research Ethics Committee (REC) to work with and store modern human tissues at the University of York (Appendix E). It was accepted subject to working within the guidelines and SOPs of the York Tissue Bank under HTA licence. At FAC, all the humans donate their bodies for research, and the conditions on which the modern individuals are kept after death ensures that no diagenetic alteration by environmental elements takes place. When an individual dies and donates their body to the Forensic Anthropology Center, they are placed outside at the facility to decompose naturally on the ground surface with a loose plastic covering over them to protect their anonymity. Once they are skeletonised, the remains are recovered and cleaned of any remnant soft tissue with tap water and a toothbrush. The time between recovery and processing may be a few weeks, a year, or even longer for the individuals who died before 2012. However, since 2012, the time between recovery and curation is weeks to months. Once cleaned, the bones are air-dried, individually labelled, and then placed in an acid-free cardboard box, where they are securely stored in the William M. Bass Donated Skeletal Collection in the Department of Anthropology at the University of Tennessee.

4.5.2 The Medieval Populations

Two of the medieval populations, Southwell and St Peter's Cemetery, were studied as part of a PhD thesis analysing dental calculus microdebris (Radini 2016). Southwell Cemetery was dug by a team of archaeologists from Pre-Construct Archaeological Services Ltd in preparation for the redevelopment of existing structures in 2012 (Keal 2012). Forty-two skeletons were recovered and the dating of this assemblage, 6th to 9th centuries, was established through radiocarbon dating results from two skeletons interpreted as belonging to the same cemetery but excavated in previous excavations in 2009 and 2011 (Keal 2012). Excavations from St Peter's Cemetery were undertaken by the University of Leicester Archaeological Services (ULAS) between 2003 and 2009 prior to developments in Leicester. In total, about 1,553 skeletons dating between the 10th and 16th centuries were uncovered from St Peter's and St Michael's Cemeteries, however, only individuals from St Peter's are included in this thesis (Morris et al. 2011). Excavations of the Nun's Field individuals by Earthworks Archaeology took place in 2007 prior to the construction of a large council building on the southwestern edge of the historic town of Chester, between Nun's Road and Grosvenor Road. One hundred articulated skeletons and 1,411 fragments of disarticulated human bone dating between the 12th and 16th centuries were recovered at the site, relating to a nunnery (Holst 2016).

4.6 Summary

All post-medieval human samples described above are accompanied by osteoarchaeological data such as age, sex, and pathology, allowing these factors to be considered in the isotopic analyses where relevant. This dataset allows for social status, regional, gender, race and age-based trends to be explored as part of the study. While there are a number of sites with fewer than ten samples, most of the sites have sizeable numbers that allow exploration of intrapopulation variations. Finally, the geographical spread of areas from which the animal sample were derived will help provide a valuable picture of possible variation in their diets during the post-medieval period. With the study sites identified, the methods by which the bones were analysed for diagenesis and stable isotope analysis will be described in the following chapter.

Chapter 5: Methods

This chapter will detail the human and faunal material that will form the basis for palaeodietary reconstruction in this thesis. As previously discussed, three chapters (Chapters 6, 7, and 8) of this thesis are written in paper publication format. This chapter outlines the common analytical methods (collagen, FTIR-ATR, and carbonate analysis) used in these papers in detail. Descriptions of the carbonate isotopic models used to analyse bone carbonate results are also briefly covered in this chapter. The details of the procedures for enamel carbonate and dental calculus carbonate analysis that are only relevant for that work will be covered in Chapter 8, therefore they are not included in this chapter. Additionally, the statistical analysis relevant to each paper will be discussed in the respective chapters. A full list of all the animal and human individuals studied in this thesis, providing skeletal numbers, location, age, sex, and bone elements used can be found in Appendices A and B respectively.

5.1 Human sample selection

The bone samples utilised in this thesis derived from eleven skeletal populations, representing one rural and ten urban populations from different socioeconomic statuses from the post-medieval period in England. Of these, seven were from the manufacturing northern towns and four from London (see Chapter 4). Bone samples from 187 individuals, reflecting a range of ages, and both sexes were selected for bone collagen analysis and 216 for FTIR-ATR and carbonate analysis in this thesis (Table 5.1). Fourteen children were included in the bone carbonate analysis to have a general view of any C_4 signals in their diet, however, the number was limited here because of possible complications of breastfeeding/weaning signals. The age and sex of the remains were assessed osteoarchaeologically by other researchers (see Chapter 4). Ribs were mostly used but where it was not possible to sample them, any bone element available for analysis was utilised.

5.2 Faunal sample selection

The faunal remains were recovered from nine sites mostly during development or redevelopment excavations in the North of England (see Chapter 4). For London human-faunal offsets, animal data from Bleasdale et al. (2019) was utilised. The bone samples selected for all analysis include cattle, pigs, sheep, and domestic fowl. All faunal remains were assigned to specific species by employing morphological criteria with the help of zooarchaeologists in the Department of Archaeology at the University of York (Appendix B). Sheep and goat samples were distinguished on the basis of ZooMS (Zooarchaeology Mass Spectrometry) following the biomolecular methods

used in Buckley et al. (2009) and Buckley et al. (2010) by Krista McGrath at the University of York. A total of 133 animals were selected for bone collagen analysis and 27 for bone carbonate and FTIR-ATR analysis in this thesis. Collagen data for 35 animal remains were provided by Sean Doherty (Table 5.2; See also Chapter 4).

Table 5-1: Human remains sampled for bone collagen, bone carbonate, and FTIR analysis from all sites, with associated dates.

Location	*Site*	*Date*	*Collagen analysis*	*Carbonate and FTIR analysis*	*Previous Collagen data*
Manchester	Cross Street	18th - 19th c	92	54	0
Manchester	Hazel Grove	1794-1910	34	31	0
Rotherham	Rotherham Minster	1780s-1854	21	21	0
Halifax	Square Chapel	1772-1857	5	32	27
Harrogate	Fewston	18th-19thc.	0	7	7
Leeds	St George Crypt	1840-1911	9	9	0
Leeds	Victoria Gate	1796-1850s	0	3	3
London	St Brides Lower	1770-1849	15	15	0
London	Royal London Hospital	1825-1841	11	11	0
London	Queen's Chapel of the Savoy	16th - 19th c.	0	10	10
London	St Barnabas/St Mary Abbots, Kensington	1760-1853	0	23	23
		Total sampled	187	216	70

Table 5-2: Faunal remains sampled for bone collagen, bone carbonate, and FTIR analysis from all sites, with associated dates.

Location	*Site*	*Date*	*Collagen analysis*	*Previous Collagen data*	*Carbonate and FTIR analysis*
Manchester	Cross Street	18th - 19th c	37		12
Runcorn, Cheshire	Norton Priory	16th - 20th c	74		6
Halifax	Square Chapel	18th - 19th c	7		6
Leeds	Otley	Unknown	8		3
Leeds	Oulton	Unknown	4		0
Tunstall	Sand-le-Mere	16th - 19th c	3		0
York	Hungate	16th - 19th c		21	0
York	The Bedern	15th - 19th c		11	0
York	Walmgate	18th - 19th c		3	0
		Total sampled	133	35	27

5.3 Collagen analysis

Collagen extraction of bone samples was performed according to the standard operating procedure for BioArCh at the University of York which is based on the Longin (1971) method modified by Brown et al. (1988).

5.3.1 Cleaning

Between 300 and 400mg of each bone sample was cleaned using a sterile blade to remove contaminants of the outer layer of bone. Once cleaned, bone samples were broken into thin shards that ran parallel to the 'grain' of bone. In cases where it was impossible to break the bone by hand, a bone crusher was used, cleaning it with ethanol between samples to avoid cross-contamination. The samples were then weighed and transferred into screw-top glass culture tubes, pre-labelled with name, sample ID, and date. For each run, about 100 mg of crushed modern homogenised bovine bone was also weighed. The bovine sample served as a quality control for the sample processing exercise as its isotopic composition had been previously determined (Budzikiewicz and Grigsby 2006).

5.3.2 Demineralisation

In order to remove minerals or mineral salts, the samples were demineralised by immersing them in a weak acid solution (8ml 0.6M Hydrochloric acid (HCl)). They were then covered loosely with foil without the lids and placed in a fridge (at ~4°C) to allow carbon dioxide from any effervescence caused by the treatment to escape. After an hour, the samples were withdrawn from the fridge and the foil was removed. The caps were then replaced and secured using parafilm. The tubes were then placed on the roller rocker in a cold room (+4°C). The cooling of the demineralisation reaction minimises hydrolytic damage to the collagen protein within the bone (Collins and Galley 1998). Demineralisation was considered to be complete when the samples became spongy and rubbery when prodded with a glass Pasteur pipette and for some, floated to the top of the tube and there was no more effervescence of the acid. The period of demineralisation varied between samples with some lasting for around two days and others more than a week. For the samples that did not demineralise within two days, the hydrochloric acid was replenished to ensure the effectiveness of the acid to demineralise and maintain the pH level. When demineralisation was complete, the spent acid was decanted into a waste beaker and each sample was rinsed three times with distilled water. For the bovine control sample, which was in powder form, centrifugation was performed in between washes to minimise the loss of sample.

5.3.3 Gelatinisation

Samples were then gelatinised - breaking down molecular bonds between collagen strands by hydrolysis making them soluble and therefore allowing the removal of any insoluble particles. Initially, the samples were immersed in 8 ml of pH3 HCL, and the tubes sealed with caps to prevent evaporation. They were then put on a heat block for 48 hours at 80°C and tinfoil was placed around the tubes to insulate them. Gelatinisation was considered complete when all the collagen had dissolved.

5.3.4 Ezee™ Filtering and Ultrafiltration

Two falcon tubes per sample, one for the filtrate and the other for the retentate, were weighed and labelled and placed aside. Additionally, ultrafilters (one per sample) were pre-cleaned by filling them with 0.1M NaOH and centrifuging for 8 minutes at 4000 x g to remove possible contaminants. The NaOH was then discarded from the ultrafilter into a beaker. The process was repeated three more times with ultrapure water, discarding the filtrate after every clean. Firstly, each sample was filtered using Ezee™ filters to remove large unwanted insoluble particulate matter prior to ultrafiltration. The supernatants (filtered gelatinised samples) were then transferred into labelled clean ultrafilters using a glass Pasteur pipette. Ultrafiltration was applied to remove contaminants of low molecular weight (<30 kDa) such as humic acid, degraded collagen fragments, and salts from the sample and isolate high molecular weight components of the gelatinised collagen (>30 kDa) using Amicon® Ultra centrifugal filters, 30 kDa (Brown et al. 1988; Brock et al. 2013). Only a portion of each sample was transferred into the ultrafilters initially. Samples were centrifuged for an initial two minutes, in order to assess the rate at whichdifferent samples passed through the filter. This then determined the period each sample was tobe left in the centrifuge. The liquid collected at the bottom of the tube (filtrate) was then removedusing a clean pipette and transferred into the clean pre-weighed and pre-labelled filtrate falcon tube. The remainder of the sample was then transferred into the ultrafilter and the whole process was repeated until the liquid remaining above the filter (retentate) was above the 500 µL line. The remaining liquid (retentate) was then transferred by a pipette into clean pre-weighed and pre-labelled retentate falcon tube. Ultrapure water was poured into the filter to just about the 500 µL level and pipetted into the retentate sample to ensure the collection of as much of the collagen as possible. The tubes were then covered with parafilm and a couple of holes were punched on the parafilm with a pair of pointed tweezers to allow gas to escape during the freeze-drying process.

5.3.5 Freeze-drying (lyophilisation)

This stage aims to remove the organic solvents and water from the sample by sublimation (i.e., solid turns into a gas without going through a liquid stage). The tubes with the samples were frozen for 24 hours at -20°C before being lyophilised in a freeze dryer for 48 hours leaving behind a solid, candy floss-like, white, or tan coloured collagen in the tubes. The tubes containing the collagen were reweighed and the original weight of the empty tube was subtracted to obtain the weight of the collagen. The collagen yield (wt.%) was then calculated as follows:

*Weight of collagen/original sampled bone weight *100*

The collagen yield was indicative of the quality of collagen (see Section 5.3.8).

5.3.6 Elemental Analyser-Isotope Ratio Mass Spectrometer

Between 0.4 and 0.6mg of the freeze-dried retentate were weighed out in duplicate into 4x3.2mm tin capsules and combusted alongside international standards in an Elemental Analyser Isotope Ratio Mass Spectrometer (EA-IRMS): a Sercon 20-22 mass spectrometer coupled with a Sercon GSL Sample Preparation System module at BioArch, University of York (Figure 5.1).

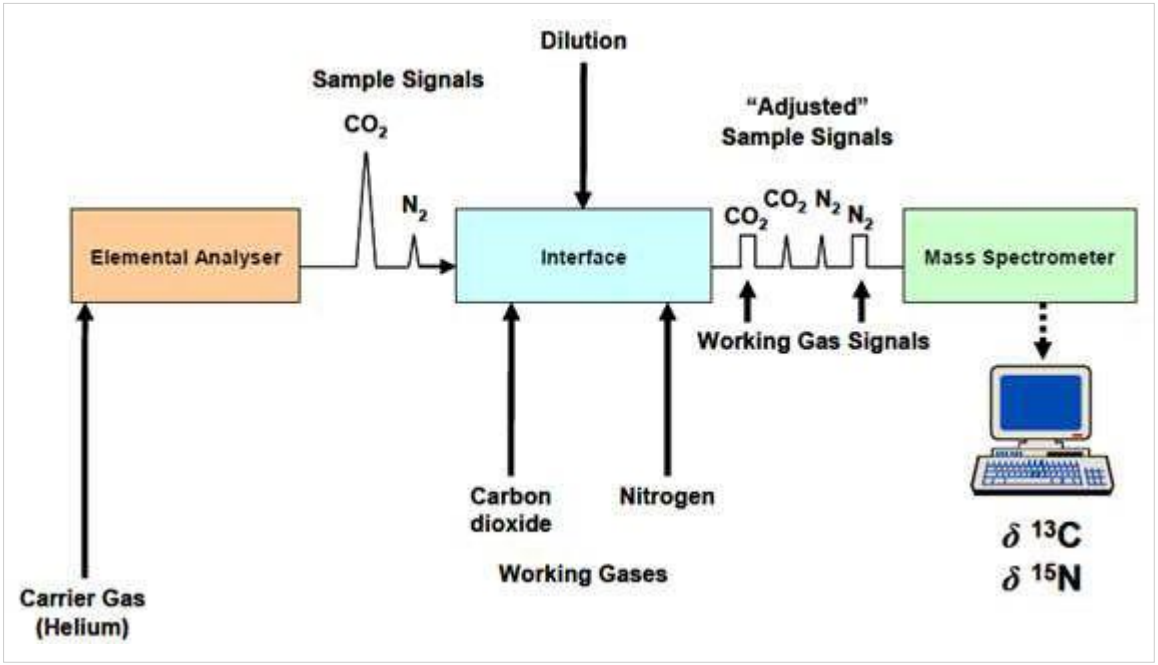


Figure 5-1: Simple schematic diagram of an ER-IRMS for the determination of $\delta^{13}C$ and $\delta^{15}N$ (Carter and Barwick 2011).

During this stage of analysis, the collagen samples within the tin foil are completely combusted in an oxygen-rich atmosphere in a quartz reactor maintained at 1000°C in the elemental analyser to produce N_2, CO_2, NO_x, and H_2O. The resultant gases are transported in a helium stream through the reactor containing CuO and Cr_2O_3 oxidation catalysts and silver wool to bind unwanted sulphur and halogen products. The gaseous products are then swept in the reduction reactor with high purity copper where excess oxygen is removed, and NO_x is reduced to N_2 at 600°C. Water is then removed via a water trap containing magnesium perchlorate ($Mg(ClO_4)_2$). Nitrogen and carbon dioxide are separated by their molecular weight via a column packed with a stationary phase held at 70°C. Finally, they are then delivered sequentially to the mass spectrometer, where they undergo ionisation (Carter and Barwick 2011, 4).

5.3.7 Standards and analytical error

Precision was controlled by duplicate analysis of samples and the use of the following standards: IAEA 600 (Caffeine), IAEA N-2 (Ammonium sulphate), IA CANE (Cane sugar), Fish Gelatin, and blank tin capsules. Accuracy for collagen analysis was determined by measurements of international standard reference materials within each analytical run. These were IAEA 600 $\delta^{13}C_{raw}$ = -27.65 ±0.09 ‰, $\delta^{13}C_{true}$ = -27.77 ±0.043 ‰, $\delta^{15}N_{raw}$ = 0.92 ±0.21 ‰, $\delta^{15}N_{true}$ = 1 ±0.2 ‰; IAEA N2 $\delta^{15}N_{raw}$ = 20.35 ±0.13 ‰, $\delta^{15}N_{true}$ = 20.3 ±0.2 ‰; IA Cane, $\delta^{13}C_{raw}$ = -11.77 ±0.09 ‰; $\delta^{13}C_{true}$ = -11.64 ±0.03 ‰. The overall uncertainties on the measurements of each sample were calculated based on the method of Kragten (1994) by combining uncertainties in the values of the international reference materials and those determined from repeated measurements of samples and reference materials. These are expressed as one standard deviation. The maximum uncertainty for all samples across all runs was <0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. In addition, a homogenised bovine bone extracted and analysed within the same batch as the samples produced the following average values; $\delta^{13}C$ = -23.01 ±0.13; $\delta^{15}N$ = 6.21 ±0.44. This was comparable to the overall mean value from 50 separate extracts of this bone sample, which produced values of $\delta^{13}C$= -22.97 ±0.19 and $\delta^{15}N$ = 6.19±0.30.

5.3.8 Criteria to assess collagen preservation

There are well-established quality control indicators for isotopic measurements of collagen which enable detection of post burial degradation in ancient materials (DeNiro 1985; Ambrose 1990). These criteria were determined based on observations of isotopic compositions of ancient and modern materials which revealed that there was only a small variation in the concentrations and ratios of carbon and nitrogen from collagen obtained from different taxa and tissues (Neuman

1949; van Klinken 1999; Szpak 2011). The established criteria used include the percentage collagen yield, elementary mass percentages of carbon (%C) and nitrogen (%N) as well as the atomic ratio of percent carbon to nitrogen (C:N).

The percentage of collagen yield (the percentage by mass of collagen produced from the original sample) in modern unaltered bone is about 22%. It has been recommended that samples with a collagen yield of less than 5% should be verified by other quality controls and those with a yield of 1% should be excluded (Ambrose 1990; van Klinken 1999). However, it should be noted that the use of ultrafilters can influence collagen yield (see section 5.3.4). The accepted ranges for atomic C/N ratio are 2.9-3.6 (DeNiro 1985) or 3.1-3.5 (van Klinken 1999) and the carbon and nitrogen concentrations are ca. 5.5-17.3%, and ca. 15.3-47% by weight respectively (Ambrose 1990). Lower percentages of carbon and nitrogen can indicate the presence of inorganic contamination while higher ones can be due to non-collagenous organic substances (van Klinken 1999). The choice of atomic C/N ratio in palaeodietary studies is at the discretion of the researcher, and in this thesis, DeNiro (1985)'s range was utilised. All the results for assessing collagen preservation are in the results of Chapters 6, 7, and 8.

5.4 Bone carbonate preservation

Reconstruction of palaeodiet through isotope analysis relies on the analysis of well-preserved biogenic tissues. However, the isotopic and chemical compositions of archaeological tissues are often diagenetically modified after burial. As a result, it is essential to assess the presence and extent of these alterations prior to subjecting archaeological tissues to isotope analysis. In this study, Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) was employed, prior to bone carbonate isotope analysis, to assess diagenesis in bone samples to ensure that only those samples that are preserved enough to yield results that are close to pristine isotope compositions are utilised. For this thesis, the use of FTIR-ATR to assess bone preservation is particularly relevant because it is fast, inexpensive, requires a small amount of sample therefore minimal sample destruction, and is straightforward to carry out (Dal Sasso et al. 2018). Although FTIR-ATR does not provide the level of quality assurance provided by the quality indicators in collagen analysis, it detects aberrant materials, such as calcite, adsorbed from the burial environment (Webb et al. 2014). Moreover, it can also provide information on the organic and inorganic structures present in a given sample (Figueiredo et al. 2012) and has been widely applied (Thompson et al. 2011; Hollund et al. 2013; Snoeck and Pellegrini 2015; Lebon et al. 2016; Kontopoulos et al. 2019a; Kontopoulos et al. 2020).

5.4.1 FTIR-ATR Spectroscopy

FTIR is based upon the absorption of infrared radiation during vibrational transitions in covalently bound atoms (Figueiredo et al. 2012, 315). When an infrared beam is brought in contact with the sample, the beam penetrates a very short distance (a few micrometres) into the sample as a result of the total internal reflection that occurs at the interface between the ATR prism which is a high-density medium, and the sample, a low-density medium (Hollund et al. 2013, 508). Absorption at a given wavelength/wavenumber is then obtained as a result of a correlation between the incident radiation and the frequency of vibration of a particular bond. These absorption peaks, obtained at agiven combination of characteristic wavenumbers, allow a known identification of the functional group characteristics of a sample. Absorbance characteristics of a sample at wide-ranging infrared wavelengths can be measured simultaneously and rapidly within seconds, rather than making a series of measurements of different monochromatic wavelengths -radiations of only one wavelength (Wright and Schwarcz 1996; Fredericks 2012; Pestle et al. 2014). Consequently,it generates characteristic bands that are typical for specific chemical bonds which can be used as a fingerprint to identify the material type, for example, bands of the protein infrared spectrum - Amide I and Amide II bands (Gonzalez and Wess 2008). FTIR-ATR can therefore be used to characterise the bone matrix because the main bone components (carbonated hydroxyapatite and collagen) absorb infrared radiation at distinct, almost complementary regions within the 400-4000 cm^{-1} range (Figueiredo et al. 2012).

5.4.2 FTIR-ATR analysis

Sample preparation and analysis were executed according to the method of Kontopoulos et al. (2018). Prior to chemical pretreatment, the bones were cleaned using a sterile scalpel blade to remove dirt and contaminating material. Once cleaned the samples were ground using an agate mortar and pestle. The powdered samples were then sieved through Endecotts woven stainless steel mesh sieves with an aperture size of 20μm and 50μm so that only grains between 20μm and 50μm particle size would be used. Spectral analyses were performed using OPUS software (Bruker). Spectra were collected in 144 scans, in the 4000–400 cm^{-1} wavenumber range, with a spectral resolution of 4cm^{-1} and zero-filling factor of 4. Each sample was measured in triplicate. The instrument's crystal and arm's tip were cleaned with tissue paper soaked in propanol before each measurement. Baseline correction and spectra normalisation were carried out using the OPUS software. For each sample, the background reading was measured, and then ~ 2-3 mg of

bone powder was pressed onto the diamond crystal and measured. Pressure was applied using a pressure control spot on the applicator to ensure good contact between the diamond crystal and the sample. Integration was performed on each of the spectra by defining the left and right edges for the peaks, troughs, and curves for baseline correction. Two common diagenetic parameters namely (i) the infrared splitting factor (Weiner and Bar-Yosef 1990) and (ii) the carbonate/phosphate ratio (Wright and Schwarcz 1996) as well as the presence of the calcite ($CaCO_3$) band at 712 cm^{-1}.peak (Hunt et al. 1950; Baxter et al. 1966) were utilised in assessing diagenesis.

5.4.2.1 Infrared Splitting Factor (IRSF)

The infrared splitting factor (IRSF) evaluates the crystallinity (structural order) within the mineral component of the bone (Weiner and Bar-Yosef 1990; Wright and Schwarcz 1996). In bone carbonate analysis, the splitting factor is calculated as the sum of the two infrared peak intensities of the $v_4PO_4^{3-}$ band at 565cm^{-1} and 605cm^{-1} divided by the intensity of the valley between them at 590cm^{-1} following Weiner and Bar-Yosef's (1990)- equation.

$$IRSF = [565_{ht} + 605_{ht}]/590_{ht} \quad \text{(Equation 1)}$$

High IRSF values point to a regularly organised lattice and large crystal size, whereas low IRSF values indicate small crystal size and irregular lattice structure (Surovell and Stiner 2001). In fresh bone, the crystal structure is poorly ordered, contains small crystals, and as a result has a greater strain resulting in a low splitting factor value. The average IRSF value in modern unaltered bone was found to be 3.357 ± 0.007 (Kontopoulos et al. 2019a). A bone that has been altered either due to age-related diagenesis or heat-induced transformation has an increasingly ordered crystal structure with larger crystals and less strain, which, therefore, results in a measurably higher IRSF value (Thompson et al. 2011, 168). The values increase with an increase in crystallinity such that archaeological bone samples exhibit higher IRSF values ranging from 3.5 - 4.8. Previous studies have indicated that IRSF values higher than 4.3 suggests extensive recrystallisation and therefore poor bioapatite preservation (Wright and Schwarcz 1996; Beasley et al. 2014, 19; Lachowicz et al. 2017, 57). However, the values in this thesis are not comparable to these as a different FTIR procedure was followed. The Kontopoulos et al. (2020) study, which used the same method as applied for this thesis, indicated that archaeological samples with IRSF values greater than 4.2 did not preserve DNA, therefore as a precaution, all samples with IRSF values higher than 4.2 (n=6) were excluded from further bone carbonate isotope analysis on the assumption that they may also have poor bioapatite preservation.

5.4.2.2 Carbonate to Phosphate ratio (C/P)

The carbonate to phosphate ratio (C/P) is a measure of diagenesis that reflects the changes to the carbonate in bioapatite crystals relative to the phosphate content ratio in a bone sample. The C/P ratio also allows for a close estimation of the total carbonate content in the bioapatite (Iliopoulos et al. 2010; Snoeck and Pellegrini 2015). The C/P ratio is calculated by dividing the intensity of the $v_3CO_3^{2-}$ band at 1415 cm^{-1} by the intensity of the $v_3PO_4^{3-}$ band at 1035 cm^{-1} following Wright and Schwarcz's (1996) equation

$$C/P = 1415_{ht}/1035_{ht}. \quad \text{(Equation 2)}$$

An increase in IRSF is generally coupled with a decrease in C/P ratio reflecting carbonate losses (Sillen 1989). The average C/P value has been reported to be 0.24 ± 0.003 for modern bone (Kontopoulos et al. 2019a). Archaeological samples exhibiting low C/P are assumed to have lost carbonate to the burial environment whereas those with higher C/P values suggest the introduction of secondary carbonate from the burial environment.

5.4.2.3 Calcite ($CaCO_3$)

High diagenetic carbonate content in bone samples is also associated with the significant presence of the calcite ($CaCO_3$) peak at 712cm^{-1} in the samples (Hunt et al. 1950; Baxter et al. 1966). During the dissolution and recrystallisation processes calcite may be incorporated into the crystal lattice of a bone (LeGeros 1981; Lee-Thorp et al. 1989). Hassan and Ortner (1977) revealed that the presenceof calcite shows a likely relationship between organic preservation and mineral in samples, suggesting that calcite contamination occurs when collagen has been destroyed. In light of this, for this thesis the calcite peak was checked before and after treatment. In cases where the peak was removed by chemical pretreatment, the samples were included in the analysis.

5.5 Carbonate analysis

Carbonate extraction of bone samples followed the standard operating procedure used at the University of York adapted from Snoeck and Pellegrini (2015) and Pellegrini and Snoeck (2016).

5.5.1 Cleaning

Firstly, the bones were cleaned using a sterile blade to remove dirt and contaminating material. The cleaned bones were crushed using an agate mortar and pestle that had been cleaned with

distilled water. Approximately 7.5 mg of bone powder was required for each sample. The agate mortar and pestle were also cleaned with ethanol between samples to prevent cross-contamination. The bone powder for each sample as well as the bovine control samples were transferred into their respective weighed fresh 15ml centrifuge tubes pre-labelled with name, sample ID, and date.

5.5.2 Secondary carbonate sample pre-treatment

Exogenous carbonate can contaminate part of the mineral phase (see Chapter 2), therefore, in order to ensure that pristine or acceptably near to pristine isotope compositions of bioapatite carbonate are obtained, the exogenous carbonate should be removed. Separation of the collagen and the mineral has also been said to be essential because stable isotopes are differentially fractionated in the organic and inorganic fractions of skeletal tissues (Snoeck and Pellegrini 2015). To ensure that data obtained from bone mineral stable isotope analyses are not from the diagenetic carbonate, a pre-treatment protocol involving removal of non-biogenic carbonates without significantly destroying the bone carbonate content must be used. In addition to this, it is important to follow suggested methods to ensure the preservation of the mineral as the accuracy of bone carbonate data is strongly dependent on the degree of alteration of the materialbeing measured (Snoeck and Pellegrini 2015; Pellegrini and Snoeck 2016). Below, a brief background of the chemical methods used in previous studies is provided first, then a description of the method followed in this thesis is given.

There are two oxidising agents that are commonly used to remove organic matter - sodium hypochlorite (NaOCl), commonly known as bleach, and hydrogen peroxide (H_2O_2), used over a range of concentrations: 1 - 5% for NaClO and 10 - 30% for H_2O_2 (Garvie-Lok et al. 2004; Metcalfe et al. 2009; Grimes and Pellegrini 2013; Snoeck and Pellegrini 2015; Pellegrini and Snoeck 2016). Other oxidizing agents such as sodium chlorite ($NaClO_2$) and hydrazine hydrate ($N_2H_4{\cdot}H_2O$) have also been sparingly used. Sodium chlorite was used in the pre-treatment of calcined bone for radiocarbon dating (Brock et al. 2010) and hydrazine hydrate in organic removal before isotopic measurements of bioapatite carbonates (Termine et al. 1973; Nielsen-Marsh and Hedges 1999). Despite considerable literature on this subject, there is still no consensus on the best way to remove the organic matter in bone carbonate isotope studies. In the last few years, a study was conducted to investigate inter-laboratory variability generated by differences in sample preparation (Pestle et al. 2014). The authors determined that the use of hydrogen peroxide produced significantly enriched apatite $\delta^{13}C$ values relative to the use of sodium hypochlorite. Recently, several organic matter removal methods have been reviewed by Snoeck and Pellegrini (2015) and Pellegrini and Snoeck (2016). Snoeck and Pellegrini (2015) utilised hydrogen

peroxide, sodium hypochlorite, hydrazine hydrate solution, and sodium chlorite to pre-treat their samples. They concluded that the use of sodium hypochlorite induces the adsorption of exogenous carbonates onto bioapatite. However, the authors indicated that despite its problems, sodium hypochlorite remained the most efficient at removing organic matter (Snoeck and Pellegrini 2015, 401). On the other hand, sodium chlorite is partially efficient in removing organic matter while hydrogen peroxide does not remove organic matter even at high temperatures. In contrast, hydrazine hydrate solution seemed to have the least chemical effects on the bioapatite carbonates and almost all the organic matter from bone and dentine samples, and all from enamel samples were removed. The authors concluded that both hydrazine hydrate and sodium hypochlorite could be used to remove organic matter if subsequent treatment with acetic acid (CH_3COOH) is undertaken (Snoeck and Pellegrini 2015, 401). However, in their continuation of the study, Pellegrini and Snoeck (2016) concluded that all the agents, including hydrazine hydrate and sodium hypochlorite adversely affected the isotope composition of bioapatite samples. Due to the uncertainties brought about by the use of these pre-treatments on $\delta^{18}O$ and $\delta^{13}C$ values of bone carbonate, the authors proposed that samples should not be subjected to any organic matter removal pre-treatment procedures.

The acid normally used to remove exogenous carbonates from bone is acetic acid. Application of dilute acetic acid to remove the diagenetic carbonate was pioneered by Sullivan and Krueger (1981) and has since been applied in several other studies (Koch et al. 1997; Garvie-Lok et al. 2004; Nielsen-Marsh and Hedges 2000b; Pestle et al. 2014; Snoeck and Pellegrini 2015; Pellegrini and Snoeck 2016). Carbonated apatites are known to be more soluble than those with less carbonate (LeGeros 1981; LeGeros and LeGeros 1983) and this property is exploited when preparing bone samples for carbonate analysis. The acetic acid treatment has been shown to remove secondary carbonate minerals and apatite that has been diagenetically enriched in carbonates (Krueger 1991). However, the observations from different chemical pretreatments carried out in previous palaeodietary studies using bone carbonate confirm that there are several issues associated with the acetic acid chemical treatment. Lee-Thorp (1989) and Lee-Thorp and van der Merwe (1991) observed the formation of brushite in modern bones treated with 1M acetic acid which was not present before treatment. Koch et al. (1997) also reported brushite in modern dentine samples despite those samples being treated by a dilute acetic acid (0.1M). However, the authors suggested that brushite formation was associated with prolonged soaking (72 hours) of the dentine samples in acetic acid treatment. Nielsen-Marsh and Hedges (1997) observed changes in crystallinity in modern and archaeological bones soaked in a variety of chemical treatments for long periods of time. The authors suggested that the crystallinity changes were a consequence of sample recrystallisation in pretreatments, therefore treatments that

minimised crystallinity should be selected. In addition, several studies have demonstrated the presence of recrystallised apatite that cannot be removed by the acid treatment (Wright and Schwarcz 1996; Nielsen-Marsh and Hedges 2000a). Several authors have also observed that unbuffered 1 M and 0.1M acetic acid solutions induced different isotopic shifts in samples (Koch et al. 1997; Nielsen-Marsh and Hedges 2000b; Garvie-Lok et al. 2004). However, Pestle et al. (2014) concluded that concentration of acetic acid and the use of buffered or unbuffered acetic acid to remove diagenetic carbonate did not have a significant effect on apatite $\delta^{13}C$ values (Pestle et al. 2014, 11) contrary to previous studies (Koch et al. 1997; Nielsen-Marsh and Hedges 2000b;Garvie-Lok et al. 2004). Pellegrini and Snoeck (2016) observed that the use of 0.1M acetic acid and 1M buffered calcium acetate/acetic acid ($(CH_3COO)_2Ca/CH_3COOH$) treatments affected the $\delta^{18}O$ and $\delta^{13}C$ values of apatite-carbonates in a similar way but the buffered solution resulted in dissolution occurring in a more moderate extent. The unbuffered 0.1 M acetic acid resulted in damage on the bone inorganic fraction and considerable mass loss in some of the modern bone samples -over 65% of mass loss on average after treatment with 0.1 M unbuffered acetic acid vs.an average mass loss of 29% after using buffered 1 M acetic acid solution (Snoeck and Pellegrini 2015, 397). Therefore, the authors proposed that in order to preserve the original isotopecomposition of bioapatite, 1 M buffered calcium acetate/acetic acid solution was appropriate forsecondary carbonate sample pre-treatment, and consequently, for this thesis 1M acetate bufferedsolution was used without any prior organic removal cleaning procedure.

In order to remove secondary minerals from the samples, the weighed samples were dissolved in 15 ml of calcium acetate ($(CH_3COO)_2Ca$) buffered 1 M solution and placed on a roller rocker for 30mins. The calcium acetate ($(CH_3COO)_2Ca$) buffered 1 M solution was prepared by mixing two solutions - the first was prepared by adding 24 ml of acetic acid (CH_3COOH) 100% into 500 ml deionised water and the second by adding 39.54 g of $(CH_3COO)_2Ca$ into a separate 500 ml deionised water. A pH of 4.7 was recommended (*Pellegrini in email correspondence*). In Pellegrini and Snoeck (2015; 2016), about 30 ml of acetic acid was used to make up their buffer, however, the pH of 4.7 could not be achieved using that amount of acetic acid. Only 24ml resulted in the recommended 4.7 pH. It is possible that there were differences in the alkalinity of the water used between the University of York laboratory (where this study was carried out) and the laboratory where the Pellegrini and Snoeck (2015; 2016) study was undertaken. After treatment, the samples were rinsed six times with deionised water, centrifuging between washes to separate the powder and water before pipetting off the water out to avoid loss of powder. The samples were placed in the freezer for 24 hours and freeze-dried for 24 hours to remove all the water and isolate apatite. The 15ml centrifuge tubes containing the treated samples were reweighed and the mass loss generated by the treatment was measured by subtracting the original weight of the tube. The

weighed samples were submitted for analysis to the Iso-Analytical laboratory at Crewe Cheshire.

5.5.3 Continuous Flow-Isotope Ratio Mass Spectrometry

Samples were weighed into clean Exetainer™ tubes and then flushed with 99.995% helium (He). After flushing, phosphoric acid was added to the samples and they were left to react in the acid overnight to allow complete conversion of carbonate to carbon dioxide (CO_2). Reference and control materials were prepared the same way. The CO_2 gas liberated from samples was then analysed by Continuous Flow-Isotope Ratio Mass Spectrometry (CF-IRMS). Carbon dioxide was sampled from the Exetainer™ tubes into a continuously flowing He stream using a double holed needle. The CO_2 was resolved on a packed column gas chromatography and the resultant chromatographic peak carried forward into the ion source of a Europa Scientific 20-20 IRMS where it was ionised and accelerated. Gas species of different mass were separated in a magnetic field then simultaneously measured using a Faraday cup collector array to measure the isotopomers of CO_2 at m/z 44, 45, and 46.

The phosphoric acid used for digestion had been prepared for isotopic analysis in accordance with Coplen et al. (1983) and was injected through the septum into the vials. Acid preparations of samples and controls were measured directly against acid preparations of Iso-Analytical working calcium carbonate standard. This procedure removes the need to apply separate corrections for temperature-dependent isotope fractionation.

5.5.4 Standards and analytical error

The reference material used during carbonate analysis was IA-R022 (Iso-Analytical working standard calcium carbonate, $\delta^{13}C_{V\text{-}PDB}$ = -28.63 ‰, and $\delta^{18}O_{V\text{-}PDB}$ = -22.69 ‰). IA-R022, NBS-18 (carbonatite, $\delta^{13}C_{V\text{-}PDB}$ = -5.01 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -23.20 ‰), IA-R066 (chalk, $\delta^{13}C_{V\text{-}PDB}$ = +2.33 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -1.52 ‰) and ILC-1 (limestone) were run as quality control check samples during analysis of the samples. IA-R022 has been calibrated against and is traceable to NBS-18 and NBS-19 (limestone, $\delta^{13}C_{V\text{-}PDB}$ = +1.95 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -2.2 ‰). IA-R066 has been calibrated against and is traceable to NBS-18 and IAEA-CO-1 (carrara marble, $\delta^{13}C_{V\text{-}PDB}$ = +2.5 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -2.4 ‰). ILC-1 has been analysed multiple times in the Isoanalytical laboratory over a number of years. The current long term mean value for those analyses is $\delta^{13}C$ = 2.17‰. NBS-18, NBS-19, and IAEA-CO-1 are inter-laboratory comparison standard materials distributed by the International Atomic Energy Agency (IAEA). The equivalent calcium carbonate content values (%) were derived by comparing the total ion beam data for the samples against the pure calcium carbonate references. The results obtained for the NBS18 and IA-R066 controls were used to check and

correct the data as required. The analytical precision, calculated from repeated analysis of internal and international standards, was better than 0.2‰ (1σ) for $\delta^{13}C$.

It should be highlighted that the mass spectrometer utilised for bone carbonate analysis in Chapter 8 differed and is described in that chapter.

5.5.5 Bone carbonate isotope modelling

To explore dietary patterns using bone carbonate isotope values ($\delta^{13}C_{carb}$), two related modelling procedures were utilised: (i) a simple carbon isotope model and (ii) a multivariate isotope model. These modeling techniques improve on the bivariate plotting of collagen results ($\delta^{13}C_{coll}$, $\delta^{15}N$), which are biased toward dietary protein sources. The inclusion of $\delta^{13}C_{carb}$ values permits the incorporation of the whole diet in the analysis and provides more detailed paleodiet reconstructions. The models are described briefly here and will be referred to in Chapters 7 and 8.

5.5.5.1 A simple carbon isotope model

Kellner and Schoeninger (2007) proposed a diet reconstruction model based on a $\delta^{13}C_{carb}$ *vs* $\delta^{13}C_{coll}$ plot with three regression lines corresponding to C_3, C_4, and marine protein diet and the position on each line indicating the energy source. However, Froehle et al. (2010) argued that there was considerable overlap between the C_4 and marine protein groups, therefore, by utilising linear regression statistics results, they proposed a simple graphic model consisting of two parallel regression lines distinguishing between C_3 and C_4/marine diets (Figure 5.2).

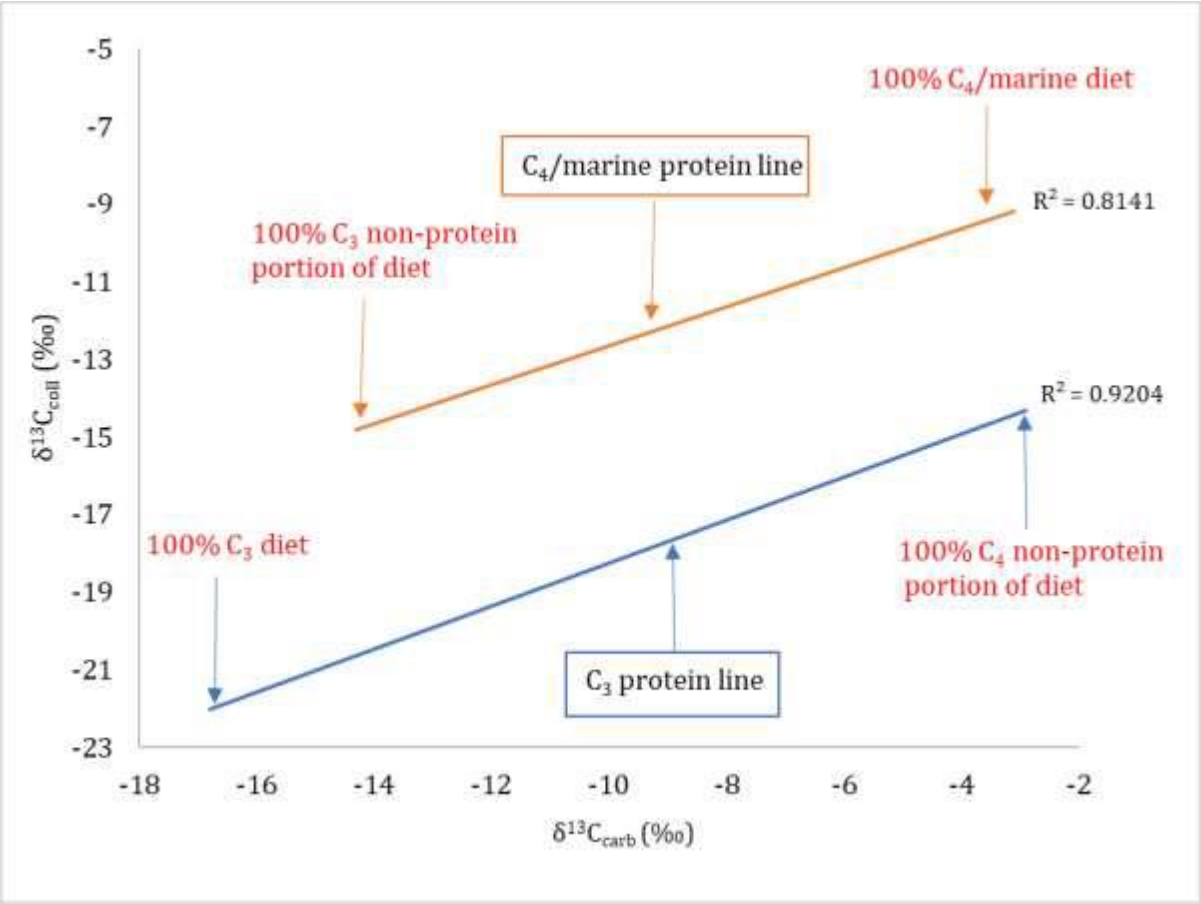


Figure 5-2: Simple isotope plot of $\delta^{13}C_{collagen}$ vs. $\delta^{13}C_{carbonate}$ (‰) produced from experimental animals fed controlled diets created by Froehle et al. (2010).

The regression lines were derived from data from experimental animals fed on controlled diets with known $\delta^{13}C$ values both in dietary protein and in the energy fraction of the diet - carbohydrate, lipid, and excess protein. Two distinctive groups according to protein sources (C_3 vs. C_4/marine) occur when a plot of carbon stable isotope ratios of bone collagen ($\delta^{13}C_{coll}$) and bone carbonate ($\delta^{13}C_{carb}$) is generated. The resulting $\delta^{13}C_{coll}$ vs. $\delta^{13}C_{carb}$ regression lines for the two protein-specific groups are parallel but are separate along the $\delta^{13}C_{coll}$ axis (Froehle et al. 2010). One line represents animals fed on C_4/Marine protein only while the other represents those fed C_3 protein only. The proximity of the values to the poles of the regression lines denotes the ratio of C_3 foods to C_4 foods in the diet whereas primary sources of dietary protein are indicated by the proximity of the values to the regression lines. Those that consumed a mixture of the two sources plot in between endpoints of the line ascribed to their specific dietary protein (Froehle et al. 2010). The authors deemed the model appropriate for archaeological human population mapping because the tissue-diet isotopic relationships found from experimental animals fed on controlled diets did not differ significantly from that of humans.

5.5.5.2 Multivariate isotope model

The regression analysis model, however, does not differentiate the consumption of C_4 and marine resources. As a result, using published archaeological data, Froehle et al. (2012) introduced the multivariate isotope model which incorporates $\delta^{15}N$ collagen results using cluster and discriminant analysis to resolve this issue (Figure 5.3).

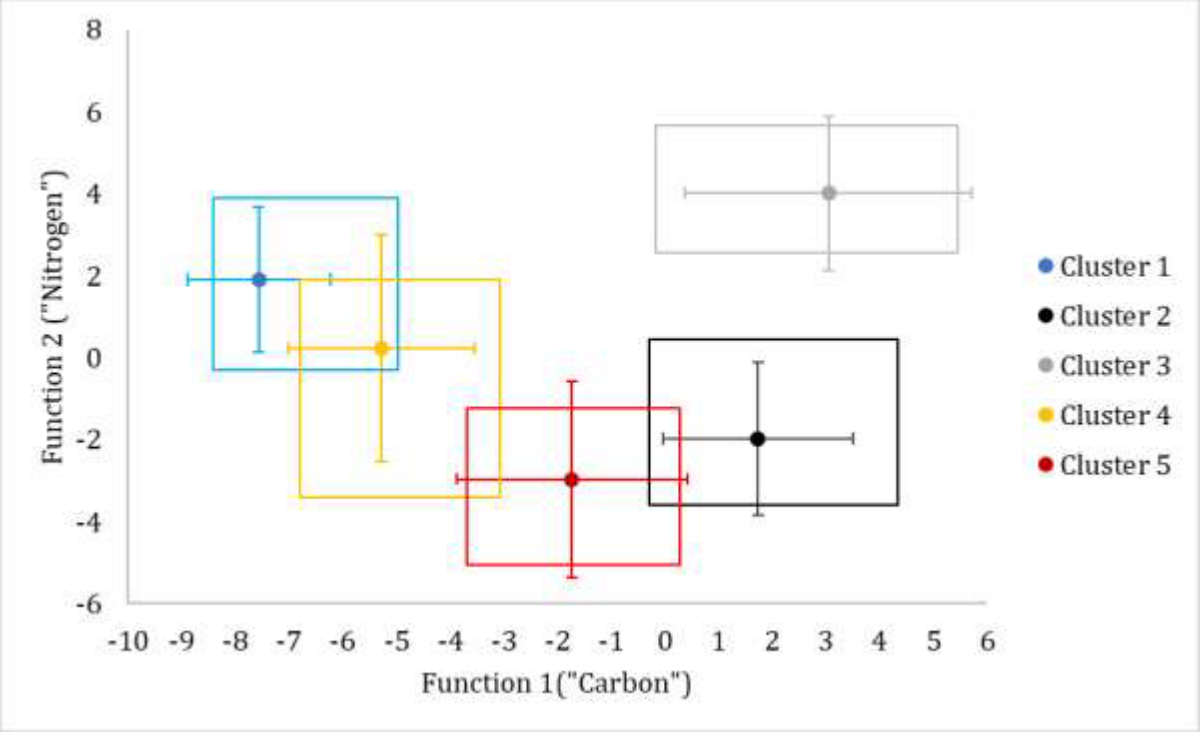


Figure 5-3: Multivariate analysis model created by Froehle et al. (2012).

The analysis carried out by Froehle et al. (2012) generated two functions that describe how the test samples varied in terms of isotopic data with the function scores produced from these 2 functions enabling the plotting of data into five clusters of dietary types.

Function 1 (Carbon) = $(0.322*\delta^{13}C_{carb}) + (0.727*\delta^{13}C_{coll}) + (0.219*\delta^{15}N_{coll}) + 9.354$

Function 2 (Nitrogen) = $(-0.393*\delta^{13}C_{carb}) + (0.113*\delta^{13}C_{coll}) + (0.622*\delta^{15}N_{coll}) - 8.703$

The five discrete dietary clusters produced are:

i) Cluster 1- 100% C_3 diet;100% C_3 protein,
ii) Cluster 2 - 30% C_3:70%C_4 diet >50%C_4 protein,
iii) Cluster 3 - 50% C_3:50%C_4 diet; Marine protein,
iv) Cluster 4 - 70% C_3:30%C_4 diet ≥65%C_3 protein and
v) Cluster 5 - 30% C_3:70%C_4 diet ≥65%C_3 protein.

Function 1 is heavily influenced by $\delta^{13}C_{carb}$ and $\delta^{13}C_{coll}$ whilst function 2 is most influenced by $\delta^{15}N_{coll}$.

Please note that although the following three chapters will ultimately be submitted for publication as multi-author articles, none of the other collaborators has contributed to the writing of any of the sections in the chapters on my behalf and I have carried out all of the research and writing relevant to the aims and objectives of this thesis. As previously discussed in Chapter 4, ZooMS analysis to separate sheep and goats was performed by the University of York technician Krista McGrath for Chapters 6 and 7 and collagen data for 70 out of 216 human and 35 out of 168 animal remains for Chapter 7 were also generated elsewhere. I generated all the remaining collagen data as well as all carbonate and FTIR data included in this thesis. I also personally analysed all the data, including data generated by other individuals but included in this thesis. These chapters can therefore be taken as publication drafts, but the final edits will involve the other authors prior to submission for publication, but only after I have handed in my thesis. In addition, some information in the chapters has been covered in detail in previous background chapters but has been included here to maintain future publication structure.

Chapter 6: Exploration of diet in post-medieval Manchester through stable isotope investigation of human and faunal remains

6.1 Introduction

The Industrial Revolution of 17th -19th century England was one of the most dynamic periods in British history. This era was a time of great population growth, scientific invention, and rapid urbanisation (O'Brien and Quinault 1993; Burnett 2005). Prior to the 17th century, a self-sustaining ecosystem existed whereby food was largely derived from a small-scale peasant type of agriculture. However, improvements in agriculture in the 17th century meant that there was greater agricultural output achieved with fewer workers than before (O'Brien and Quinault 1993; Burnett 2005). Inventions of new machinery also led to rapid changes in the manufacturing industry which subsequently increased the movement of the rural poor to developing towns and cities for work (O'Brien and Quinault 1993; Burnett 2005). Consequently, populations grew rapidly in these transforming towns and urban centres. This ever-expanding urbanisation in turn placed heavy demands on food supplies and contributed to the creation of distinct food consumption patterns based on social classes, and the city of Manchester was no exception (Scola 1992).

In contrast to earlier periods in Britain, archaeologically speaking, individuals' dietary habits in the post-medieval period have previously received little attention in archaeological studies until relatively recently. However, with an increase in urban development, a great number of post-medieval skeletal remains have been recovered around the country (Powers et al. 2013) and these remains are generally very well preserved with cases of soft tissue and hair preservation and can occasionally be identified through the use of coffin plates or grave markers. The availability of archaeological skeletal populations from this period coupled with the opportunities presented by a rich historical resource to draw upon e.g., Lightfoot et al. (2020) means that the dynamics of post-medieval diets are increasingly being explored using scientific methods such as stable isotope analysis. Within the last few decades, isotope analyses onpost-medieval sites in Britain have grown in number e.g. Richards (2006), Trickett (2006), Nitsch et al. (2010), Nitsch et al. (2011), Roberts et al. (2012), Beaumont (2013), Beaumont et al. (2013a), Beaumont et al. (2013b), Brown and Alexander (2016) and Bleasdale et al. (2019). These studies have demonstrated the ways in which stable isotope analysis data can enhance the deciphering of dietary habits in post-medieval populations. However, these studies typically relate to the sites in London or cities in the south of England, primarily because of the increase in the number of excavations as a result of developmental proposals in the south of England (for instance, over

5000 post-medieval burials were excavated between 2008 and 2013 in London alone (Powers et al. 2013, 126). This study, however, focuses on two sites from the Manchester urban community in the north-west of England.

Manchester was Britain's most important industrial city after London after 1750 (Glinert 2009). Historical records indicate that in the second half of the 18th century, a number of factors transformed the city into a major industrial conurbation, with some naming it the "Western world's first industrialised city" (Douglas et al. 2002, 236). The combination of the number of coal mines in the city, the construction of the Bridgewater canal to supply coal to cotton mills, as well as new technological innovations in the long-established wool and linen textile industry all contributed to the major industrialisation of the city. Manchester also pioneered the development of the world's first inter-city passenger railway to Liverpool which increased trade without restrictions and massive imports of cotton in the city. This industrial dynamism led to an extensive population growth from nearly 77 thousand in 1801 to 316 thousand in 1851 with 54% coming from elsewhere in the British Isles and 1% from overseas (Douglas et al. 2002, 237). Extensivepopulation growth in Manchester increased the local demand for food (Douglas et al. 2002). Furthermore, in addition to the expanding local demand for food, improved communications also meant that food in the city was sourced from increasingly distant parts of Britain and other countries (Chaloner 1959; Scola 1992). As Manchester expanded, the city developed well demarcated zones that divided people into classes, the working class, which included farmers and low-skilled factory workers who did not own any means of production; and the middle/upper class, who owned most of the means of production (Scola 1992, 9). Availability and access to foodbecame wage dependent (Engels 1845). Certain foods were exclusive to the middle- or upper- class individuals only. Social groupings such as age and sex within these communities also governed dietary habits (Scola 1992). Archaeological material from two populations from this historical city is available, making Manchester ideal for a dietary study of a northern urban population in post-medieval England. The two populations deriving from different social groups,middle/upper Cross Street Unitarian Chapel and low-class Chapel Street, Hazel Grove were sampled in this research to provide a comparison between the diets of different socioeconomic classes. This study provides new insights into the diet of these two populations through carbon and nitrogen isotope analysis of human and animal bone collagen ($\delta^{13}C$, $\delta^{15}N$) with a focus on possible age, status, race and sex variation in diet.

6.2 Diet in Manchester

Historic accounts of typical food consumed by the 18th to 19th century Manchester population indicate a large proportion of C_3 foods such as rye, wheat, and oats in the form of bread and porridge (Chaloner 1959; Scola 1992). The Corn Laws of 1815, however, had a disastrous effect

on grain prices which particularly affected the poorer classes who were heavily dependent on bread as a staple food. As a result, people turned to potatoes as a food source (Pickering and Tyrell 2000). In addition to C_3 foods, minor contributions from C_4 resources such as cane sugar and maize formed part of these populations' diet (Chaloner 1959; Scola 1992). Cane sugar was mostly afforded by the rich until after the abolition of Sugar Tax in 1874 when it became available to all classes (Walvin 2017) and maize had been provided to the Irish who had migrated to Englandas relief food during the Great Irish Famine of 1845 to 1852 (Ó'Gráda 1989). Other sources of food included dairy products such as milk, cheese and butter, fruits, and vegetables which were mostly afforded by the rich (Scola 1992).

Meat and fish were consumed especially by the middle and upper classes (Chaloner 1959; Scola 1992). Meat consumed consisted of veal, pork, mutton, sheep, poultry, and beef. John Holt's 1794 survey of agriculture in Lancashire revealed that except for veal and pork, very little of the meat consumed within the county was from local produce (Holt 1794). The poor could rarely afford beef, mutton, and veal as they were expensive luxuries (Booth 1977; Scola 1992). Pork and poultry, including wildfowl of various kinds, however, were available in large quantities in the city and were commonly consumed by all people (Chaloner 1959). Sheep and cattle were typically brought to the city from North Wales, the Welsh borders, Lincolnshire, and the neighbouring counties (Redhead et al. 1792; Marshall 1818; Scola 1992). Furthermore, after the 1759 abolishment of the Cattle Acts which previously banned the importation of livestock from Ireland, and the end of the Napoleonic Wars (1793-1815), the importation of cattle from Ireland into the city commenced (Blackman 1975). By the mid-19th century, market reports showed that about 80 to 90% of cattle were sourced either directly from Ireland or indirectly from Ireland via Liverpool (Scola 1992, 47). Although Manchester is inland, it received sea fish such as cod, lobsters, and turbot from the Yorkshire coast and soles from the Lancashire coast. These were again mainly afforded by the middle and upper classes. However, the poor could often afford mackerel and cured herring from the Isle of Man and the east coast herring fleets from Scotland, mostly in times of glut (Aikin 1785; Chaloner 1959, 44). The exact quantity of freshwater fish available for consumption is unknown but it has been indicated that farming of freshwater fish was limited and had gone into decline from the time of the Reformation in the 16th century (North1714).

6.3 Isotopic Analysis Background

The principles of using stable isotope analysis of carbon and nitrogen to quantify dietary practices of ancient populations are well established e.g., Schwarcz and Schoeninger (2012). Body tissues such as bones, used in this study, incorporate stable isotopes of carbon and nitrogen ($\delta^{13}C$, $\delta^{15}N$) from foods consumed by individuals during their lifetime and are retained after death (Schwarcz and Schoeninger 2012). In palaeodietary studies, changes in the ratio of the heavier to the lighter

isotope are expressed in delta notation (δ) in parts per thousand (per mil or ‰) relative to an internationally agreed sample material (McKinney et al. 1950; Craig 1957). Due to turnover, bone tissues represent an individual's average diet over 10 years prior to death, or over much longer period of time than 10 years, depending on the skeletal element (Cox and Sealy 1997; Hedges et al. 2007; Meier-Augenstein 2017). Bone collagen $\delta^{15}N$ provides information on the main protein sources of the diet. While bone collagen $\delta^{13}C$ values also represents the protein sources in the diet, about 25% of bone collagen (non-essential amino acids) can be synthesised from lipids and carbohydrates (Fernandes et al. 2012).

The $\delta^{13}C$ value of consumers is primarily affected by the photosynthetic pathway (C_3 or C_4) of plants such that C_3 plants, such as wheat and barley, have lower $\delta^{13}C$ values than those of C_4 plants, such as maize and sugarcane (Schwarcz and Schoeninger 2012). In addition to being valuable in distinguishing between C_3 and C_4 plant intake, $\delta^{13}C$ can be utilised in differentiating marine and terrestrial protein diets. This is because the $^{13}C/^{12}C$ ratio used by plants differs in terrestrial and oceanic ecosystems such that the $\delta^{13}C$ values of the former tend to be ~8‰ higher than that of the later (Chisholm et al. 1982; Mays 1997). Most C_4 plants are not indigenous to Britain but as already indicated, cane sugar and maize contributed to the diet in the Greater Manchester area in the 18th to 19th century (Barker et al. 1970; Scola 1992). Nitrogen isotope ratios provide information about the consumer's trophic position in the food chain and differentiate terrestrial and marine food contributions to the consumer's diet (Schoeninger et al. 1983; Minagawa and Wada 1984). It has also been established that $\delta^{15}N$ values can be elevated due to nutritional stress within the body being examined. Hobson et al. (1993) reported elevated levels of $\delta^{15}N$ in the body tissues of birds under controlled feeding strategies and in the wild, undergoing nutritional stress and fasting. In addition, isotopic studies of pregnant women's hair found that $\delta^{15}N$ values increased during periods of nutritional stress caused by morning sickness (Fuller et al. 2004). Furthermore, $\delta^{15}N$ values in plants can be increased if soils are depleted of nitrates through anthropogenic manuring input which in turn elevates consumers' $\delta^{15}N$ values (Hoefs 2015).

6.4 Materials

The human and animal samples were collected from the Cross Street, Manchester; Hazel Grove, Manchester; and Norton Priory, Cheshire (Figure 6.1). In total, 54 adults, 36 nonadults, and two of unknown age from Cross street were sampled for analysis. Of these, 27 were females,25 were males and 40 were of unknown sex. In addition, a total of 20 adults and 14 nonadults from Hazel Grove were selected for collagen analysis. Of these, 7 were females, 6 were males and 21 were of unknown sex. Age and sex for both sites were determined osteologically by Newman and Holst (2016) and Keefe and Holst (2017). The possible females (?F) and possible males (?M) were treated as females and males respectively. A total of 111 faunal remains were sampled, 37 from

Cross Street and 74 from Norton Priory.

Figure 6-1: Map of Victorian Greater Manchester showing the locations of the sites of study and other post-medieval sites mentioned in the text.

6.4.1 Cross Street Unitarian Chapel

Archaeological excavations at Cross Street Unitarian Chapel, Manchester took place between 2014 and 2015 in advance of the construction of the Second City Crossing tramway along Cross Street in the northern half of Manchester city centre. The Unitarian Chapel was originally erected in 1694 by Reverend Henry Newcome to accommodate his growing congregation. Like many other non-conformist groups, the chapel was attended by the poorer classes in the beginning, however, with time the chapel recruited more prosperous members from manufacturing classes who were artisans or merchants (Baker 1884). Around 241 individual skeletons and 17,679 fragments of disarticulated bone were recovered. Among the individuals recovered at this site, were 16 individuals who were buried with legible nameplates, 5 of whom were nonadults, and 11 were adults (7 males and 4 females). Nine of these individuals were analysed in this study (Table 6.1).

Table 6-1: Named individuals from Cross Street sampled in this thesis and their potential dates of death.

Sample	*Named individual*	*Sex*	*Age (years)*	*Date of death*
2.03	William Crane	M	85	1 January 1808
2.21	Ann Goodier	F	MA 46+	Unknown
2.31	Elizabeth Potter	F	MA 46+	Unknown
2.40	Harriet Slater	F?	YJ 6	10 August 1819
2.52	Ellen Rylande	F	62	12 July 1835
4.38	Sarah Baron	F?	MA 46+	Unknown
5.16	John Blinkhorn	M	64	17 April 1838
5.23	Mary Mason	F	64	24 August 1809
61.03	Joseph Guildford	M	OMA 26-45	1829

Osteological analysis carried out by York Osteoarchaeology Ltd on all 241 skeletons as well as on fragments of disarticulated bone recovered at this site revealed that the proportion of nonadults recovered (28%) was lower than expected from historical documents for this period. The London Bills of Mortality suggest that around 50% of the population died before the age of twenty years between the early 18th and mid-19th centuries (Roberts and Cox 2003, 304). Among the sexed adults, there was a slightly higher proportion of males (51.1%) than females (48.9%) (Keefe and Holst 2017). In terms of osteological indicators of diet and nutrition, dental health at this site was generally poor; females showed high levels of caries, with 20.7% of their teeth affected compared to 18.5% of male teeth. Also observed was antemortem tooth loss for both males and females. Just over three-quarters of the adults with at least one observable tooth position (94/122, 77.0%) had experienced antemortem tooth loss and 27.5% of their teeth had been lost antemortem. Thisis considered as consistent with a diet of refined sugars and processed carbohydrates. Conditions associated with lack of specific nutrients such as scurvy (lack of vitamin C which can be found in fresh fruit, vegetables, and marine fish) and rickets (lack of vitamin D, which is produced by the

body during exposure to sunlight as well as other sources like oily fish, eggs, and milk) were observed in non-adults. Children with these conditions also had evidence of higher rates of enamel defects than adults (Keefe and Holst 2017).

Excavations at this site also uncovered unstratified animal remains. Industrial services during the 19th century onwards disturbed the burial ground; therefore, these animal bones might be from a later date than the graveyard. However, in the absence of any other suitable animal material from the city itself, these remains were sampled for an animal baseline. Similarities with other post-medieval animal remains from England will be considered to determine if Cross Street faunal samples can be reliably utilised as baselines.

6.4.2 Chapel Street, Hazel Grove

In 2016, CFA Archaeology excavated the site of a former Wesleyan Chapel on Chapel Street, Hazel Grove, Greater Manchester. Hazel Grove is presently a suburb located within the Metropolitan Borough of Stockport, approximately 9 miles from Manchester city centre (Figure 6.1). The site is located at a former Wesleyan Chapel dated at around 1785 and a Sunday school in place between c.1823-1912. Most of the population living in Hazel Grove were those of the poor classes (Errington 2001; Newman and Holst 2016). Osteological analysis was carried out on 38 skeletonsrecovered in the southwest and northwest of the rear of the church by York Osteoarchaeology Ltd in 2016 (Newman and Holst 2016). The nameplates recovered during the excavations (Table6.2) reveal that individuals recovered were representatives of the 18th and early 19th century members of the congregation (Newman and Holst 2016).

Table 6-2: Named individuals from Hazel Grove sampled in this study and their potential dates of death.

Sample	*Named individual*	*Sex*	*Age (years)*	*Date of death*
12	G.T	M?	38	1831
26	Mary Penney	F	48	1827
28	Edward Penney	M?	6.25	1834
36	Margaret To-	F	88	1832

Sexing was carried out using standard osteological techniques involving examination of the shape of the skull and the pelvis and could only be carried out in individuals whose sexual characteristics had developed - during late puberty and early adulthood (Newman and Holst 2016). For the named individual 28, sex estimation could not be carried out as that individual was a juvenile, therefore, sex was assigned as probably male (M?) due to the name Edward. The burial population comprised a slightly higher proportion of females than males, which is common in non-conformist burial grounds (Newman and Holst 2016)., however, the small sample size should

also be taken into account. Half of the population was made up of non-adults, with a high proportion of infants(1 to 11 months, 52.6%) and young juveniles (1 to 6 years, 31.6%) within the sample, which corresponds with historical documentation that states that a large proportion of deaths occurred within the first five years of life during this time (Cherryson et al. 2012; Renshaw and Powers 2016). Four individuals tentatively exhibited cranial morphology that could be suggestive of African/mixed ancestry (Skeletons 1, 10, 14 & 21) within the Hazel Grove sample (Newman and Holst 2016, 24–25). Enamel defects, *cribra orbitalia* (anaemia), and residual rickets, all evidencefor childhood stress, were also identified among the adults. As was the case for Cross Street, six infants and young juveniles suffered from scurvy and rickets. Osteological analysis also revealed poor dental health at this site (Newman and Holst 2016). Unlike Cross Street, there wereno animal remains available from Hazel Grove.

6.4.3 Norton Priory, Chester

To supplement the animal remains sampled from Cross Street, further animal material was sampled from Norton Priory Runcorn, Cheshire, approximately 27.7 miles from Manchester, which represented a broadly contemporaneous dataset in the North West (Figure 6.1). Documentary evidence indicates that meat consumed in Manchester was supplied from regions outside the city, including Cheshire (Scola 1992). Excavations at Norton Priory were undertaken between 1970 and 1987 by the Runcorn Development Corporation and focussed on the buildings of Priory of St Mary (1134 - 1536) and some of the areas occupied post-dissolution (Wright et al. 2017). Animal remains were sampled from the post-dissolution Period 2 between 1536-c1730, representative of the suppression of the priory, and the Tudor and later occupation of Norton Hall, as well as Period 3 between c 1730-1928, representative of the Occupation of the Georgian Mansion (Wright et al. 2017), contemporaneous with the humans from Greater Manchester. The Priory was founded in 1134 by orders of the Catholic Church, Augustinian Canons, and its dissolution occurred in 1536 (Greene 2004). After the dissolution of the Priory, the Brooke family purchased it in 1545 and converted the buildings into a large family home, the Norton Hall estate. Around 1730, the Brooke family who by then were still living at the site destroyed the medieval buildings and built an even larger mansion, the new Palladian house, possibly resided in by the family together with servants carrying out manual work and farm labourers working the land. Although the house was finally demolished in 1928, there are indications that the house became gradually less occupied by the late 19th century (Brown and Howard-Davis 2008), so that the animal remains found at this site are unlikely to date beyond this period. The majority of the samples came from a large midden, assigned to Period 2 (1536-c1730) which was located near the kitchens (Brown and Howard-Davis 2008). Dating of the faunal assemblage in Period 3 (c 1730-1928) proved difficult because of landscaping and the disturbance surrounding the

demolition of the house in 1928 as the remains originated from undifferentiated topsoil deposits during excavations (Brown and Howard-Davis 2008; Wright et al. 2017), such that they could actually originate from Period 2. As a result, in this study, all the remains from Periods 2 and 3 were combined for analysis.

6.5 Methods

6.5.1 Bone collagen isotope analysis

Collagen extraction of bone followed the standard operating procedure for BioArCh at the University of York, based on the Longin (1971) method modified by Brown et al. (1988). Each bone sample was cleaned using powder abrasion, to remove contaminants of the outer layer of bone. Following this, bone samples (300-400mg) were demineralised in 8ml of 0.6M hydrochloric acid (HCl) for 1-5 days. The demineralised samples were rinsed thrice using deionised water and then gelatinised using pH3 HCl on a heat block for 48 hours at 80°C. Following this, the soluble collagen solutions were Ezee™ filtered to remove unwanted particulate matter from the collagen solution, and then the supernatants were then ultrafiltered to isolate the high molecular weight > 30 kDa fraction. They were then frozen for a minimum of 12 hours at -20°C before being lyophilized in a freeze dryer for 48 hours. Collagen yields (wt.%) were calculated to distinguish well-preserved from poorly preserved collagen using the formula [(bone mass (mg)/bone collagen mass(mg)) x 100]. Between 0.9 and 1.1mg of the freeze-dried retentate were weighed out in duplicate into 4x3.2mm tin capsules and combusted alongside international standards in an Elemental Analyser/Isotope Ratio Mass Spectrometer EA/IRMS: a Sercon 20-22 mass spectrometer coupled with a Sercon GSL Sample Preparation System module at BioArch, University of York.

All $\delta^{13}C$ and $\delta^{15}N$ ratios are expressed using the delta notation (δ) in parts per thousand (‰) relative to the international standards, VPDB for $\delta^{13}C$ and atmospheric N_2 (AIR) for $\delta^{15}N$, using the following equation: [δ (‰) = ($R_{sample}/R_{standard}$ -1) x 1000]. Accuracy was determined by measurements of international standard reference materials within each analytical run. These were IAEA 600 $\delta^{13}C_{raw}$ = -27.65 ±0.09 ‰, $\delta^{13}C_{true}$ = -27.77 ±0.043 ‰, $\delta^{15}N_{raw}$ = 0.92 ±0.21 ‰, $\delta^{15}N_{true}$ = 1 ±0.2 ‰; IAEA N2 $\delta^{15}N_{raw}$ = 20.35 ±0.13 ‰, $\delta^{15}N_{true}$ = 20.3 ±0.2 ‰; IA Cane, $\delta^{13}C_{raw}$ = -11.77 ±0.09 ‰; $\delta^{13}C_{true}$ = -11.64 ±0.03 ‰. The overall uncertainties on the measurements of each sample were calculated based on the method of Kragten (1994) by combining uncertainties in the values of the international reference materials and those determined from repeated measurements of samples and reference materials. These are expressed as one standard deviation. The maximum uncertainty for all samples across all runs was <0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. In addition, a homogenised bovine bone extracted and analysed within the same batch as

the samples produced the following average values: $\delta^{13}C$ = -23.01 ±0.13; $\delta^{15}N$ = 6.21 ±0.44. This was comparable to the overall mean value from 50 separate extracts of this bone sample, which produced values of $\delta^{13}C$ = -22.97 ±0.19 and $\delta^{15}N$ = 6.19±0.30.

6.5.2 Statistical analysis

Statistical analysis was undertaken using R statistics, PAST, and SPSS Statistics Version 26 to compare dietary profiles of all remains in this study using an alpha level of 0.05 for all statistical tests (Hammer et al. 2001; IBM 2019; R Core Team 2020). Non-parametric statistics were used to compare diagenetic parameter values between the population groups and the variations within them because of the non-normal distribution of data as indicated by Kolmogorov-Smirnov and Shapiro-Wilk tests. The tests were executed using the non-parametric equivalent of a one-way analysis of variance (ANOVA) and the non-parametric equivalent of the independent T-test: Kruskal Wallis Test and Mann Whitney test respectively.

6.6 Results

6.6.1 Collagen Preservation

Preservation of collagen samples was assessed through collagen yields, elemental (%C, %N) data, and atomic C:N ratios. The collagen yields from Cross Street and Hazel Grove sites were in the range between 1.1 and 22.6% and 3.0 and 16.1% respectively (Appendix A). Good bone collagen preservation for faunal remains was indicated by collagen yields (faunal range 3.7% to 25.3%; See Appendix B). A decrease in collagen yield is to be expected because of the effects of using ultrafilters, therefore samples with a collagen yield of less than 1% were not excluded but subjected to further quality checks. For both human and faunal remains, elemental (%N, %C) data from all samples exceeded acceptable standards of at least 4.8% and 13% for nitrogen and carbon, respectively (Ambrose 1990, 447). The atomic C:N ratios from both the human and faunal remains fell within the acceptable range of between 2.9 and 3.6 (DeNiro 1985).

6.6.2 Animal data

Individual results of faunal remains are presented in Appendix B. The faunal results provide baseline values for interpreting the diet of individuals from both sites. The isotopic data for animals from both Cross Street and Norton Priory are very similar. Data from individual species reveals different ranges for the different animals (Table 6.3). There is no statistical difference between $\delta^{13}C$ values for cattle, pigs, and sheep and $\delta^{15}N$ values for cattle and pigs between both sites, although sheep are statistically different (Table 6.4). The sheep from Norton Priory are notable for having considerably higher $\delta^{15}N$ values than those at Cross Street.

Table 6-3: Descriptive statistics for $\delta^{13}C$ and the $\delta^{15}N$ values of faunal remains from Cross Street Manchester (CSM) and Norton Priory, Runcorn (NP).

Site/Species	*N*	*Range*	*Minimum*	*Maximum*	*Mean*	*Std. Deviation*
CSM Cattle $\delta^{13}C$	14	2.2	-22.7	-20.5	-22.0	0.6
NP Cattle $\delta^{13}C$	20	1.3	-22.8	-21.5	-22.2	0.3
CSM Mallard $\delta^{13}C$	2	2.1	-21.9	-19.8	-20.9	-
NP Domestic Fowl $\delta^{13}C$	12	1.2	-21.8	-20.5	-21.0	0.4
CSM Pigs $\delta^{13}C$	8	6.1	-22.3	-16.2	-21.0	2.0
NP Pigs $\delta^{13}C$	20	1.2	-22.1	-20.8	-21.4	0.4
CSM Sheep $\delta^{13}C$	13	1.0	-22.4	-21.4	-22.0	0.4
NP Sheep $\delta^{13}C$	23	1.4	-22.7	-21.3	-22.1	0.4
CSM Cattle $\delta^{15}N$	14	2.1	5.4	7.5	6.3	0.6
NP Cattle $\delta^{15}N$	20	2.4	4.9	7.3	6.2	0.8
CSM Mallard $\delta^{15}N$	2	0.8	10.9	11.7	11.3	-
NP Domestic Fowl $\delta^{15}N$	12	3.5	8.1	11.6	9.4	1.1
CSM Pigs $\delta^{15}N$	8	3.3	5.5	8.8	6.8	1.3
NP Pigs $\delta^{15}N$	20	4.4	5.1	9.5	7.6	1.4
CSM Sheep $\delta^{15}N$	13	3.5	3.3	6.8	5.4	1.0
NP Sheep $\delta^{15}N$	23	5.9	4.6	10.5	7.9	1.4

*Table 6-4: Statistical comparison between the $\delta^{13}C$ and the $\delta^{15}N$ values of faunal remains between Cross Street and Norton Priory sites. *Statistically significance is observed between Norton Priory and Cross Street sheep.*

Site	*N*	*Independent samples Mann-Whitney U Test $\delta^{13}C$ (‰)*	*Independent samples Mann-Whitney U Test $\delta^{15}N$ (‰)*
Norton Priory Cattle	14	U=123.500, p=0.569	U=131.000, p=0.769
Cross Street Cattle	20		
Norton Priory Pigs	20	U=95.000, p=0.469	U=114.000, p=0.089
Cross Street Pigs	8		
Norton Priory Sheep	23	U=110.500, p=0.202	U=278.500, p=0.000*
Cross Street Sheep	13		

The $\delta^{13}C$ results indicate that, except for one pig from Cross Street, all animals exhibit low values indicating that C_3 fodder dominated the diet at both sites (Figure 6.2). The $\delta^{13}C$ value pig sample CSM 6217 is less negative (-16.20‰) implying that the pig might have been foddered on C_4 plants.

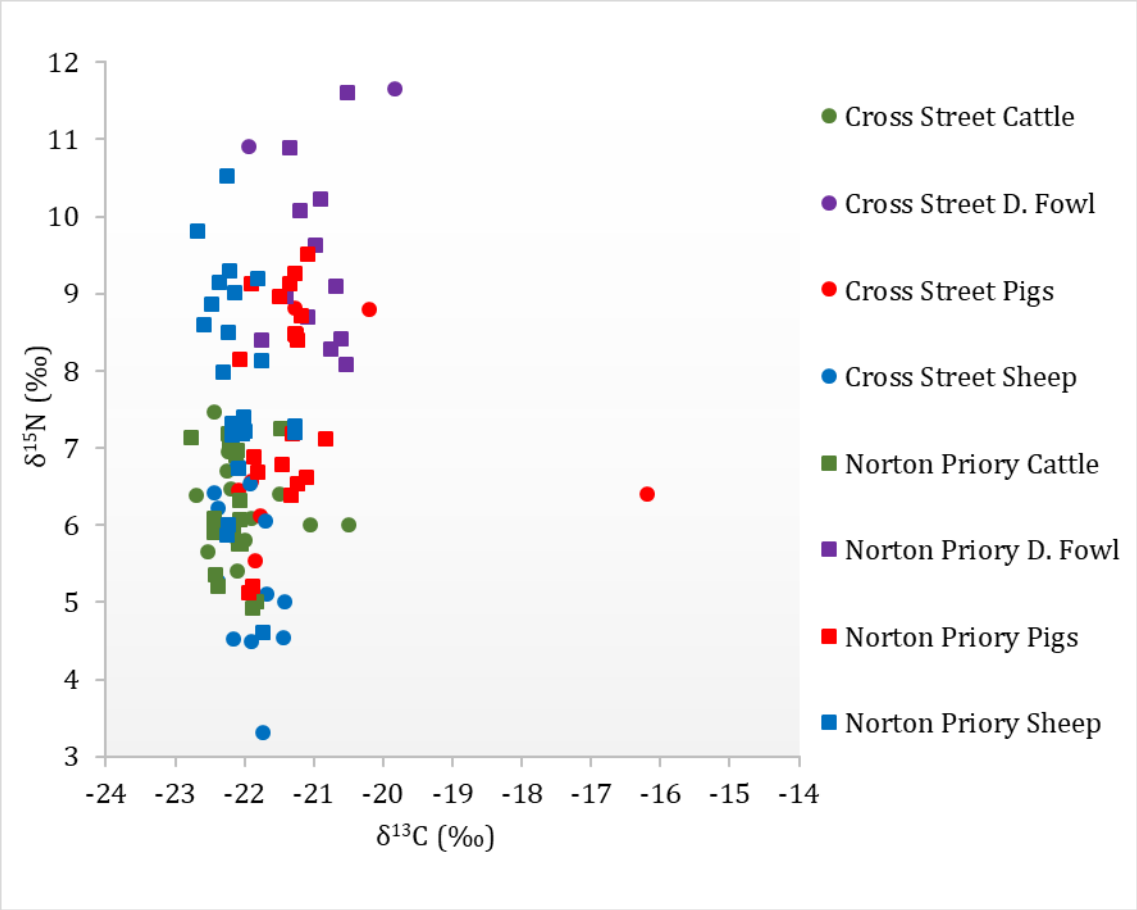


Figure 6-2: $\delta^{13}C$ and the $\delta^{15}N$ values of faunal remains across both sites in this study.

6.6.3 Comparisons with other post-medieval faunal remains

Compared to other animals from across England, $\delta^{13}C$ values of the cattle, pigs, and sheep from this study generally fall within the range of values reported for post-medieval Queen's Chapel Savoy and Prescot Street sites in London as well as in Durham (Millard et al. 2015; Bleasdale et al. 2019; see Figure 6.3).

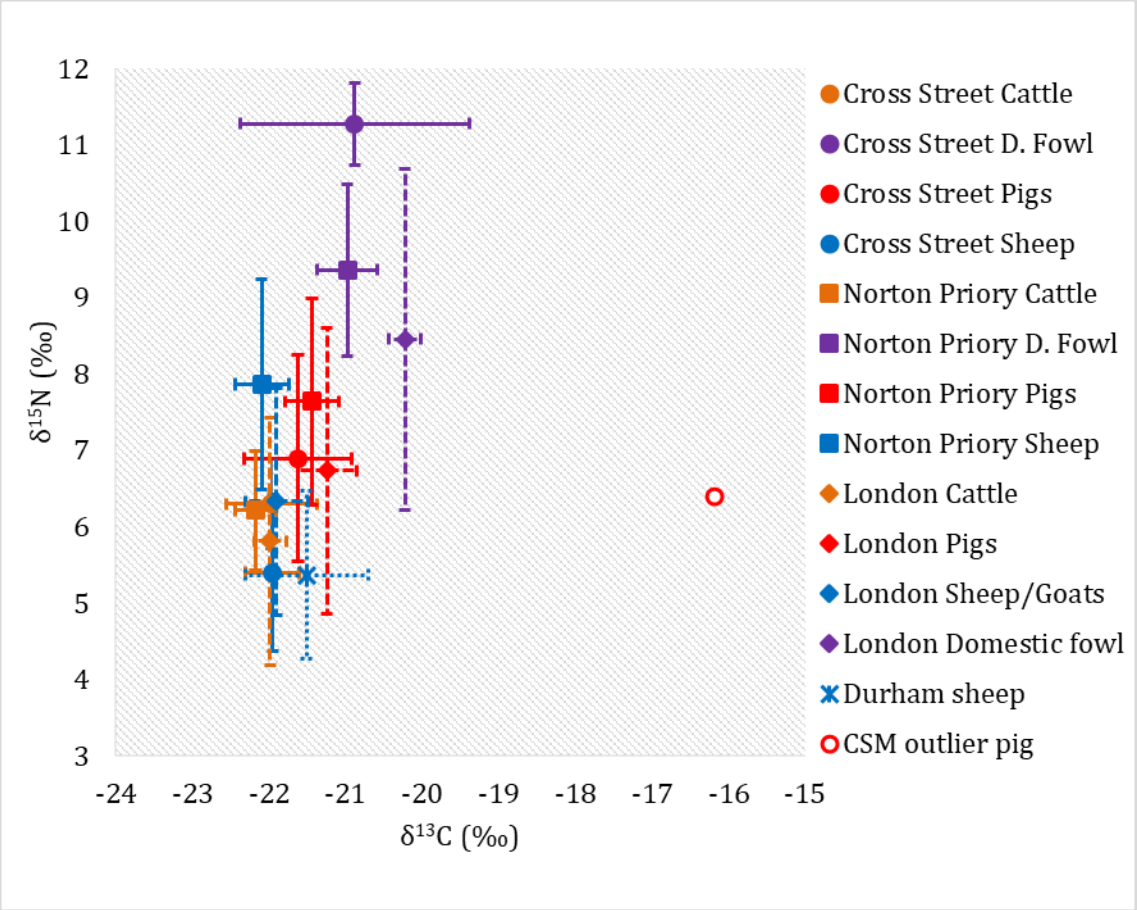


Figure 6-3: Faunal mean $\delta^{13}C$ and $\delta^{15}N$ values to 1σ from Cross Street Manchester (CSM) and Norton Priory Chester (NP). There are only 2 Cross Street domestic fowl, therefore they have not been averaged. Dotted error bars are for reference from data collected in Bleasdale et al. (2019) at Queen's Chapel Savoy and Prescot Street sites in London and in Millard et al. (2015) at Durham.

There are no statistically significant differences in the cattle and pigs from the Cross Street, Norton Priory and London sites [Cattle - (Kruskal-Wallis H test, $\delta^{13}C$ $X^2(2) = 3.913$, $p = 0.141$); Kruskal-Wallis H test, $\delta^{15}N$ $X^2(2) = 1.026$, $p = 0.599$); all pigs including CSM 6217 with ^{13}C enrichment - (Kruskal-Wallis H test, $\delta^{13}C$ $X^2(2) = 2.162$, $p = 0.339$); Kruskal-Wallis H test, $\delta^{15}N$ $X^2(2) = 3.744$, $p = 0.154$)]. Comparisons between sheep from this study and the London and Durham sheep showed no significant differences in the $\delta^{13}C$ values among the four groups [Kruskal-Wallis H test, $\delta^{13}C$ $X^2(3) = 7.544$, $p = 0.056$) but statistically significant differences in the $\delta^{15}N$ values [Kruskal-Wallis H test, $\delta^{15}N$ $X^2(3) = 27.734$, $p = 0.000$). The posthoc analysis produced during that test revealed that the Norton Priory sheep cause the significant variation in this group (Table 6.5).

Table 6-5: Post hoc pairwise results for the sheep from Cross Street, Norton Priory, London, and Durham. The significance level is 0 .05 and the significance values have been adjusted by the Bonferroni correction for multiple tests.

Sample 1-Sample 2	$\delta^{15}N_{coll}$
Durham-Cross Street sheep	1.000
Durham-London sheep	0.532
Durham-Norton Priory sheep	0.000
Cross Street-London sheep	0.901
Cross Street-Norton Priory	0.000
London-Norton Priory	0.027

6.6.4 Human data

The results of stable isotope analysis for all the human remains are presented in Appendix A.

6.6.4.1 Inter-population diet variations due to age

In order to examine dietary variations in these two populations, comparisons were made between the adults (18+ years), older nonadults (7-17 years for Cross Street vs. 4-14 years for Hazel Grove) and infants (0-6 years for Cross Street vs. 0-4 years for Hazel Grove) from both sites (Figure 6.4; Table 6.6).

Table 6-6: Mean $\delta^{13}C$ and $\delta^{15}N$ values for both adult and children individuals sampled for bone collagen from Cross Street and Hazel Grove Manchester sites.

Site	Age Group	Mean δ^{13}Ccollagen (‰)	1 σ	Mean δ^{15}Ncollagen (‰)	1 σ
Cross Street Manchester	0-6years old (n=22)	-19.8	0.5	12.1	1.5
	7-17years old (n=14)	-19.9	0.4	11.5	0.9
	Adults over 18 years old (n=54)	-19.8	0.6	11.6	0.9
Hazel Grove Manchester	0-4years old (n=9)	-19.8	0.3	12.4	1.2
	4-14years old (n=5)	-20.4	0.3	9.9	0.3
	Adults over 18 years old (n=20)	-20.2	0.3	10.9	0.6

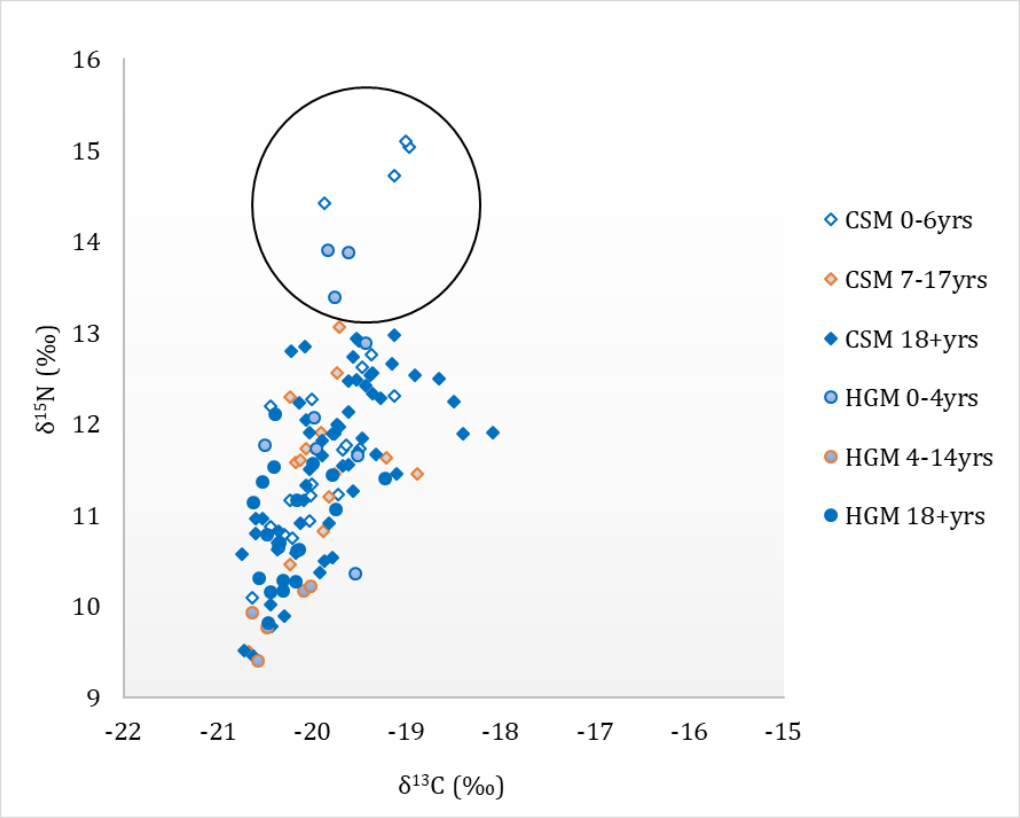


Figure 6-4: Cross Street (CSM) and Hazel Grove (HGM) Non-adult and adult bulk collagen results. The individuals circled were aged between 0-6 years old and showed remarkably high $\delta^{15}N$ values relative to the rest of the individuals sampled.

Hazel grove adults have statistically significantly lower $\delta^{13}C$ and $\delta^{15}N$ values than the Cross Street adults ($\delta^{13}C$ Mann-Whitney U test U=826.5, p=0.000; $\delta^{15}N$ Mann-Whitney U test U=813, p=0.001). There are no significant differences in $\delta^{13}C$ values between the older children at Cross Street and Hazel Grove [$\delta^{13}C$ Mann-Whitney U test U=14.000, p=0.056] but there are significant differences in $\delta^{15}N$ values ($\delta^{15}N$ Mann-Whitney U test U=4.000, p=0.002). Cross Street infants were categorised from 0-6 years and Hazel Grove from 0-4 years old (Newman and Holst 2016; Keefe and Holst 2017). Since weaning age in the post-medieval period occurred around the age of 1 year old (Lewis and Gowland 2007), it is assumed that those groups included infants who were being breastfed or had just been weaned when they died. Infants from both populations tend to have higher $\delta^{15}N$ than adults (Figure 6.4; Table 6.6). In particular 7 infants (3 from Hazel Grove and 4 from Cross Street) showed $\delta^{15}N$ values that could be considered to be representative of ^{15}N enrichment due to breastfeeding (Figure 6.4). However, $\delta^{15}N$ values can become elevated due to physiological disruptions from conditions such as malnutrition that cause negative nitrogen balance (Katzenberg and Lovell 1999; Fuller et al. 2005; Mekota et al. 2006). Infants from both sites presented with conditions such as scurvy and rickets, associated with inadequate nutrition, therefore, malnutrition may also be a contributing factor. No statistically significant difference was observed between the mean carbon and nitrogen isotope values between Cross Street Manchester infants and Hazel Grove Manchester infants ($\delta^{13}C$ Mann-Whitney U test U=109.000; p=0.685; $\delta^{15}N$ Mann-Whitney U test U=120.000; p=0.379).

6.6.4.2 Intra-population diet variations due to age, sex, and ancestry

To further explore whether any age-related differential access to dietary sources existed within each population, statistical analysis was undertaken. A Kruskal-Wallis H test showed that there was no statistically significant difference in $\delta^{13}C$ and $\delta^{15}N$ values between the different age groups for the Cross Street population [$\delta^{13}C$ $X^2(2) = 0.798$, $p = 0.6709$ and $\delta^{15}N$ $X^2(2) = 0.567$, $p = 0.753$]. However, a statistically significant difference was observed between the age groups at Hazel Grove site [$\delta^{13}C$ $X^2(2) = 9.929$, $p = 0.007$ and $\delta^{15}N$ $X^2(2) = 19.204$, $p = 0.000$]. Posthoc pairwise tests produced during the analysis show that there was very strong evidence ($p < 0.05$, adjusted using the Bonferroni correction) of significant differences in $\delta^{13}C$ values and $\delta^{15}N$ values between (i) the infant group and the older nonadult group ($\delta^{13}C$: p=0.006 and $\delta^{15}N$: p=0.000), (ii) the infant group and the adult group ($\delta^{13}C$: p=0.007 and $\delta^{15}N$: p=0.003) as well as in the (iii) $\delta^{15}N$ values between the older nonadult group and the adult group (p=0.018). However, there are no significant differences between the older nonadult group and the adult group in the $\delta^{13}C$ values (p=0.380).

It was also possible to compare bone collagen isotopic results from Cross Street to explore whether there was any differential access to dietary resources based on sex (Figure 6.5). There are no significant differences between the bone collagen stable isotope ratios of Cross Street males (mean $\delta^{13}C = -19.7 \pm 0.6‰$; mean $\delta^{15}N = 11.6 \pm 1.0‰$) and females (mean $\delta^{13}C = -19.8 \pm 0.6‰$; mean $\delta^{15}N = 11.7 \pm 0.8‰$), indicating that there was no differential access to dietary resources on the basis of sex at this site ($\delta^{13}C$ Mann-Whitney U test U=367, p=0.589; $\delta^{15}N$ Mann-Whitney U test U=347 p=0.862). Only a few individuals from Hazel Grove were sexed, therefore, differences in $\delta^{13}C$ and $\delta^{15}N$ values were not statistically determined for this site, instead they were display graphically (Figure 6.6). Visually, there seems to be no difference in diet between the males and females at this site.

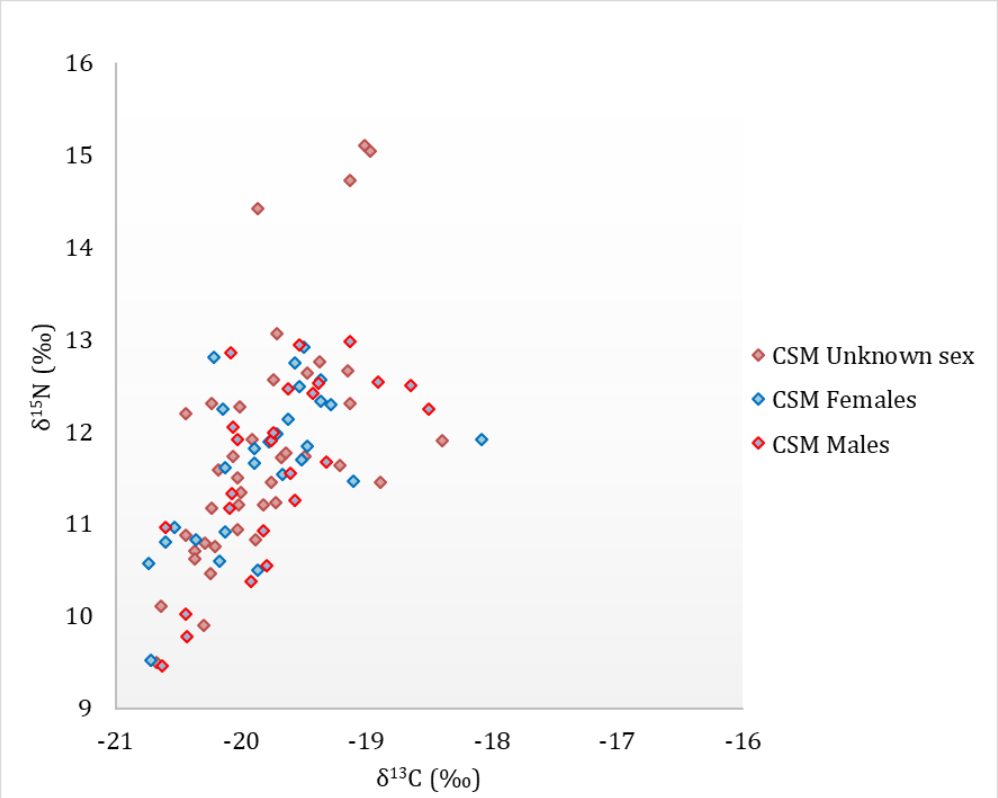


Figure 6-5: Bone collagen $\delta^{13}C$ and $\delta^{15}N$ values for Females (n = 27), Males (n = 25) and Unknown sex (n=40) from Cross Street.

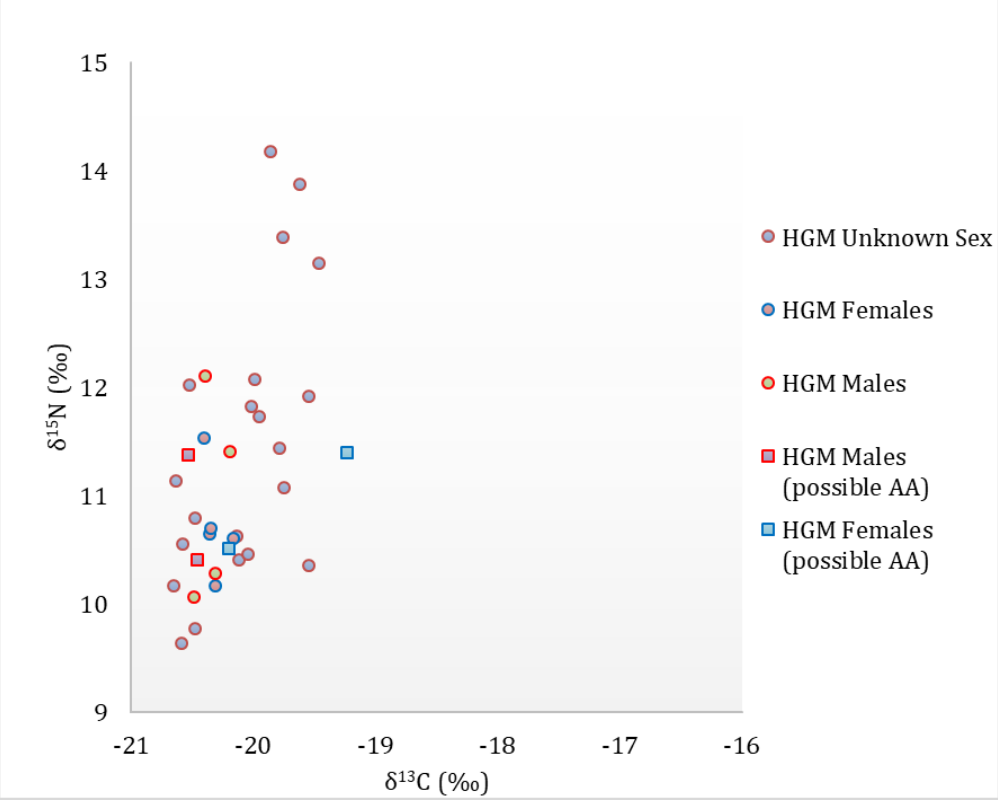


Figure 6-6: Intrapopulation diversity (sex and possible African/mixed Ancestry (AA)) Bone collagen $\delta^{13}C$ and $\delta^{15}N$ values for Females (n = 7), Males (n = 6) and Unknown sex (n=21) from Hazel Grove.

No statistically significant differences in both $\delta^{13}C$ and $\delta^{15}N$ values were found between individuals who exhibited traits of African/mixed ancestry and the rest of the adults at Hazel Grove site suggesting that access to food was the same ($\delta^{13}C$ Mann-Whitney U test U=64.000, p=0.857; $\delta^{15}N$ Mann-Whitney U test U=69.500 p=0.624). However, one of the individuals of African/mixed ancestry, HGM 14, has a slightly higher $\delta^{13}C$ value suggesting potential consumption of some C_4 or marine resources (Figure 6.6).

6.6.5 Human-animal isotope offsets

When the faunal and non-infant human sample populations are plotted together (Figure 6.7), older nonadult and adult $\delta^{13}C$ and $\delta^{15}N$ values from both sites are consistent with the consumption of locally available fauna from both Cross Street and Norton Priory. It is also evident that the domestic fowl are closer to humans.

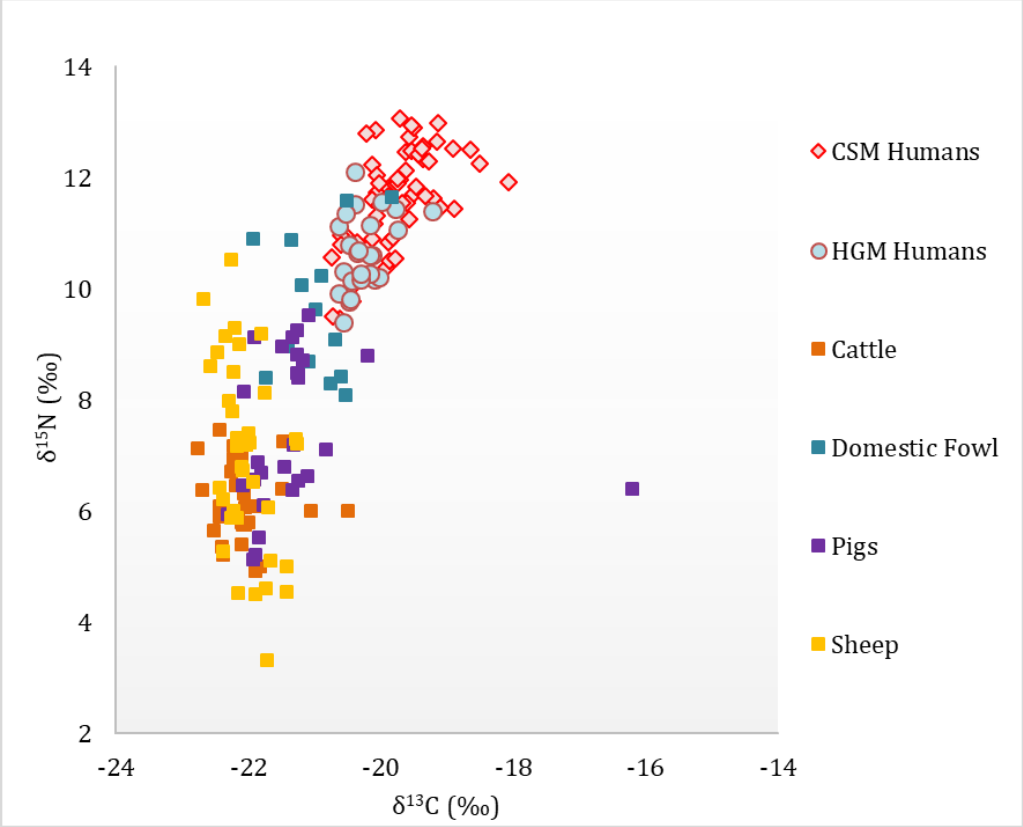


Figure 6-7: Human Hazel Grove, Manchester (HGM) and Cross Street, Manchester (CSM) and combined faunal bulk collagen results from Cross Street, Manchester and Norton Priory, Chester, bone collagen results. Infants are excluded from this comparison.

The $\delta^{13}C$ offsets of humans against omnivores ($\delta^{13}C_{Human\text{-}omnivores}$) for both the Cross Street and Hazel Grove populations (1.4‰ and 0.9‰ respectively) and against the herbivores for Hazel Grove population ($\delta^{13}C_{Hazel\ Grove\text{-}herbivores}$ = 1.8‰) are within the range of trophic level shift of 0-2‰ (Lee-Thorp et al. 1989; Bocherens and Drucker 2003), and just above the upper limit for the Cross Street

population ($\delta^{13}C_{Cross\ Street\text{-}herbivores}$= 2.3‰). Regarding the $\delta^{15}N$ human-fauna offsets, the offsets between the Cross Street and the Hazel Grove populations and herbivores (5.0 ‰ and 4.0 ‰ respectively) and Cross Street omnivores (3.5‰) fall within the expected trophic level enrichment of 3-5‰(Schoeninger 1985; Bocherens and Drucker 2003) but those from Hazel Grove fall below the lower limit of 3‰ ($\delta^{15}N_{Hazel\ Grove\text{-}omnivores}$ = 2.5‰).

6.7 Discussion

6.7.1 Animal management

The herbivore (cattle and sheep) carbon and nitrogen stable isotope data from this study indicate that they consumed a diet mainly consisting of C_3 terrestrial resources, conforming to the expectations that livestock was foddered on C_3 forage during the post-medieval period. However, the omnivores (pigs and domestic fowl) have slightly higher mean $\delta^{15}C$ and $\delta^{15}N$ values than herbivores suggesting diets enriched in ^{13}C and ^{15}N. One pig in particular (CSM 6217), exhibited a very high $\delta^{13}C$ value (-16.2‰) and low $\delta^{15}N$ (6.4‰) implying that it might have been foddered on C_4 plants most likely to be maize, used for fodder in industrial Britain as has been suggested by other authors (Brassley 2000; Martin 2012). Another possibility is that the pig might have originated from a different country where it was fed a C_4 diet. There is evidence that some pigs were imported from the Americas, Australia, Africa, and Canada during the 18th century (Capie and Perren 1980; Plumb 2010). The differences between herbivores and omnivores are probably indicative of omnivores being backyard animals that consume food scraps discarded by humans resulting in higher nitrogen values as has been reported in other studies (Müldner and Richards 2007; Hammond and O'Connor 2013).

Compared to the livestock from London and sheep from Durham, differences are only observed in the $\delta^{15}N$ values in sheep. The contrast in $\delta^{15}N$ values between different sites suggests that the sheep were managed differently and probably were sourced from different geographical areas. Durham, Cross Street, and London sheep are similar but Norton Priory sheep, in particular, exhibit a wide range of $\delta^{15}N$ values (+4.6‰ to 10.5‰) which may suggest that they originate from different locations, which is probably reflecting the provisioning of urban settlements during this period throughout which animals were being supplied from a wide range of sources (Drummond and Wilbraham 1939). Historical documents suggest that in order to satisfy the demand for sheep in Cheshire during the 18th century, they were brought from the Welsh borders (Redhead et al. 1792; Marshall 1818; Scola 1992) or urban settings like London where sewage and animal dung were being utilised for intensive manure, which would result in high $\delta^{15}N$ isotope values (Drummond and Wilbraham 1939; Beaumont et al. 2013b; Bleasdale et al. 2019). However, when compared to London sheep (Figure 6.3), the Norton Priory sheep still have very high values. It is possible that they may have been sourced from areas where sheep were probably being stocked in high densities resulting in greater ^{15}N enrichment

than those in London. Several archaeological studies have found a link between high stocking density or grazing rate with enrichment in $\delta^{15}N$ values (Schwertl et al. 2005; Britton et al. 2008; Fraser et al. 2011; Müldner et al. 2014). Nineteenth-century accounts indicate high stocking densities ranging from 1,000 to 3,200 sheep per acre, in areas that were being extensively manured in England and the Welsh borders (Marshall 1818; Ellman 1831; Woodward 2008).

6.7.2 Socioeconomic influence on Manchester diet

In general, the $\delta^{13}C$ and $\delta^{15}N$ values of individuals from post-medieval Manchester indicate that their diet consisted of C_3 terrestrial plants as well as meat and/or other animal products from animals foddered on C_3 resources. However, diet and social life are inextricably linked in post-medieval England (Bleasdale et al. 2019), therefore great inter-population diversity in the $\delta^{13}C$ and $\delta^{15}N$ values consistent with their diverse social origins is expected between the Cross Street and Hazel Grove populations. High $\delta^{13}C$ and $\delta^{15}N$ values for some of the Cross Street adults may be due to the consumption of more ^{13}C enriched foods, marine sources or high trophic level animal protein (Schoeninger and DeNiro 1984), which were unavailable or in lower quantities in the lower class Hazel Grove population (Scola 1992). The amount of meat consumed during this period varied according to class such that the middle and upper classes could afford to eat meat daily whereas eating meat for the lower classes was a rare luxury (Beeton 1901; Oddy 2000; Thirsk 2007). Engels's description of food consumption in Manchester emphasised that access to food such as meat in the city was wage dependent (Engels 1845, 73). Nonetheless, the human-animal offsets in $\delta^{15}N$ values at Cross Street were not very high for both herbivores and omnivores (5.0 ‰ and 3.5 ‰ respectively). These values fall within the expected trophic level enrichment of 3-5‰ (Schoeninger 1985; Bocherens and Drucker 2003) which suggests that marine resources were not a significant component of the diet. The $\delta^{13}C$ offsets of Cross Street humans against herbivores (2.3‰) is just above the upper limit of the trophic level shift of 0-2‰ (Lee-Thorp et al. 1989; Bocherens and Drucker 2003) whereas that of Hazel Grove is lower and within the trophic level shift of 0-2‰ suggesting consumption of more ^{13}C enriched foods or animals foddered on C_4 resources for Cross Street individuals (Schoeninger and DeNiro 1984). The Cross Street population is a middle/upper-class population, therefore, the C_4 foods were most likely to be sugar from sugarcane rather than maize, which was mostly consumed by the Irish poor during this time. One individual with possible African or mixed ancestry from Hazel Grove has a slightly higher $\delta^{13}C$ value (Figure 6.6), implying that there is the potential that she may have consumed food slightly enriched in ^{13}C (e.g., maize or animals foddered on sugarcane waste) compared to the rest of the population. This individual was aged between 26 and 45 years, therefore, it cannot be ruled out that she may have originated from a place where she consumed a C_4 crop-rich diet, and their bone collagen may have later shifted towards the C_3 local diet. This possibility could be tested by using strontium and oxygen isotope analysis to

ascertain whether they had a different geographical origin in childhood or dentine serial sectioning to explore childhood diet (Lamb et al. 2012; Bleasdale et al. 2019).

Comparison between the older nonadults from both sites shows that despite having similar $\delta^{13}C$ values, the Cross Street older nonadults were consuming more animal protein than the Hazel Grove population, in line with the results obtained for the adults. On the other hand, diet does not seem to vary among infants between different socio-economic classes, which might seem surprising. However, Newman and Gowland's (2017) study of different status infants from Chelsea Old Church (high status), St Benet Sherehog (middle status), Bow Baptist (middle status), and Cross Bones (low status) demonstrated that during the 18th and 19th century, all children suffered high rates of similar food-related disorders regardless of class. The age at which infants were weaned during this period seems to have been the same regardless of class but for different reasons. Infants from middle or upper status were weaned early because breastfeeding was considered inconvenient or unfashionable. As a result, infants were given artificial feeds such as pap (a mixture of flour or breadcrumbs cooked in milk or water), panada (a bread broth, or milk flavoured with sugar, eggs, or spices), or cow's milk which were all not nutritious. On the other hand, those from lower classes were weaned early and given similar foods because food allocation prioritised men (Drummond and Wilbraham 1939; Wickes 1953; Fildes 1995; Nitsch et al. 2011; Britton et al. 2018).

An assessment of access to food resources within the two populations themselves yields interesting differences between the two sites. The $\delta^{13}C$ and $\delta^{15}N$ results indicate that food access was similar across all age groups at the Cross Street site. This is expected as middle and upper-class children had access to a lot of the same food as the adults (Turner 1995). On the other hand, the nonadults from Hazel Grove consumed foods less enriched in ^{15}N when compared to adults, suggesting consumption of more animal protein in adults which is expected in lower-class populations (Rowntree 1901).

In terms of gender, no differences were observed in access to food between the sexed individuals in the Cross Street population. An account by Davidoff and Hall (2018) of men and women of the English Middle Class 1780–1850 suggested that during this time, middle-class gender roles were flexible such that some women were working in businesses and other occupations as much as men. Therefore, one would not necessarily expect differences between the sexes in a middle/upper-class population such as the one at Cross Street. On the other hand, although only a few individuals were sexed in the Hazel Grove population and, therefore, sex differences cannot statistically be examined, visually, there also seems to be no differences in diet between the working-class individuals sexed at Hazel Grove (Figure 6.6). This is unexpected as various sources indicate that there were differences in the food consumed by the working-class man and women during the industrial period (Broomfield 2007; Clayton and Rowbotham 2008; Clayton and Rowbotham 2009). These authors have indicated that, with men forming a larger group of working-class people, the hours they endured on physical activity meant

they required more calories than women. Since men were supposedly getting a larger share of the family's food and better-quality meat, men in this population would be expected to have higher carbon and nitrogen values. However, as stated earlier, there is no isotopic difference between different tissues (and milk) of an animal, therefore, differences would only show up if the women had a lower proportion of animal protein in the diet. Additionally, it cannot be ruled out that this was the case as there are only a few individuals sexed in this population.

6.7.3 Comparison of Manchester sites with contemporary English sites

Bone collagen isotope data from this study were compared with data from nine contemporaneous sites in England from St Barnabas, Kensington and Queen Chapel Savoy (Bleasdale et al. 2019); Lukin Street (Beaumont et al. 2013b); Spitalfields, London (Nitsch et al. 2010); St Luke's Chelsea and Holy Trinity, Coventry (Trickett 2006), Churchyard of St. Martin's-in-the-Bull Ring, Birmingham (Richards 2006), Plymouth and Gosport (Roberts et al. 2012).

The Hazel Grove site displays the lowest mean $\delta^{13}C$ and $\delta^{15}N$ values in this group while St Barnabas has the highest (Figure 6.8). Except for the Gosport population, all the other populations display higher $\delta^{13}C$ and $\delta^{15}N$ values when compared to both Cross Street and Hazel Grove sites. However, it should be noted that although the Cross Street and Hazel Grove $\delta^{15}N$ values fall within the range of results obtained for the Gosport and Plymouth populations and mean $\delta^{13}C$ lies within the range of results obtained for the Gosport population, comparisons between these populations are very problematic because the Gosport and Plymouth populations were naval sites (Roberts et al. 2012), therefore, have different contexts from the Manchester sites. In addition, comparisons between the Queen's Chapel and either Cross Street or Hazel Grove populations is also difficult because the Queen's Chapel site is a mixed-status population (Bleasdale et al. 2019). Considering this, Hazel Grove and Cross Street were only compared with populations of similar status. Individuals from St Luke's, St Barnabas, Spitalfields and St Martin Birmingham were classified as being from higher socioeconomic class whereas those from Lukin Street and Coventry were classified as being from low economic class (Richards 2006; Trickett 2006; Nitsch et al. 2010; Beaumont et al. 2013b; Bleasdale et al. 2019).

*Table 6-7: Statistical comparison between the $\delta^{13}C$ and the $\delta^{15}N$ values of human remains between Cross Street and the middle/upper-class comparative sites as well as Hazel Grove and the lower-class comparative sites. *No statistically significant differences observed between Cross Street and Churchyard of St. Martin's-in-the-Bull Ring, Birmingham populations.*

Site	*N*	*Independent samples Mann-Whitney U Test $\delta^{13}C$ (‰)*	*Independent samples Mann-Whitney U Test $\delta^{15}N$ (‰)*
Cross Street Chelsea	68 32	U=256.000, p=0.000	U=341.500, p=0.000
Cross Street St Barnabas	68 25	U=325.000, p=0.000	U=68.000, p=0.000
Cross Street Spitalfields	68 164	U=990.500, p=0.000	U=741.000, p=0.000
Cross Street St. Martin's, Birmingham	68 18	U=203.500, p=0.000	U=439.000, p=0.066*
Hazel Grove Lukin Street	25 119	U=155.000, p=0.000	U=112.500, p=0.000
Hazel Grove Coventry	25 13	U=18.000, p=0.000	U=14.500, p=0.000

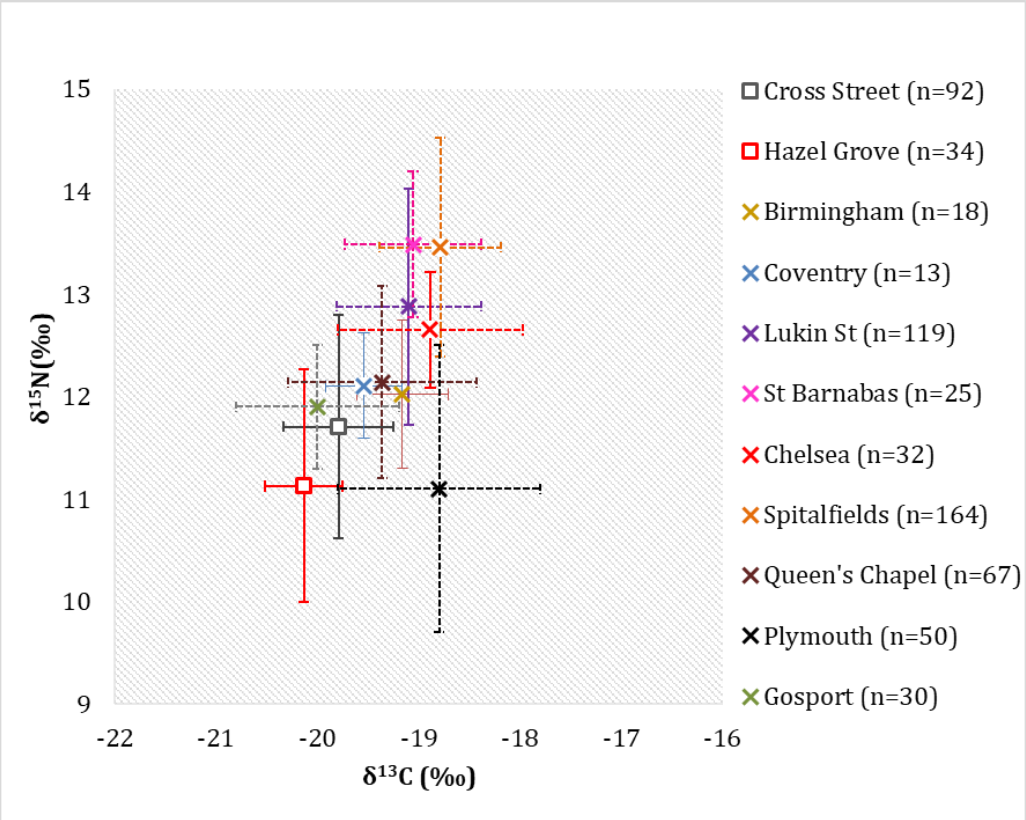


Figure 6-8: A biplot of $\delta^{13}C$ and $\delta^{15}N$ values, showing mean and 1σ for individuals from Cross Street and Hazel Grove Manchester and seven contemporary English sites St Barnabas and Queen Chapel Savoy (Bleasdale et al. 2019); Lukin Street (Beaumont et al. 2013b); Spitalfields, London (Nitsch et al. 2010); St Luke's Chelsea and Holy Trinity, Coventry (Trickett 2006), Churchyard of St. Martin's-in-the-Bull Ring, Birmingham (Richards 2006), Plymouth and Gosport (Roberts et al. 2012).

Except between the Cross Street and Churchyard of St. Martin's-in-the-Bull Ring, Birmingham populations' $\delta^{15}N$ values, the rest of the $\delta^{13}C$ and $\delta^{15}N$ value comparisons show significant differences suggesting variations in diet despite similarities in socioeconomic status (Table 6.7). For the Cross Street and Churchyard of St. Martin's Birmingham populations, despite having similar $\delta^{15}N$ values, their $\delta^{13}C$ values are significantly different. It is possible that, because the city of Birmingham was wealthier than Manchester during the 19th century (Wise and O'Thorpe 1950; Wise 1951), St. Martin's individuals had more access to cane sugar compared to the Cross Street individuals, which may account for the differences in the $\delta^{13}C$ between the two populations despite their similar status. However, although this difference is significant, it is only 0.8‰, which is not very large and therefore, not necessarily a result of differences in diet. It is most likely that the isotopic variability between these two sites is from analysis having been undertaken in different laboratories as has been observed in other studies (Pestle 2014). Cross Street and St Martin's are the only sites that do not show a statistically significant difference in $\delta^{15}N$ values and this similarity, most likely stems from the greater availability of terrestrial animal or aquatic resources protein to Londoners when compared to the rest of the country. All the London sites have higher mean $\delta^{13}C$ and $\delta^{15}N$ values suggesting that London's access to ^{13}C enriched foods such as cane sugar or maize (Beaumont 2013; Beaumont et al. 2013b; Bleasdale et al. 2019) and/or high trophic level protein was different from the other cities in England (Spencer 2000; Picard 2006; Trow-Smith 2013; Metcalfe 2015). The $\delta^{13}C_{coll}$ offsets of humans against omnivores for London populations ($\delta^{13}C_{London\text{-}omnivores}$ = 1.8‰) are within the range of trophic level shift of 0-2‰ (Lee-Thorp et al. 1989; Bocherens and Drucker 2003), and just above the upper limit for herbivores ($\delta^{13}C_{London\text{-}herbivores}$ = 2.9‰). The $\delta^{15}N$ human-fauna offsets between the London populations fall outside the upper limit of 5‰ ($\delta^{15}N_{London\text{-}herbivores}$ = 6.7‰ and $\delta^{15}N_{London\text{-}omnivores}$ = 5.5‰; see Chapter 7) reflecting the addition of other resources such as omnivores and marine/freshwater sources (Lee-Thorp et al. 1989; Bocherens and Drucker 2003). For the London sites, meat and fish would have been an important part of their diet due to improvements in transportation and interventions from the government that lowered fish prices and increased the number of livestock brought to the city (Drummond and Wilbraham 1939; Stead 1985; Burnett 2004). In addition, the higher $\delta^{15}N$ values in London also resulted from agricultural practices in the city such as sewage waste being used as manure (Drummond and Wilbraham 1939) and market gardens using intensive manure (Strype 1720).

6.8 Conclusions

This paper presents the stable isotopic carbon and nitrogen ratios of two Manchester city populations of different socioeconomic statuses, allowing a novel insight into the diet of post-medieval populations in Greater Manchester, England. The data from this study has significantly added to the animal dietary baseline for post-medieval England. The diet of most individuals from Manchester was

predominantly based on plants and animal protein from C_3 terrestrial environments, common for the whole of England during this time. The hypothesis that the diet of middle/upper classes was different from that of lower classes in the post-medieval period was supported in the older nonadults and adult populations from both sites. The infants showed no significant differences in the isotope values between the two sites, despite socio-economic status. Moreover, this study revealed that individuals from London had more access to ^{13}C enriched foods such as cane sugar or marine protein than those from Manchester despite similar socioeconomic status. Although the elevated $\delta^{13}C$ values from Cross Street could suggest that these individuals had minor contributions of C_4 resources, there is a need to conduct further analysis such as bone carbonate or combined stable isotope analysis of bulk bone collagen and single amino acids analysis for clarity. The enrichment of ^{13}C values as a result of the consumption of cane sugar or maize (carbohydrates) is difficult to ascertain using only bone collagen analysis as 75% of the carbon in bone collagen is derived from dietary protein whereas only about 25% is derived from fats and carbohydrates (Fernandes et al. 2012). While the slightly elevated $\delta^{13}C$ value for the Hazel Grove site individual who might have been of African/mixed ancestry could be a result of her originating from another area or country; strontium and oxygen isotope analysis of this individual and the three other individuals with traits of African/mixed ancestry is therefore needed to investigate this possibility comprehensively. Expanding these analyses to additional post-medieval sites, especially in the North, would assist in developing a detailed picture of the diversity in diet and lifeways during 17th to 19th century England.

Chapter 7: Reconstruction of diets in industrialised London and Manufacturing Northern towns in England from the 17th to 19th centuries using bone carbonate and collagen stable isotope analysis.

7.1 Introduction

Analysis of stable carbon isotopes in bone carbonate ($\delta^{13}C_{carb}$) has been routinely employed to examine the adoption and spread of maize, a C_4 plant, across the Americas (Tykot et al. 1996; Tykot 2006). In northwest Europe, however, where C_3 plants (e.g., wheat, barley, oats), tend to predominate as dietary staples throughout the archaeological record, isotope analysis of bone carbonate has rarely been applied. Here, this study focuses on what is arguably another dramatic addition of C_4 crops to human diets, namely the uptake of cane sugar and potentially maize, in the diets of 17th to 19th century populations in England, in a significant phase of food globalisation. In addition, the addition of C_4 diets in animal populations from this period is also explored here. A large new multi-isotope dataset deriving from several post-medieval sites representing major regional centres in England to consider diets in terms of geographical location and socioeconomic status is presented. This study represents the first application of carbon isotope analysis of bone carbonate ($\delta^{13}C_{carb}$) to English skeletal material alongside the more traditional collagen-based isotopic analysis ($\delta^{13}C_{coll}$, $\delta^{15}N$).

The period from the seventeenth to the nineteenth century in England, witnessed unprecedented changes in almost all aspects of daily life, among them, shifts in food acquisition and consumption across both rural and urban environments linked to both the Agricultural and Industrial Revolutions although the role of each in these transformations is hotly debated (Crafts 1985; Overton 1996; Burnett 2005). Agricultural accounts reveal huge increases in arable output as well as improvements in animal husbandry that sustained an increasingly urbanised economy during this period (Turner et al. 2001; Clayton and Rowbotham 2008). The number of individuals employed in agriculture declined from about 55% of the whole population in 1700 to nearly 36% by 1801 (Overton 1996). The expanding numbers of urban dwellers required an industrial provisioning system reliant upon new food technologies (Oddy and Burnett 1992; Allen 1994). The political and social systems in England also had to contend with various wars during this period (e.g., the outbreak of the French wars in 1793) that frequently resulted in food shortages. With the expanding population, growth in urban or industrial regions such as London and the manufacturing northern towns, increased competition and social imitation among different classes led to differences in purchasing power and eating habits as well as distinctive tastes across all classes (Shammas 1984; Burnett 2005). Dietary surveys conducted in the 19th-century by Dr. Edward Smith revealed marked regional differences in diet (Smith 1864;

Barker et al. 1970). It is the intention, therefore, of this paper to explore these differences through a study of comparative dietary consumption among eleven populations of different socioeconomic statuses from different cities in the manufacturing north and London to explore structured relations and dietary trends in 17th-19th century industrial England.

7.2 Diet in 17th to 19th century England

Diet during this period was predominantly cereal based with a small meat component, this difference being more pronounced in lower socioeconomic classes who consumed considerably more bread relative to meat (Oddy 2000). Post-medieval England was characterised by a high degree of socioeconomic stratification and social status was clearly reflected in the different dietary patterns of the rich and the poor throughout this period. The rich enjoyed greater access to foods such as meat, fish, and cane sugar due to the privilege of wealth which also provided them with greater access to non-local food markets (Barker et al. 1970; Oddy 1990; Gumerman 1997). In general, food for the upper classes included white wheaten bread, vegetables, fruits, cane sugar, tea, cheese, eggs, meat, and fish (Oddy 2000; Clayton and Rowbotham 2009). For the poor, certain foods such as cane sugar, cheese, and occasional supplies of meat were prioritised to the males who supposedly required more energy to work and as a consequence, females and children subsisted on mainly cheap rye or barley bread and/or potatoes (Burnett 2005; Horrell and Oxley 2012). On the other hand, for the middle/upper classes, women worked in similar jobs as men, therefore, both sexes in these populations consumed similar foods (Davidoff and Hall 2018), Due to preservation and transport restrictions, most fish consumed up to the end of the 18th century were locally sourced. Fish consumption was higher in London than in the northern manufacturing towns (Mayhew 1967; Burnett 2004; Panayi 2008) and was generally not consumed in rural areas except those located close to the sea or local rivers (Thirsk 2007). Fish were consumed sparingly by the lower classes, with the northern poor only affording to eat mackerel from the Isle of Man and/or cured herring from the herring fleets in Scotland and the London poor eating oysters and smoked herring (Aikin 1785; Chaloner 1959; Coull 1996; Broomfield 2007; Clayton and Rowbotham 2009). The London middle/upper-class individuals, on the other hand, consumed fish such as cod, haddock, whelks, salmon, halibut, lobsters, mullet, bream, pike, and tench from the Billingsgate fish market and garden pond cultures and those in the northern manufacturing towns consumed cod, lobsters, turbot, and soles from the Yorkshire coast, Lancashire coast and the East coast (Mayhew 1967; Coull 1996; Dyer 1988; Burnett 2004; Panayi 2008; Thirsk 2007). Similarly, the lower-class individuals from both London and the manufacturing northern towns consumed little or no meat or offcuts (Chaloner 1959; Stead 1985; Thirsk 1990; Scola 1992). However, it must be noted that, as is the case with fish, Londoners of all social classes, consumed more meat than those in similar classes in the rest of the country during this period (Trow-Smith 2013). Although the available meat included beef, poultry,

pork, and sheep, poultry and pigs were locally available in great quantities in the country (Chaloner 1959; Scola 1992; Burnett 2005; Broomfield 2007; Clayton and Rowbotham 2009). Livestock such as cattle and sheep were brought to London from as far as Inverness in Scotland, the west of England, the chalk hills and Breckland (Spencer 2000; Picard 2006; Trow-Smith 2013; Metcalfe 2015) while in the northern manufacturing towns, they came from the Welsh Borders, North Wales, Lincolnshire, Scotland and the neighbouring counties (Redhead et al. 1792; Marshall 1818; Scola 1992). It must be noted, however, that there were a few pigs and cattle that were not indigenous to Britain or Western Europe but were imported from places such as the Americas, Australia, Africa, and Canada (Capie and Perren 1980; Plumb 2010).

7.2.1 C_4 input in diet

This study's main focus is on investigating the increase in consumption of C_4 resources during this period. While crops consumed in England were predominantly C_3 cereals such as wheat, rye, barley, oats, and increasingly potatoes, a small number of other C_4 species such as maize and cane sugar found their way into the country through improved trade links with the Americas, Asia and Africa (Mintz 1986; Oddy 2000; Thirsk 2007). Maize appeared in the country after it was introduced as relief food to the Irish poor who migrated to Britain to work in workhouses during the Great Irish Famine of 1845 to 1849 (Dudley-Edwards and Williams 1956; Ó'Gráda 1989). Historical sources, however, indicate that although maize became relief food for the Irish poor, until the 20th century, maize was not widely accepted by the English regardless of class except as animal fodder (Holland 1919; Messer 2000; Martin 2012). Cane sugar consumption increased due to Britain's colonisation of the West Indies in the 17th century, which led to average per capita cane sugar intake in the country to increase by 11.8 kg per year between 1700 and 1850 (Figure 7.1) (Mintz 1986; Johnson et al. 2007; Walvin 2017). Cane sugar intake further increased after the 1874 abolition of sugar tax made it cheaper and affordable to all classes (Mintz 1986; Langer et al. 1991; Marshall 1998; Walvin 2017).

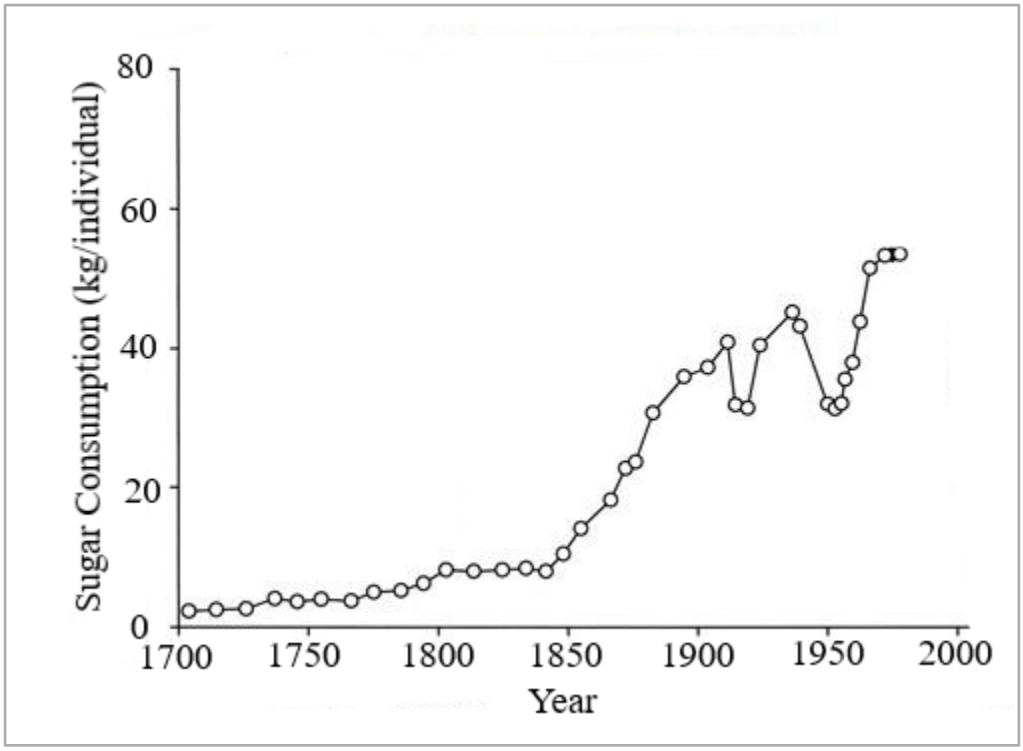


Figure 7-1: Sugar intake per capita in the United Kingdom from 1700 to 1978 (Yudkin 1972; Deer 1949; Johnson et al. 2007).

The British Naval blockade after 1700, which prevented cane sugar imported from the West Indies from being sent to other European countries such as Germany, France, Italy, and Austria resulted in the shortage of cane sugar in those countries. This led them to begin developing techniques to extract sugar from beets so that by the 19th century they were consuming more beet sugar than cane sugar (Ballinger 1974; Mintz 1986; Johnson et al. 2007). With the British consuming more cane sugar than other western European countries, and the Irish immigrants who had consumed maize migrating in the country around that time, this makes Britain the ideal place to study the consumption of cane sugar and/or maize in the 18th and 19th centuries.

7.3 Isotopic approaches to Post-Medieval diets

This paper uses multiple tissues, bone collagen, and bone mineral for isotope analysis. The mechanisms underlying the fractionation of stable isotopes within these tissues and different ecosystems have been outlined in detail elsewhere (for a detailed review see Schwarcz and Schoeninger 2012). Carbon isotopic ratios can be used to differentiate C_3 and C_4 plant foods with C_3 plants (e.g., wheat and barley) having lower $\delta^{13}C$ values than those of C_4 plants (e.g., maize and cane sugar). On the other hand nitrogen isotope ratios generally act as markers signalling the trophic level of an organism , demonstrated by a $\delta^{15}N$ value enrichment of approximately 3–5‰ with each trophic step (Deniro and Epstein 1981; Schoeninger et al. 1983; Minagawa and Wada 1984). While the $\delta^{13}C$ ranges in marine and C_4 foods overlap (higher $\delta^{13}C$ values), $\delta^{15}N$ can also be used as an indicator of

marine dietary sources because of the longer trophic chains common in marine ecosystems (Chisholm et al. 1982; Schoeninger and DeNiro 1984; DeNiro 1987; Mays 1997; van Klinken 1999).

Due to turnover, bone tissues represent an individuals' average diet over a number of years prior to death, depending on the skeletal element (Cox and Sealy 1997; Hedges et al. 2007; Meier-Augenstein 2017). Bone collagen $\delta^{15}N$ provides information on the main protein sources of the diet. While bone collagen $\delta^{13}C$ values ($\delta^{13}C_{coll}$) also represents the protein sources in the main, about 25% of bone collagen (non-essential amino acids) is typically synthesised from other dietary sources (lipids/carbohydrates) (Fernandes et al. 2012). Bone carbonate $\delta^{13}C$ values ($\delta^{13}C_{carb}$), however, reflect whole diets (carbohydrates, lipids, and proteins) because bone carbonate forms in equilibrium with blood carbonate that is itself a product of energy metabolism (Krueger and Sullivan 1984; Ambrose and Norr 1993). Bone $\delta^{13}C_{carb}$ values can reveal C_4 contributions from the whole diet that may be obscured when using bone $\delta^{13}C_{coll}$ alone. The utilisation of both $\delta^{13}C_{coll}$ and $\delta^{13}C_{carb}$ in isotopic studies therefore provides a more complete estimate of several dietary inputs. In Europe, studies that have made use of both tissues are sparse (Grupe et al. 2009; Reitsema et al. 2010; Yoder 2012; Olsen et al. 2016). Although $\delta^{13}C_{coll}$ data from these studies suggested that dietary protein was primarily derived from C_3 resources, the differences in the $\delta^{13}C_{carb}$ values among the individuals in some sites indicated that some individuals may have consumed more of the C_4 or marine foods than others e.g. Reitsema et al. (2010) and Yoder (2012), thereby showing how $\delta^{13}C_{carb}$ can augment $\delta^{13}C_{coll}$ results.

Previous studies of post-medieval diet in England have all focused on the utilisation of $\delta^{13}C_{coll}$ and $\delta^{15}N$ but the majority of studies have been conducted on populations from London, with only a few from elsewhere (Birmingham (Richards 2006) Coventry (Trickett 2006) and Plymouth and Gosport (Roberts et al. 2012)). These studies have focused on exploring the diets of different social classes e.g. lower (Trickett 2006; Beaumont 2013; Beaumont et al. 2013a; Beaumont et al. 2013b), mixed (Bleasdale et al. 2019), upper class diets (Richards 2006; Trickett 2006; Nitsch et al. 2010; Nitsch et al. 2011; Brown and Alexander 2016; Bleasdale et al. 2019), the diet of naval seamen (Roberts et al. 2012) and the dietary input of migration and famine (Beaumont 2013; Beaumont et al. 2013a; Beaumont et al. 2013b).

The results from these studies, so far, indicate that London populations have diets dominated by terrestrial C_3 resources and only one individual from Queen's Chapel of the Savoy (QCS 1123) exhibits a major C_4 component in their diet. Most individuals show enrichment in ^{15}N associated with the consumption of meat and aquatic (freshwater/marine) foods, with middle and upper social class individuals' data indicating greater access to these resources, as might be expected (Trickett 2006; Nitsch et al. 2010; Nitsch et al. 2011; Brown and Alexander 2016; Bleasdale et al. 2019). In contrast, Lukin Street, a lower-class population representing poor first-generation Irish immigrants presents high $\delta^{15}N$ values which have been posited to be due to physiological processes caused by periods of

starvation rather than high protein consumption (Beaumont 2013, 128). Populations outside London also reveal a terrestrial C_3 dominated diet, but individuals from a naval cemetery in Plymouth show ^{13}C enrichment potentially in keeping with seafaring to North America where C_4 resources were commonly consumed both directly and indirectly through animal fodder (Roberts et al. 2012). Middle class populations from Birmingham and Coventry (Richards 2006; Trickett 2006) possess $\delta^{15}N$ values that are lower than middle class populations in London indicating some differentiation between London based populations and those of equivalent status elsewhere in England. All these studies have alluded to the potential for some inclusion of C_4 resources in the diets of some individuals at each of their respective sites, visible as slightly higher $\delta^{13}C_{coll}$ values. However, C_4 sources cannot be decoupled from marine sources, particularly where $\delta^{15}N$ values are high when using bulk bone collagen isotopes alone. Bone carbonates may allow greater clarity as to the presence of C_4 carbohydrates in the diet.

7.4 Bone carbonates and diagenesis

Bone carbonates are more susceptible to diagenesis than collagen and, unlike collagen, there is a lack of established quality control criteria (Garvie-Lok et al. 2004). After burial, archaeological bone undergoes alterations driven by both biological and physico-chemical processes (Hollund et al. 2013). Diagenetic changes in bone can occur by the action of water in the burial environment which can dissolve components of bone mineral; infilling of pore spaces with cementitious material precipitating from surrounding water; recrystallisation and uptake of ions as well as hydrolysis (Monge et al. 2014). Recrystallisation is due to dissolution and reformation of crystals, with the addition of new ions such as carbonates, phosphates, calcium, and strontium into the crystal structure through adsorption and diffusion (Monge et al. 2014). As a result of diagenesis, bone mineral undergoes changes in crystallinity leading to difficulties in the interpretation of isotopic signatures as indicators of diet. Therefore, evaluating whether the original isotope compositions of bone mineral fractions are not altered by diagenesis is an essential step for stable isotope analysis interpretation (Shin and Hedges 2012).

Diagenesis is commonly assessed by determination of crystallinity of the subject samples using Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) with the parameter of interest being the infrared splitting factor (IRSF) (Weiner and Bar-Yosef 1990; Hollund et al. 2013; Sivakumar et al. 2014). However, crystallinity may fail to describe the extent of chemical and mineralogical changes; therefore, it cannot be used in isolation (Lebon et al. 2010, 2265–6). Another parameter that is used is the carbonate-to-phosphate ratio (C/P), a measure of diagenesis that reflects the changes to the carbonate in bioapatite crystals relative to the phosphate content in a bone sample (Wright and Schwarcz 1996) (Table 7.1).

Table 7-1: FTIR indices and the equations used to calculate them in this study.

IRSF	This index is calculated as the sum of the two infrared peak intensities of the $v_4PO_4^{3-}$ band at 565cm^{-1} and 600cm^{-1} divided by the intensity of the valley between them at 590cm^{-1} following Weiner and Bar-Yosef (1990)'s equation. Greater separation of these phosphate peaks is observed on apatites with larger and more ordered crystals which in turn results in higher IRSF values (Weiner and Bar-Yosef 1990). $[IRSF = [565_{ht}cm^{-1} + 600_{ht}cm^{-1}]/590_{ht}cm^{-1}]$
C/P ratio	The carbonate content (C/P ratio) is calculated by dividing the intensity of the $v_3CO_3^{2-}$ band at 1415 cm^{-1} by the intensity of the $v_3PO_4^{3-}$ band at 1035 cm^{-1} following Wright and Schwarcz (1996)'s equation $[C/P = 1415_{ht}cm^{-1}/1035_{ht}cm^{-1}]$.

In addition to these indices, the FTIR spectra are examined for indications of the presence of the calcite ($CaCO_3$) band at 712 cm^{-1} peak which indicates non-apatite mineral structures in bone (Hunt et al. 1950; Baxter et al. 1966). It has been posited that dissolution and recrystallisation processes may incorporate calcite into the crystal lattice of a bone (LeGeros 1981; Lee-Thorp et al. 1989). Price et al (1992) demonstrated that the presence of crystalline impurities in bone such as calcite shows a likely relationship between organic preservation and mineral in samples whereby calcite contamination occurs when collagen is destroyed.

This investigation, therefore, applies FTIR-ATR analysis using these two parameters and the presence of calcite to assess the extent of diagenesis in all the study samples prior to the stable isotope analysis of bone carbonate.

7.5 The sites in their historical context

The human bone remains analysed for this study were obtained from eleven sites- four sites from London and seven sites from the manufacturing northern towns (Figure 7.2) and date from the late 16th to 19th century with most, however, dating from the 18th to 19th centuries (Table 7.2). The samples were explicitly selected from contemporaneous sites but included different social status groups and regions to assess the extent, if any, of variation of diets with socio-economic status in post-medieval England.

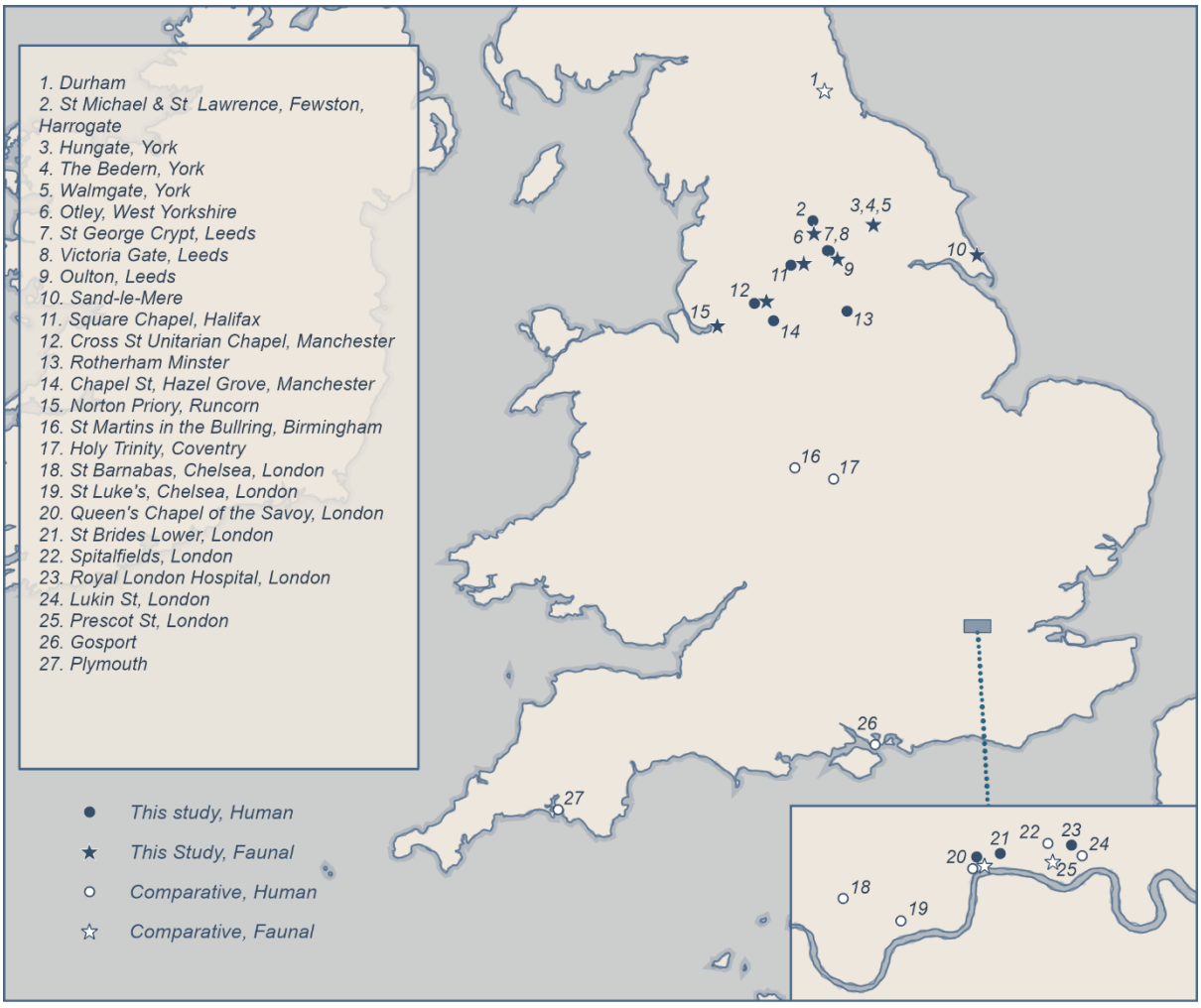


Figure 7-2: Map of post-medieval England showing the locations of the sites of study and other post-medieval sites mentioned in the text. The city of London is shaded, and sites are given numbers from 18 to 25.

*Table 7-2: Human burials sampled for bone collagen and bone carbonate analysis from all sites, with associated dates and economic status. +Difficult to confirm as there would have been people buried from many religious backgrounds, however they are more likely non-denominational or nominally Anglican *The Fewston assemblage includes rural farmers and industrial mill workers and although the individuals recovered at the site date between 1550-1908 all the 7 individuals date between the 18th and 19th centuries.*

Site name	*Location*	*Status*	*Period*	*Denomination*	*Reference*
Fewston (n=7)	Harrogate	Mixed*	18th-19thc.	Church of England	(Caffell and Holst 2010; Gowland and Caffell Forthcoming)
Rotherham Minster (n=21)	Rotherham	Low Status	1780s-1854	Church of England	(Keefe and Holst 2011)
Hazel Grove (n=31)	Stockport	Low Status	1794-1910	Non-conformist Wesleyan	(Newman and Holst 2016)
Victoria Gate (n=3)	Leeds	Low Status	1796-1850s	Non-conformist Methodist	(Caffell and Holst 2014)
St George Crypt (n=9)	Leeds	Middle/Upper Class	1840-1911	Anglican Church	(Caffell and Holst 2009)
Square Chapel (n=32)	Halifax	Middle Class	1772-1857	Non-conformist Congregationalist	(Keefe and Holst 2015)
Cross Street (n=54)	Manchester	Middle/Upper Class	18th-19thc.	Non-conformist Unitarian	(Keefe and Holst 2017)
Royal London Hospital (n=11)	London	Low Status	1825-1841	-	(Fowler and Powers 2012)
St Brides Lower (n=15)	London	Low Status	1770-1849	Church of England	(Miles and Conheeney 2005)
Queen's Chapel (n=10)	London	Mixed	1510-1854	Unknown+	(Sibun and Ponce 2018)
St Barnabas/St Mary Abbots, Kensington (n=23)	London	Upper Class	1760-1853	Church of England	(Goldsmith 2016; Bleasdale et al. 2019)

Detailed information on the historical context of each population from which human remains were sampled is provided in Chapter 4 and a brief description is provided in Table 7.2. In order to understand the human isotope data in this study, contemporaneous faunal remains from England were examined. Contemporary animal material was available from the same or nearby sites, see Table 7.3.

*Table 7-3: Faunal remains sampled for bone collagen and bone carbonate analysis. *Bone collagen analysis was performed on all the remains but only 27 were analysed for bone carbonate.*

Site name	Location	County	Site code	Period
Cross Street (n=37)	Manchester	Greater Manchester	CSM	17th-19thc.
Hungate (n=21)	York	North Yorkshire	HUN	16th-20thc.
Norton Priory (n=74)	Norton Runcorn	Cheshire	NP	16th-20thc.
Square Chapel (n=7)	Halifax	West Yorkshire	SQC	18th-19thc.
The Bedern (n=11)	York	North Yorkshire	BED	15th-19thc.
Otley (n=8)	Otley	West Yorkshire	GPMO	Unknown
Oulton (n=4)	Leeds	West Yorkshire	Flee	Unknown
Walmgate (n=3)	York	North Yorkshire	WAL	18th-19thc.
Sand-le-Mere (n=3)	Sand-le-Mere	East Yorkshire	SALM T8	17th-19thc.

7.6 Materials and Methods

In total, the analysis of archaeological diet of post-medieval English populations included isotopic data from 216 human skeletons from eleven archaeological cemeteries and 169 faunal remains from nine archaeological sites across England. Collagen data for 70 of the 216 human samples analysed for carbonate isotope analysis were already available, having been generated in Bleasdale et al. (2019) study as well as previous undergraduate and postgraduate dissertations at the University of York. Thirty-five faunal bone collagen data were derived from a previous postgraduate dissertation and the remaining 133 faunal data were generated here. All human individuals as well as 27 faunal remains were sampled for FTIR-ATR and carbonate analysis in this study.

Detailed collagen, FTIR-ATR, and carbonate analytical procedures can be found in Chapter 5. FTIR-ATR sample preparation and analysis were executed according to the method of Kontopoulos et al. (2018). Collagen extraction of bone samples followed the standard operating procedure for BioArCh at the University of York, which is based on the Longin (1971) method modified by Brown et al. (1988). The carbonate extraction of bone samples followed the standard operating procedure used at the University of York adapted from Snoeck and Pellegrini (2015) and Pellegrini and Snoeck (2016). All stable isotope ratios are reported using standard delta (δ) notation. $\delta^{13}C_{collagen}$ ($\delta^{13}C_{coll}$), $\delta^{13}C_{carbonate}$ ($\delta^{13}C_{carb}$) and $\delta^{15}N_{collagen}$ ($\delta^{15}N$) values used in the text are expressed in parts per mille (‰) relative to international standards, Vienna PeeDee Belemnite standard (VPDB) and atmospheric nitrogen (AIR) for $\delta^{13}C$ and $\delta^{15}N$, respectively using the following equation: [δ (‰) = ($R_{sample}/R_{standard}$ -1) x 1000].

Accuracy for collagen analysis was determined by measurements of international standard reference materials within each analytical run. These were IAEA 600 $\delta^{13}C_{raw}$ = -27.65 ±0.09 ‰, $\delta^{13}C_{true}$ = -27.77 ±0.043 ‰, $\delta^{15}N_{raw}$ = 0.92 ±0.21 ‰, $\delta^{15}N_{true}$ = 1 ±0.2 ‰; IAEA N2 $\delta^{15}N_{raw}$ = 20.35 ±0.13 ‰, $\delta^{15}N_{true}$ = 20.3 ±0.2 ‰; IA Cane, $\delta^{13}C_{raw}$ = -11.77 ±0.09 ‰; $\delta^{13}C_{true}$ = -11.64 ±0.03 ‰. The overall uncertainties on the measurements of each sample were calculated based on the method of Kragten (1994) by combining uncertainties in the values of the international reference materials and those determined from repeated measurements of samples and reference materials. These are expressed as one standard deviation. The maximum uncertainty for all samples across all runs was <0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. In addition, a homogenised bovine bone extracted and analysed within the same batch as the samples produced the following average values; $\delta^{13}C$ = -23.01 ±0.13; $\delta^{15}N$ = 6.21 ±0.44. This was comparable to the overall mean value from 50 separate extracts of this bone sample, which produced values of $\delta^{13}C$ = -22.97 ±0.19 and $\delta^{15}N$ = 6.19±0.30.

The reference material used during carbonate analysis was IA-R022 (Iso-Analytical working standard calcium carbonate, $\delta^{13}C_{V\text{-}PDB}$ = -28.63 ‰, and $\delta^{18}O_{V\text{-}PDB}$ = -22.69 ‰). IA-R022, NBS-18 (carbonatite, $\delta^{13}C_{V\text{-}PDB}$ = -5.01 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -23.20 ‰), IA-R066 (chalk, $\delta^{13}C_{V\text{-}PDB}$ = +2.33 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -1.52 ‰) and ILC-1 (limestone) were run as quality control check samples during analysis of the samples. IA-R022 has been calibrated against and is traceable to NBS-18 and NBS-19 (limestone, $\delta^{13}C_{V\text{-}PDB}$ = +1.95 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -2.2 ‰). IA-R066 has been calibrated against and is traceable to NBS-18 and IAEA-CO-1 (carrara marble, $\delta^{13}C_{V\text{-}PDB}$ = +2.5 ‰ and $\delta^{18}O_{V\text{-}PDB}$ = -2.4 ‰). ILC-1 has been analysed multiple times in the Isoanalytical laboratory over a number of years. The current long term mean value for those analyses is $\delta^{13}C$ = 2.17‰. NBS-18, NBS-19, and IAEA-CO-1 are inter-laboratory comparison standard materials distributed by the International Atomic Energy Agency (IAEA).

7.6.1 Statistical analysis

Statistical analysis was carried out using R statistics, PAST statistics and IBM SPSS statistics version 26 (Hammer et al. 2001; IBM 2019; R Core Team 2020). Non-parametric statistics were used to compare isotope values among groups because of the non-normal distribution of data as indicated by Kolmogorov-Smirnov and Shapiro-Wilk tests as well as small sample sizes in some cases (Appendix D). The tests were executed using the non-parametric equivalent of a one-way analysis of variance (ANOVA) and the non-parametric equivalent of the independent T-test - the Kruskal Wallis Test and Mann Whitney test respectively. Posthoc pairwise tests adjusted using the Holm's Sequential Bonferroni correction produced during the tests were also utilised.

7.7 Results

The $\delta^{13}C_{coll}$, $\delta^{13}C_{carb}$, $\delta^{18}O_{carb}$ and $\delta^{15}N_{coll}$ isotope data for the human and faunal bone samples from all the sites in this study are presented in Appendix A and B, respectively. The IRSF and C/P ratio data produced using FTIR-ATR are listed in Appendix C. The $\delta^{18}O_{carb}$ were only utilised in exploring diagenesis through examining correlations with $\delta^{13}C_{carb}$ and FTIR parameters. Using the Mann Whitney tests, there are no statistically significant differences in the $\delta^{13}C_{coll}$, $\delta^{13}C_{carb}$ or $\delta^{15}N_{coll}$ values between males and females in all the sites that had a sufficient number of individuals sexed (Table 7.4), therefore, in order to evaluate geographical and status differences, all individuals of both sexes are combined for each site.

Table 7-4: Mann Whitney tests between males and females in sites where there was a sufficient number of sexed individuals to compare. F? And M? were assumed to be F and M respectively.

Sites	*δ^{13}Ccollagen*	*δ^{13}Ccarbonate*	*δ^{15}Ncollagen*
CSM (M=25; F=27)	U = 367; p = 0.589	U = 239.5; p = 0.968	U = 347; p = 0.862
HGM (M=5; F=6)	-	-	-
FEW (M=3; F=4)	-	-	-
SQC (M=16; F=10)	U = 72.5; p = 0.712	U = 52.5 p = 0.155	U = 75; p = 0.812
STGC (M=4; F=2)	-	-	-
VGL (M=0; F=1)	-	-	-
ROM (M=10; F=10)	U = 47; p = 0.850	U = 45; p = 0.734	U = 37.5; p = 0.364
QCS (M=3; F=7)	-	-	-
SBK (M=13; F=10)	U = 45.5; p = 0.236	U = 52.5; p = 0.457	U = 46; p = 0.250
RLH (M=8; F=3)	-	-	-
SBL (M=7; F=8)	U = 23; p = 0.603	U = 19; p = 0.325	U = 21; p = 0.452

7.7.1 Sample preservation

All human and faunal samples produced collagen yields of 1% or higher and fell within accepted ranges for C/N ratio of ca. 2.9-3.6, %N of ca. 5-17%, and %C of ca. 14-48% (DeNiro 1985; Ambrose 1990; Sealy et al. 2014). Although FTIR-ATR was employed to obtain insight into bone mineral diagenesis, there are still no clear-cut quality indicators to recognise extracted bone mineral preservation levels. Rather, in line with previous studies, this study used FTIR-ATR indices infrared splitting factor (IRSF) and carbonate-phosphate ratio (C/P)) and their relationship to each other to assess diagenesis. Crystallinity, measured by the IRSF has been used as a meaningful indicator of bone preservation (Snoeck et al. 2014; Kontopoulos et al. 2019a). The IRSF values in all human and animal samples used here ranged from 3.27 to 4.23 (Mean = 3.72±0.18) and 3.50 to 3.97 (Mean = 3.71±0.12) respectively (Appendix C; Figure 7.3). Except for three samples (QCS 1998; QCS 124 and CSM 61.03), all samples IRSF are greater than that obtained for the modern bones (IRSF=3.357 ± 0.007) (Kontopoulos et al. 2019a, 145). Acceptable IRSF values for untreated archaeological bone generally

fall between 2.6 and 4.0 (Wright and Schwarcz 1996; Nielsen-Marsh and Hedges 2000; Lee-Thorp and Sponheimer 2003; Garvie-Lok et al. 2004). However, the methods used in these studies are different from the one used here in terms of sample sizes and baseline corrections. One study that utilised a similar method used here (Kontopoulos et al. 2020) demonstrated that archaeological samples with IRSF values greater than 4.2 did not preserve DNA. Consequently, all the samples with IRSF values higher than 4.2 (n=6) were arbitrarily excluded from further analysis of the isotopes of bone carbonate on the assumption that they may have poor bioapatite preservation as well.

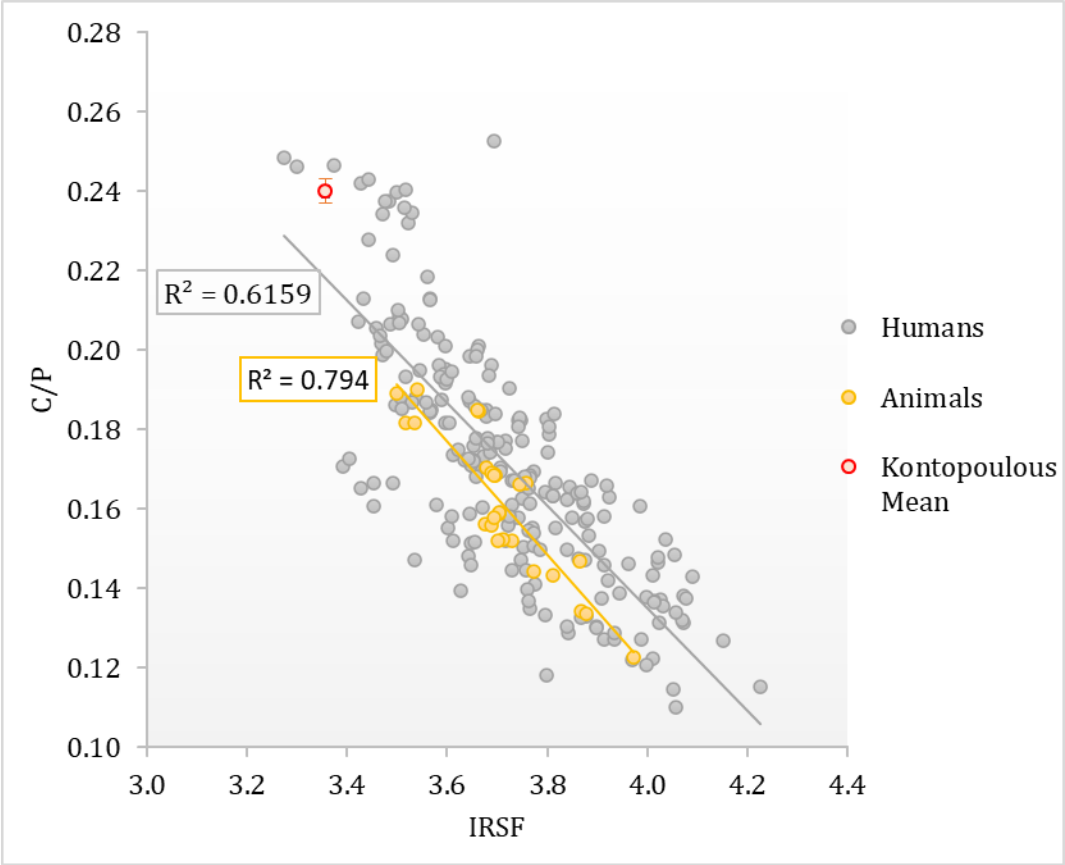


Figure 7-3: IRSF vs C/P ratio for post-medieval humans and animals in the current study. Mean IRSF vs C/P found in Kontopoulos et al. (2019a) were added for reference

The C/P absorbance ratios, which measure the relative carbonate content in bone samples ranged from 0.11 to 0.25 (Mean = 0.17±0.03) for human bones and 0.12 to 0.19 (Mean = 0.16±0.02) for animal bones (Appendix C; Figure 7.3). Except for six, all the other bones fell below the mean C/P values that were found in modern unaltered bone (Mean C/P=0.24 ± 0.003) as reported - in Kontopoulos et al. (2019a) indicating a loss of the carbonate fraction in the bone apatite. The six samples for which the C/P values fell above the modern bone range were retained within the overall analysis because they were very close to the 'normal' range. Additionally, their carbonate content reduced as a result of pre-treatment.

Calcite was identified in only nine human samples, primarily by the presence of a peak at 712 cm^{-1} (Hunt et al. 1950; Baxter et al. 1966) suggesting that for the rest of the samples the carbonate content most likely belonged to the structural carbonate of the hydroxyapatite. However, the FTIR spectra do not show this peak after chemical pre-treatment indicating that no calcite remained in the treated samples, therefore, these samples were included in the bone carbonate isotope analysis.

In addition to FTIR parameters, the relationship between $\delta^{13}C$ and $\delta^{18}O$ data (Appendix A) was examined to explore diagenesis. No correlation was observed between $\delta^{13}C$ and $\delta^{18}O$ values in both human and animal samples (Figure 7.4). It has previously been established that a correlated variation of bone carbonate $\delta^{13}C$ and $\delta^{18}O$ can occur as a result of both uptake and incorporation of light carbon and oxygen from the surrounding environment during diagenetic alteration (Heydari et al. 2001). Additionally, Ullmann and Korte (2015, 13) also noted that post depositional alteration, almost always results in a positive correlation between $\delta^{13}C$ and $\delta^{18}O$ carbonates subjected to diagenesis. Therefore, the absence of a correlation between these two isotope ratios in this study strongly suggests that the isotopic signals for the samples were generally preserved.

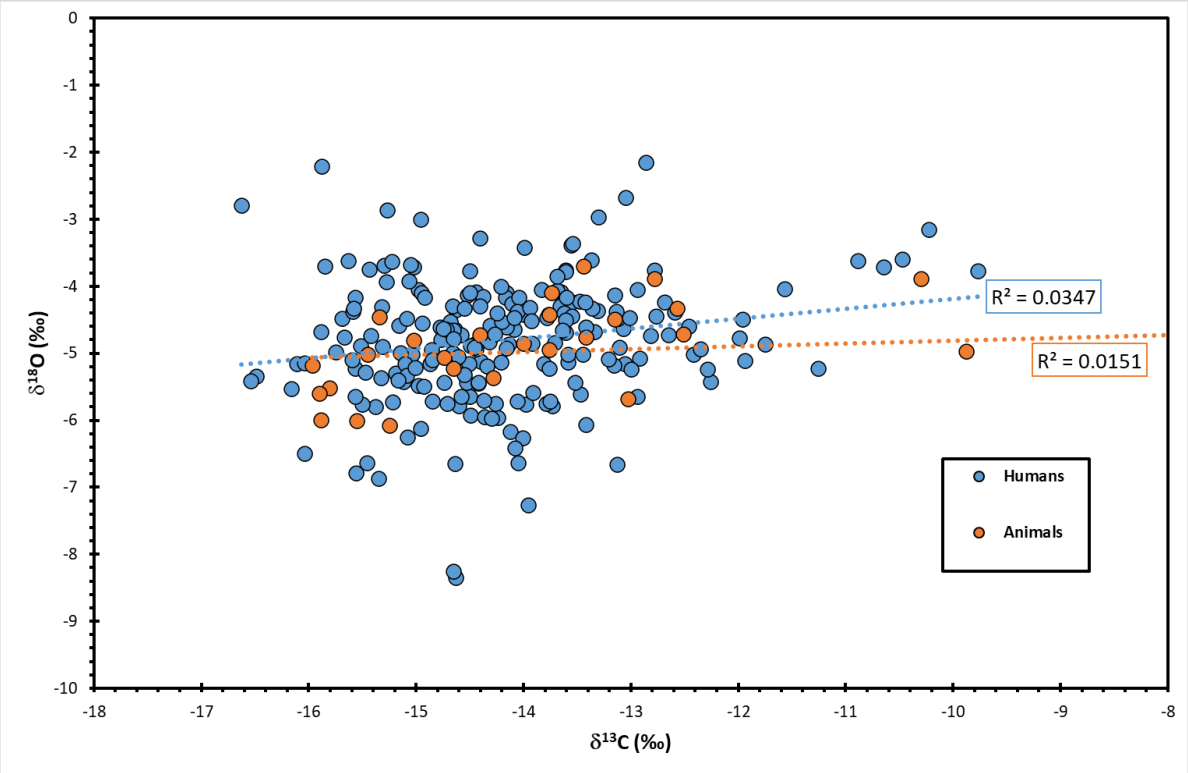
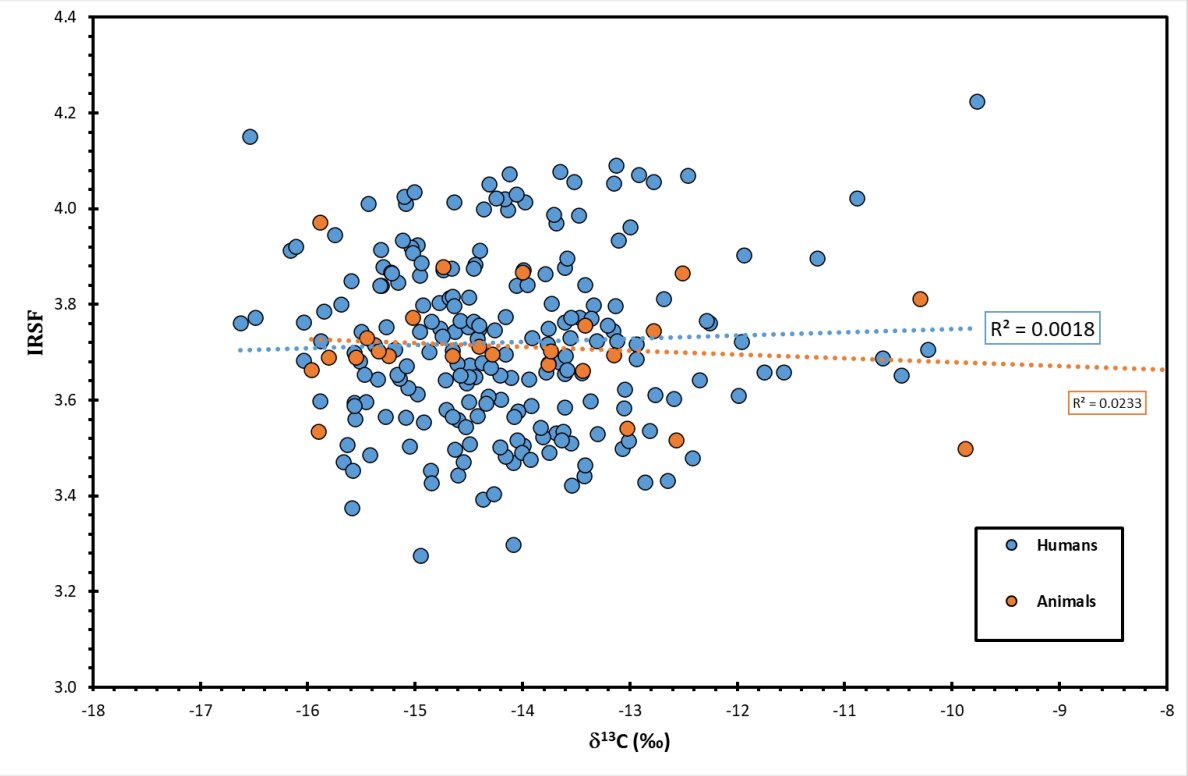
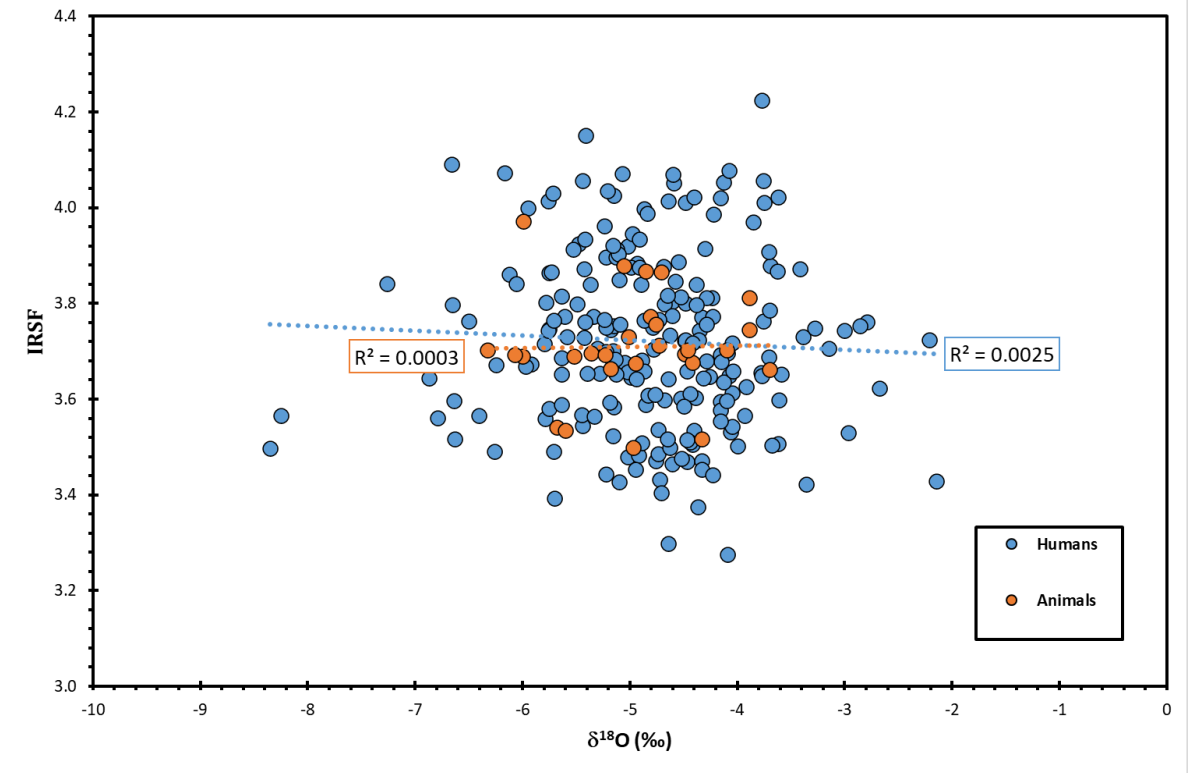


Figure 7-4: The relationship between bone carbonate $\delta^{13}C$ and $\delta^{18}O$ in humans and animals in this study

Furthermore, when FTIR data (IRSF and C/P ratio) was examined with reference to the $\delta^{13}C$ and $\delta^{18}O$ of bone carbonate, no correlations were observed between the parameters (Figure 7.5).


IRSF
δ13C (‰)
R² = 0.0018
R² = 0.0233
Humans
Animals


IRSF
δ18O (‰)
R² = 0.0003
R² = 0.0025
Humans
Animals

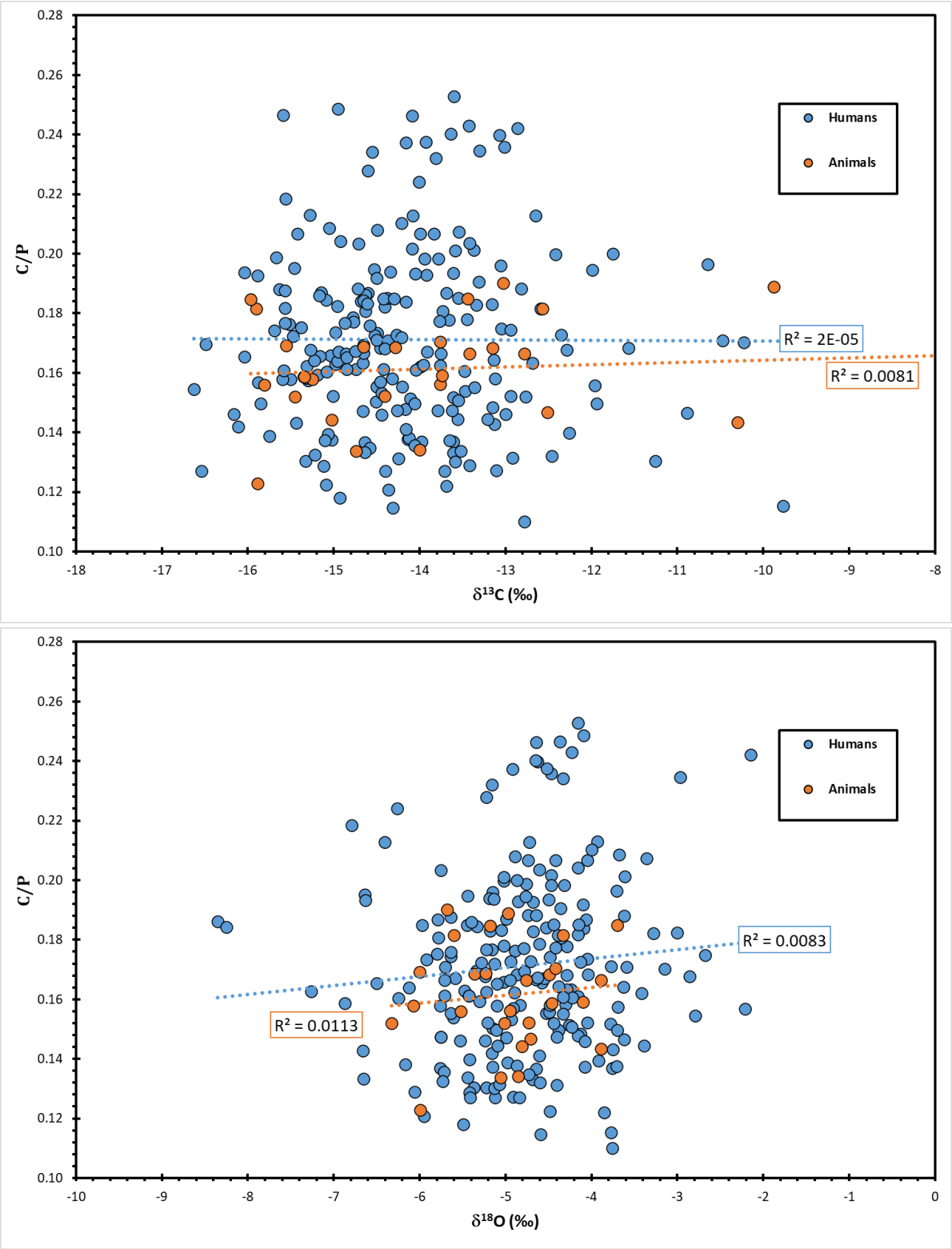


Figure 7-5: The relationship between bone carbonate $\delta^{13}C$ and $\delta^{18}O$ with IRSF and C/P ratio in humans and animals in this study

There is a good reason to believe that the absence of correlation between these parameters shows that the isotopic signals are still preserved as suggested in other studies on well preserved samples e.g., Wright and Schwarcz (1996) and Roche et al. (2010).

7.7.2 Faunal isotopic data

Summary data for faunal remains in this study are supplied in Table 7.5. There was a statistically significant difference in the $\delta^{13}C_{coll}$ and $\delta^{15}N$ values between the four different groups of faunal remains [Kruskal-Wallis H test, $\delta^{13}C_{coll}$ $X^2(3) = 58.0$, $p = 0.000$; $\delta^{15}N$ $X^2(3) = 37.0$, $p = 0.000$ but no statistically significant difference in the $\delta^{13}C_{carb}$ $X^2(2) = 4.1$, $p = 0.127$].

Table 7-5: Descriptive statistics for all faunal remains analysed in this study.

Isotope data	Species	Range	Minimum	Maximum	Mean	Std. Deviation
$\delta^{13}C_{coll}$ (‰)	Cattle (n=46)	2.8	-23.3	-20.5	-22.1	0.5
	Domestic Fowl (n=14)	2.1	-21.9	-19.8	-21.0	0.6
	Pig (n=32)	13.8	-22.3	-8.6	-20.6	3.0
	Sheep (n=77)	2.2	-23.0	-20.8	-22.0	0.4
$\delta^{15}N$ (‰)	Cattle (n=46)	5.5	3.7	9.1	6.4	1.1
	Domestic Fowl (n=14)	3.6	8.1	11.7	9.6	1.3
	Pig (n=32)	5.0	5.1	10.2	7.6	1.5
	Sheep (n=77)	7.2	3.3	10.5	6.6	1.7
$\delta^{13}C_{carb}$ (‰)	Cattle (n=6)	7.2	-14.0	-6.8	-12.3	2.8
	Pig (n=15)	6.1	-16.0	-9.9	-14.2	2.0
	Sheep (n=6)	2.5	-15.3	-12.8	-14.1	1.1

Posthoc pairwise tests were produced for the three groups for all isotopes (Table 7.6). There was very strong evidence ($p < 0.05$, adjusted using the Holm's Sequential-Bonferroni correction) of significant differences in $\delta^{13}C_{coll}$ and $\delta^{15}N$ values between omnivores (domestic fowls and pigs) and herbivores (cattle, sheep) (Table 7.6). Finally, there was a significant difference between the $\delta^{13}C_{carb}$ values in cattle and pigs probably due to two pigs within this pig group with high $\delta^{13}C_{carb}$ suggesting C_4 input in their diet.

Table 7-6: Post hoc p value results for different regions after the Holm's Sequential Bonferroni adjustment.

Isotopes	Species	Cattle	Domestic Fowl	Pigs	Sheep
$\delta^{13}C_{coll}$ (‰)	Cattle	-	0.000	0.000	0.395
	Domestic Fowl	0.000	-	0.178	0.000
	Pigs	0.000	0.178	-	0.000
	Sheep	0.395	0.000	0.000	-
$\delta^{15}N$ (‰)	Cattle	-	0.000	0.002	0.574
	Domestic Fowl	0.000	-	0.004	0.000
	Pigs	0.002	0.004	-	0.004
	Sheep	0.574	0.000	0.004	-
$\delta^{13}C_{carb}$ (‰)	Cattle	-	-	0.044	0.308
	Pigs	0.044	-	-	0.424
	Sheep	0.308	-	0.424	

7.7.3 Comparisons with other post-medieval faunal remains (collagen)

The mean $\delta^{13}C$ and $\delta^{15}N$ values for cattle, pigs, and sheep from the northern sites all fall within the range of values reported in Bleasdale et al. (2019) for post-medieval Queen's Chapel of the Savoy and Prescot Street sites in London (Figures 7.4 and 7.5). However, the mean $\delta^{13}C$ and $\delta^{15}N$ values for post-medieval sheep in this study fall outside the mean values for post-medieval sheep from Durham (Figure 7.6).

Although there is no statistically significant difference in the $\delta^{15}N$ values between the northern and London domestic fowl, there is a significant difference in the $\delta^{13}C$ values [Domestic Fowl - ($\delta^{13}C$ Mann-Whitney U test U=5.000, p=0.03; $\delta^{15}N$ Mann-Whitney U test U=25.000 p=0.391)]. However, the difference in $\delta^{13}C$ mean values between the two groups is only 0.1‰ which is too small to suggest any dietary differences. The two pigs bone collagen $\delta^{13}C$ is indicating consumption of C_4 suggesting that they have different diets from the rest of the animals which will be explored further in the discussion. On the other hand, there are no statistically significant differences in the livestock from the North and those from London [Cattle - ($\delta^{13}C$ Mann-Whitney U test U=204.5, p=0.084; $\delta^{15}N$ Mann-Whitney U test U=224 p=0.170); all Pigs including those with ^{13}C enrichment -($\delta^{13}C$ Mann-Whitney U test U=129.500, p=0.374; $\delta^{15}N$ Mann-Whitney U test U=105.000 p=0.108); Sheep-($\delta^{13}C$ Mann-Whitney U test U=530.500, p=0.223; $\delta^{15}N$ Mann-Whitney U test U=575.500 p=438)].

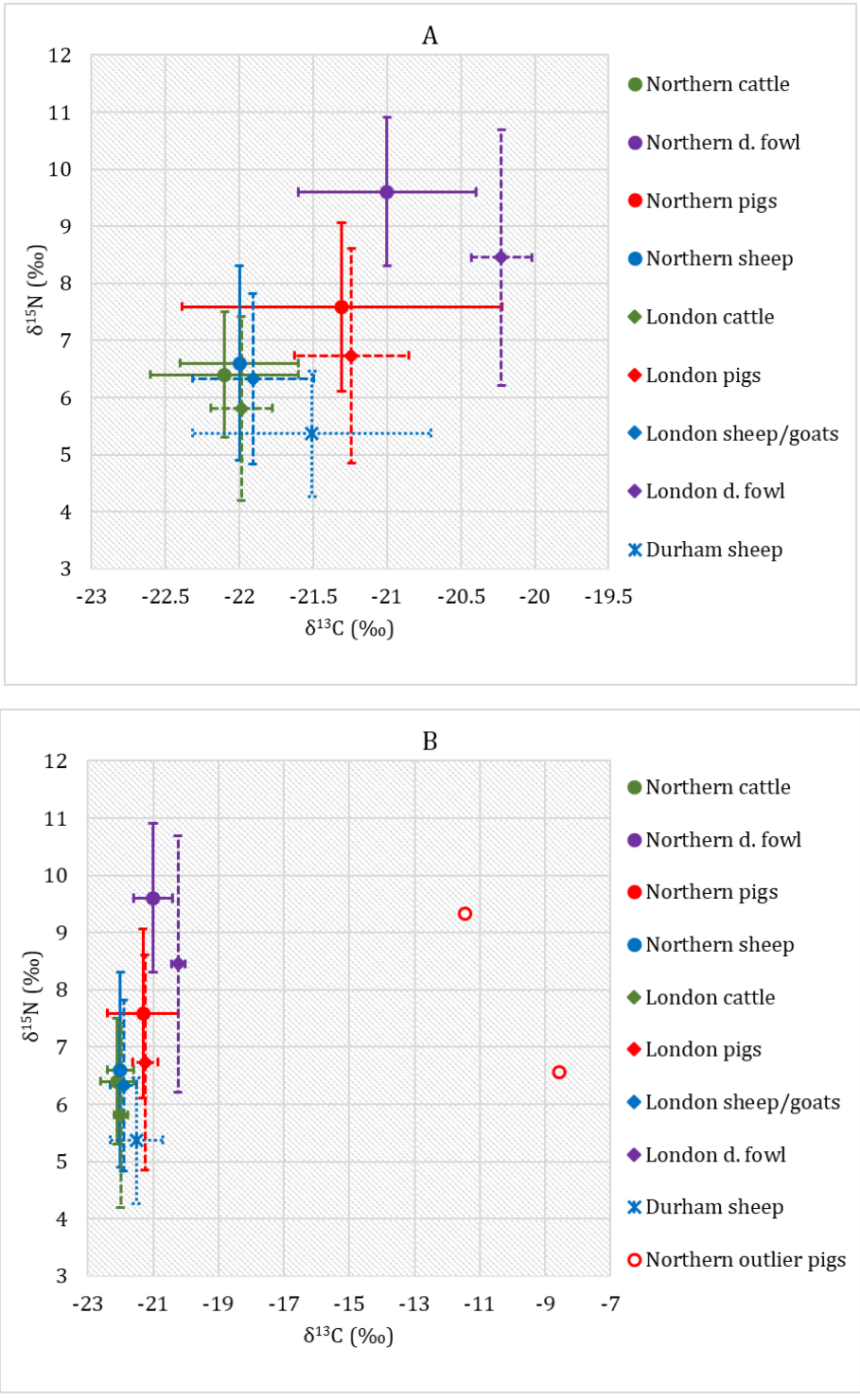


Figure 7-6: Faunal mean $\delta^{13}C$ and $\delta^{15}N$ values to 1σ from the Northern sites in this study. Dotted error bars are for reference from data collected in Bleasdale et al. (2019) at Queen's Chapel Savoy and Prescot Street sites in London and in Millard et al. (2015) at Durham (A) excluding the pig outliers (B) including the pig outliers.

This implies that, except for the two northern pigs with high $\delta^{13}C$ values, all livestock in this study consumed a generally similar diet to that of London livestock. The livestock in London originated from a wide range of areas from around the country due to improvements in transportation during this period (Bleasdale et al. 2019), therefore it is not surprising that its diet resembles that of the North. The sheep from a comparative site from Durham (Figure 7.6), however, show a statistically

significant difference to those from the other northern towns [Durham Sheep -($\delta^{13}C$ Mann-Whitney U test U=338.000, p=0.011; $\delta^{15}N$ Mann-Whitney U test U=321.000 p=0.007) suggesting that they were consuming different diet. Although it was expected that the Durham sheep would have similar values to the other northern sheep, it seems likely that the city's supply of sheep was localised rather than animals transported from elsewhere. In addition, the difference may be due to Durham sheep dating earlier (16^{th}-17^{th} century) than most northern sheep in this study.

7.7.4 Human isotopic data

The mean $\delta^{13}C_{coll}$, $\delta^{13}C_{carb}$ and $\delta^{15}N$ values for different groups for human skeletons are presented in Table 7.7. Figure 7.7 displays human and faunal bone samples in this study and a biplot of $\delta^{13}C$ and $\delta^{15}N$ values displaying mean and 1σ for individuals in this study and English post-medieval comparative sites.

A statistically significant difference was observed between the mean carbon collagen isotope values and the mean nitrogen isotope values for the northern manufacturing populations and those from London analysed in this study ($\delta^{13}C_{coll}$ Mann-Whitney U test U=754.5, p=0.000); ($\delta^{15}N$ Mann-Whitney U test U=814 p=0.000 and ($\delta^{13}C_{carb}$ Mann-Whitney U test U=3683, p=0.020). The London populations generally have more elevated values than the northern populations. This pattern extends when the published London collagen data is integrated with the collagen data from this study (Figure 7.7D). Similarly, other published sites except for Gosport, have elevated $\delta^{13}C_{coll}$ values relative to the northern populations in the current study.

Table 7-7: Summarised statistics of all stable isotope ratios according to region and sites. Stable isotope values reported as the mean ±1 sd. Sample numbers in parentheses.

Region	Regional $\delta^{13}C_{coll}$ (‰) Mean± 1σ	Regional $\delta^{15}N_{coll}$ (‰) Mean± 1σ	Regional $\delta^{13}C_{carb}$ (‰) Mean± 1σ	Site	$\delta^{13}C_{coll}$ (‰) Mean± 1σ	$\delta^{15}N_{coll}$ (‰) Mean± 1σ	$\delta^{13}C_{carb}$ (‰) Mean± 1σ
Northern towns (n=157)	-19.9 ± 0.5	11.6 ± 1.1	-14.3 ± 1.1	Cross Street, Manchester (n=54)	-19.7 ± 0.5	11.9 ± 1.2	-14.3 ± 1.0
				Hazel Grove, Manchester (n=31)	-20.2 ± 0.4	11.0 ± 1.1	-14.1 ± 1.0
				Fewston, Harrogate (n=7)	-20.1 ± 0.5	11.4 ± 0.7	-14.6 ± 1.1
				Square Chapel, Halifax (n=32)	-19.9 ± 0.5	11.8 ± 0.9	-14.8 ± 0.9
				St George Crypt, Leeds (n=9)	-19.7 ± 0.5	11.8 ± 0.5	-14.6 ± 0.6
				Victoria Gate, Leeds (n=3)	-20.2 ± 0.3	11.7 ± 0.3	-14.2 ± 0.6
				Rotherham Minster (n=21)	-19.8 ± 0.4	11.4 ± 0.8	-13.6 ± 1.1
London (n=59)	-19.1 ± 1.0	12.8 ± 1.0	-13.8 ± 1.3	Queen Chapel Savoy (n=10)	-18.4 ± 1.9	12.3 ± 1.3	-13.7 ± 1.5
				St Barnabas Chelsea (n=23)	-19.1 ± 0.7	13.6 ± 0.7	-13.6 ± 1.5
				Royal London Hospital (n=11)	-19.3 ± 0.6	12.5 ± 0.7	-13.4 ± 0.7
				St Brides Lower (n=15)	-19.5 ± 0.5	12.3 ± 0.8	-14.5 ± 1.0

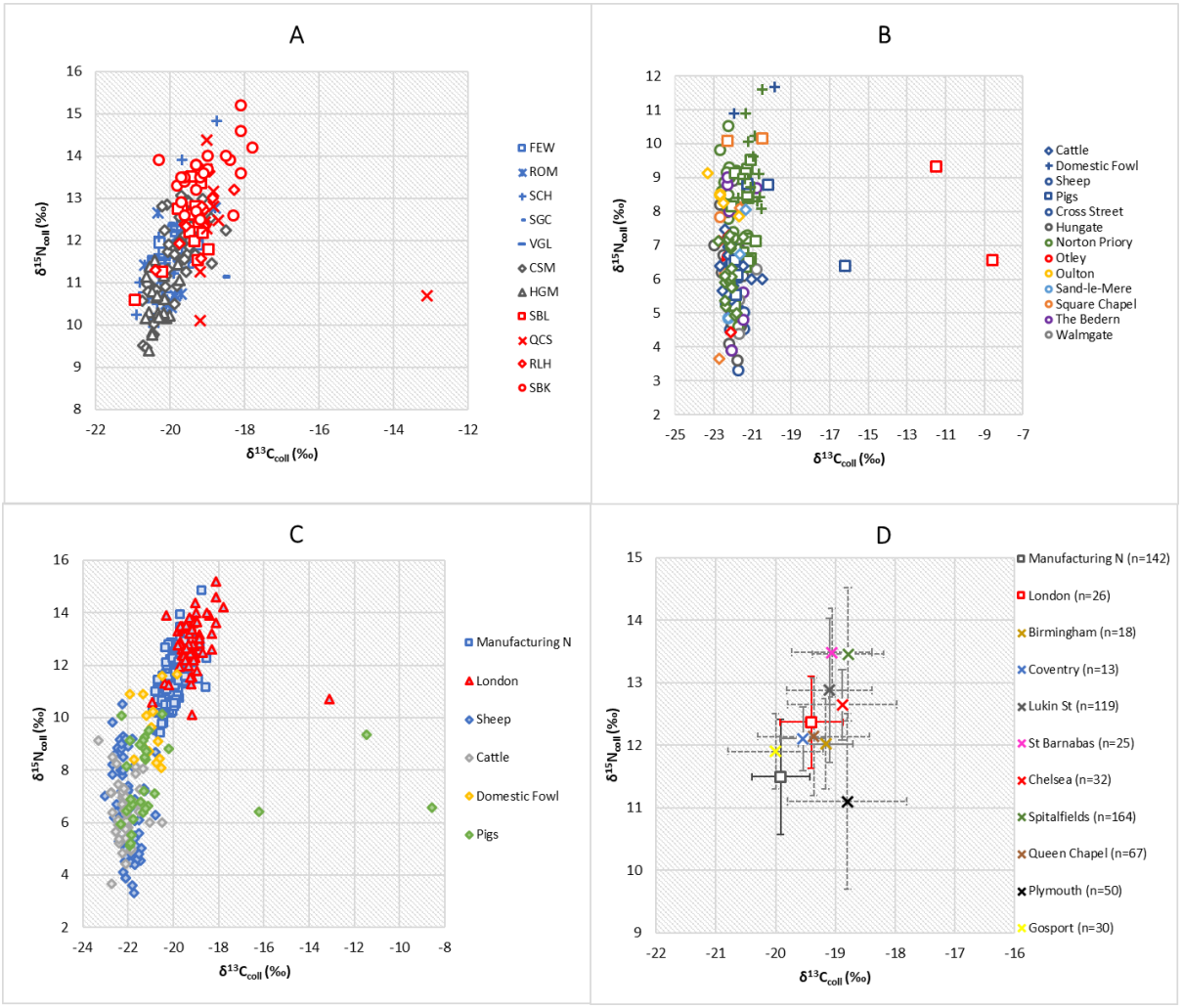


*Figure 7-7: Scatter plots of $\delta^{15}N$ and $\delta^{13}C$ results of **(A)** all human bone samples excluding infants in this study: Fewston (FEW); Rotherham Minster (ROM); Square Chapel Halifax (SCH); St George's Crypt (SGC); Victoria Gate Leeds (VGL); Cross Street Manchester (CSM); Hazel Grove Manchester (HGM); St Brides Lower (SBL); Queen Chapel Savoy (QCS); Royal London Hospital (RLH) and St Barnabas Kensington (SBK). **(B)** faunal bone samples in this study. **(C)** Human samples grouped by region and faunal samples grouped by species together **(D)** A biplot of $\delta^{13}C$ and $\delta^{15}N$ values, showing mean and 1σ for individuals from the northern and London (St Brides Lower and Royal London only) populations and published seven contemporary English sites St Barnabas and Queen Chapel Savoy (Bleasdale et al. 2019); Lukin Street (Beaumont et al. 2013b); Spitalfields, London (Nitsch et al. 2010); St Luke's Chelsea and Holy Trinity, Coventry (Trickett 2006), Churchyard of St. Martin's-in-the-Bull Ring, Birmingham (Richards 2006), Plymouth and Gosport (Roberts et al. 2012).*

All human $\delta^{13}C_{carb}$ values range from -9.8‰ to -16.6‰, with an average of -14.2± 1.2‰. The $\delta^{13}C_{carb}$ values have a wider range at each site than the $\delta^{13}C_{coll}$ that likely reflects the wider range of resources that the carbonate values pertain to. In scenarios where all individuals' diets are based either entirely on C_3 resources or entirely on C_4 resources, the anticipated $\delta^{13}C_{carb}$ values are -14.5‰ and -0.5‰ respectively (Tykot 2004; Díaz-del-Río et al. 2017). Diets that fall in between, as is the case for almost 40% of the whole population in this study, suggest mixed C_3 and C_4 dietary inputs (Figure 7.8). The carbonate data suggests some dietary variability but dependence mainly on C_3 terrestrial resources overall.

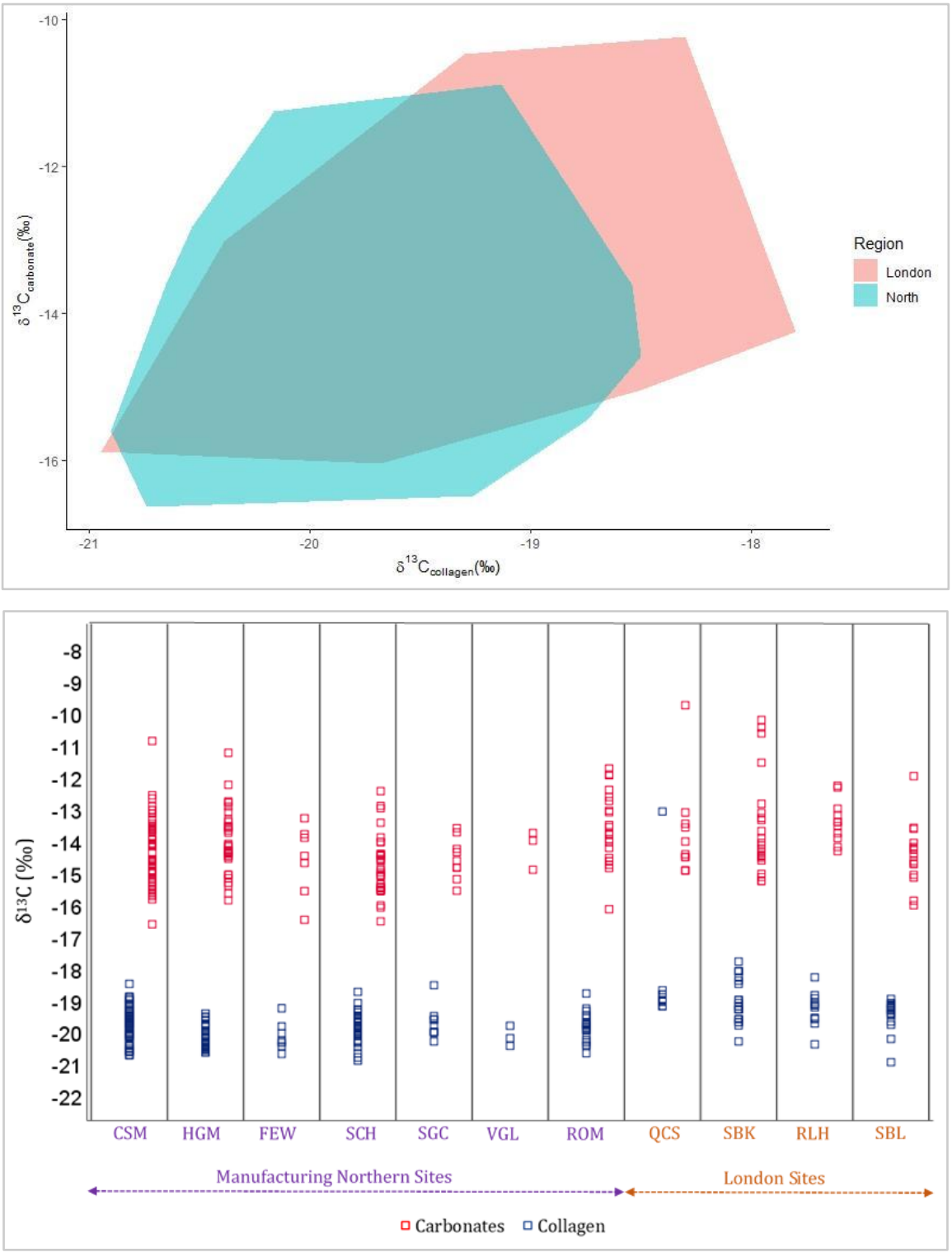


Figure 7-8: ***TOP*** *– A bagplot comparison of $\delta^{13}C_{coll}$ and $\delta^{13}C_{carb}$ across different populations from the sites in the Manufacturing Northern towns (n=7) to the sites in London (n=4). The northern populations are [Cross Street Manchester (CSM), Hazel Grove Manchester (HGM), Fewston (FEW), Square Chapel Halifax (SCH), St George's Crypt Leeds (SGC), Victoria Gate Leeds (VGL), Rotherham (ROM)]; The London populations are [Queen Chapel Savoy (QCS), St Barnabas Kensington (SBK), Royal London Hospital (RLH) and St Brides Lower (SBL)].* ***BOTTOM*** *- Comparison of the range of $\delta^{13}C_{coll}$ and $\delta^{13}C_{carb}$ between the northern sites and London (excluding QCS 1123). Collagen data for QCS and SBK sites was obtained from Bleasdale et al. 2019.*

There is, however, a greater variation in the $\delta^{15}N$ values. All individuals from the northern manufacturing towns except for three individuals (SK 39, 59 and 98) from Square Chapel fall between 9.4‰ and 13.1‰ and those from London exhibit $\delta^{15}N_{coll}$ values between 10.1‰ and 15.2‰ (Figure 7.9). The London populations appear to have consumed a diet with a relatively high animal protein component and possibly more freshwater protein than the northern populations as indicated by higher $\delta^{15}N$ values (Figure 7.9) and $\delta^{13}C_{coll}$ values (Figure 7.8) measured in the London samples. This is possible because Londoners had a good supply of freshwater fish from the Billingsgate fish market and garden pond cultures (Mayhew 1967; Dyer 1988; Burnett 2004; Thirsk 2007; Panayi 2008). Except for Plymouth, this pattern also extends to other populations when published collagen data from other post-medieval sites is included (Figure 7.7D). Plymouth, however, displays lower $\delta^{15}N_{coll}$ values compared to both the London and northern populations in this study.

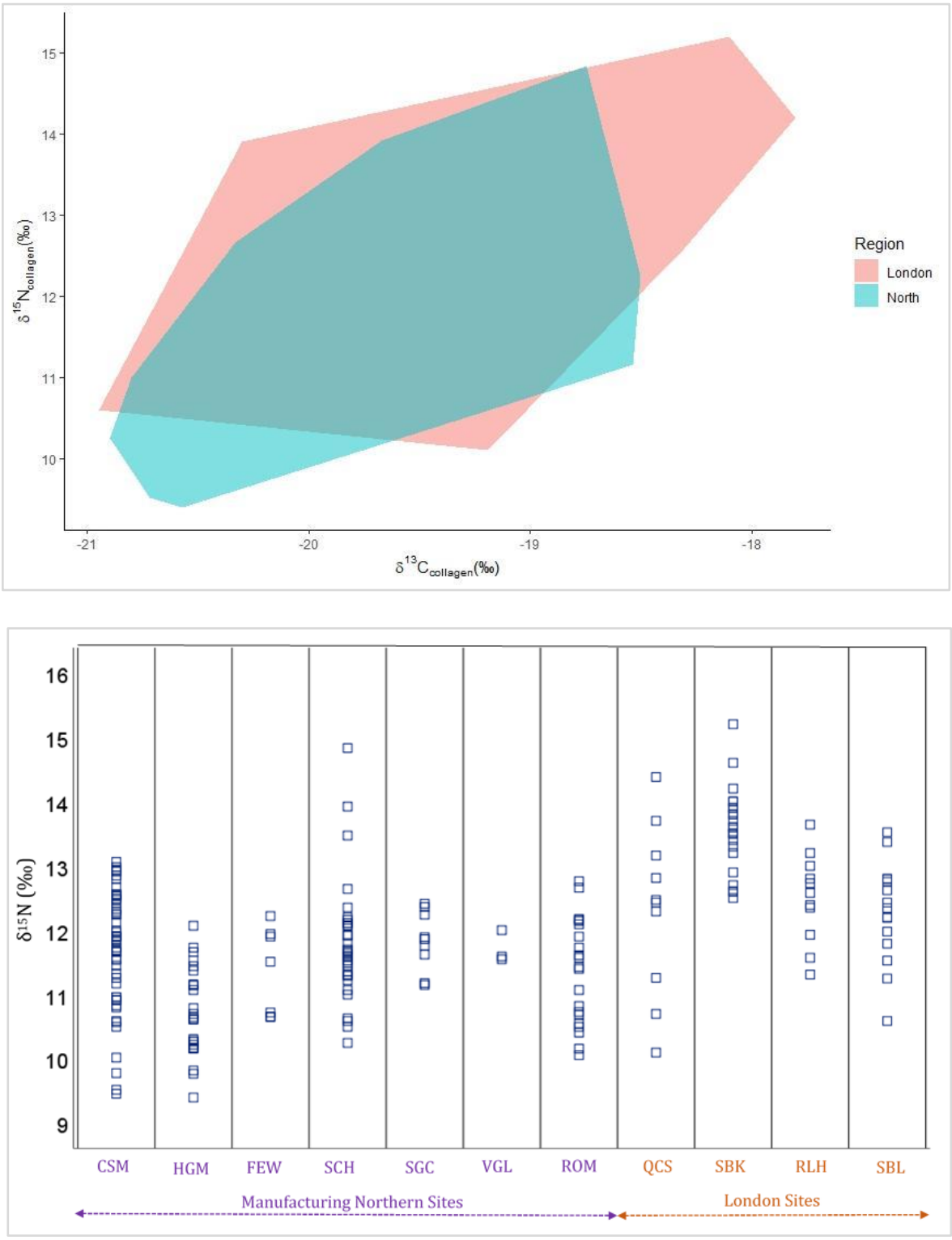


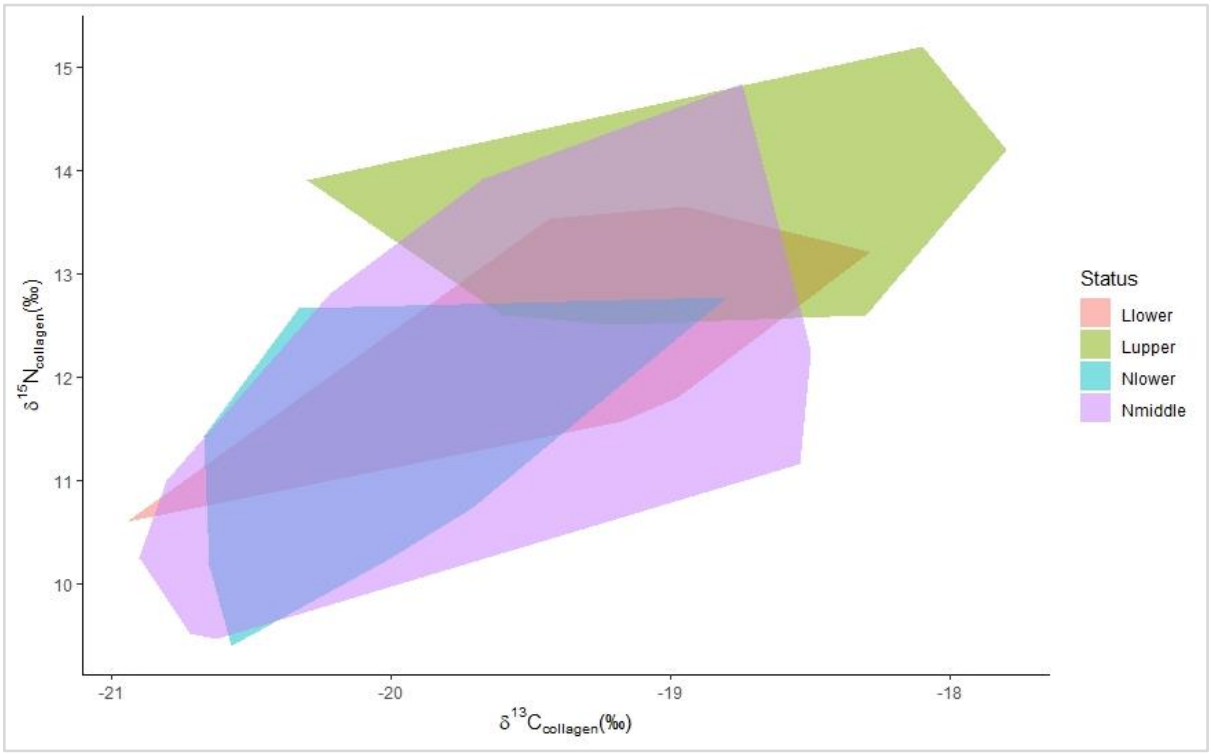
Figure 7-9: ***TOP*** *– A bagplot comparison of adult $\delta^{15}N$ across different sites from the sites in the Manufacturing Northern towns (n=7) to the sites in London (n=4). The sites studied are Northern sites [Cross Street Manchester (CSM), Hazel Grove Manchester (HGM), Fewston (FEW), Square Chapel Halifax (SCH), St George's Crypt Leeds (SGC), Victoria Gate Leeds (VGL), Rotherham (ROM)]; London sites [Queen Chapel Savoy (QCS), St Barnabas Kensington (SBK), Royal London Hospital (RLH) and St Brides Lower (SBL)]. Collagen data for QCS and SBK sites was obtained from Bleasdale et al. 2019.* ***BOTTOM*** *- Comparisons of the range of adult $\delta^{15}N$ across the northern sites and London. Collagen data for QCS and SBK sites was obtained from Bleasdale et al. 2019.*

7.7.4.1 Intra-population variations

There is a statistically significance difference for all the $\delta^{13}C_{coll}$, $\delta^{15}N$ and $\delta^{13}C_{carb}$ for the northern manufacturing populations [Kruskal-Wallis H test, $\delta^{13}C_{coll}$ $X^2(6) = 24.389$, $p = 0.000$; $\delta^{15}N$ $X^2(6) = 31.760$ $p = 0.000$ and $\delta^{13}C_{carb}$ $X^2(6) = 17.303$, $p = 0.008$]. However, the posthoc analysis produced during that test has identified the sites where these differences are primarily occurring (Appendix D). The posthoc comparisons revealed that the Hazel Grove and Rotherham populations are the only ones giving rise to the significant variation in this group (Appendix D). When both these populations are excluded from the group there is no statistically significant difference in all the isotope ratios in the rest of the northern populations. There is also a statistically significant difference between all the three isotope ratios in the London populations [Kruskal-Wallis H test, $\delta^{13}C_{coll}$ $X^2(3) = 10.046$, $p = 0.018$; $\delta^{15}N$ $X^2(3) = 22.980$ $p = 0.000$ and $\delta^{13}C_{carb}$ $X^2(3) = 8.763$, $p = 0.033$]. But unlike the northern populations, this difference is not a result of just two specific sites (Appendix D).

7.7.4.2 Socioeconomic status variations

Figure 7.10 displays graphical comparisons of all the $\delta^{13}C_{coll}$, $\delta^{15}N$ and $\delta^{13}C_{carb}$ value ranges between the northern manufacturing populations and the London populations according to socioeconomic status. Excluding Fewston and Queen's Chapel of the Savoy populations which are rural and mixed status populations respectively, there is a statistically significant difference observed between the known lower status urban northern populations and the lower status London populations for both $\delta^{13}C_{coll}$ values (Mann-Whitney U test - U= 165.000 p=0.000) and $\delta^{15}N$ values (Mann-Whitney U test- U=131.000 p=0.000). The statistical analysis indicated that the London populations tend to have higher $\delta^{13}C_{coll}$ and $\delta^{15}N$ values than the northern populations. However, no statistical difference is observed in $\delta^{13}C_{carb}$ values (Mann-Whitney U test - U=531.000 p=0.519). In addition, a Mann-Whitney U test indicated that the upper status population in London have more enriched $\delta^{13}C_{coll}$, $\delta^{13}C_{carb}$, and $\delta^{15}N$ values than the northern middle/upper status populations ($\delta^{13}C_{coll}$ (U=342.000, p=0.000); $\delta^{15}N$ (U=86.000 p=0.000) and $\delta^{13}C_{carb}$ (U=662.000 p=0.008).



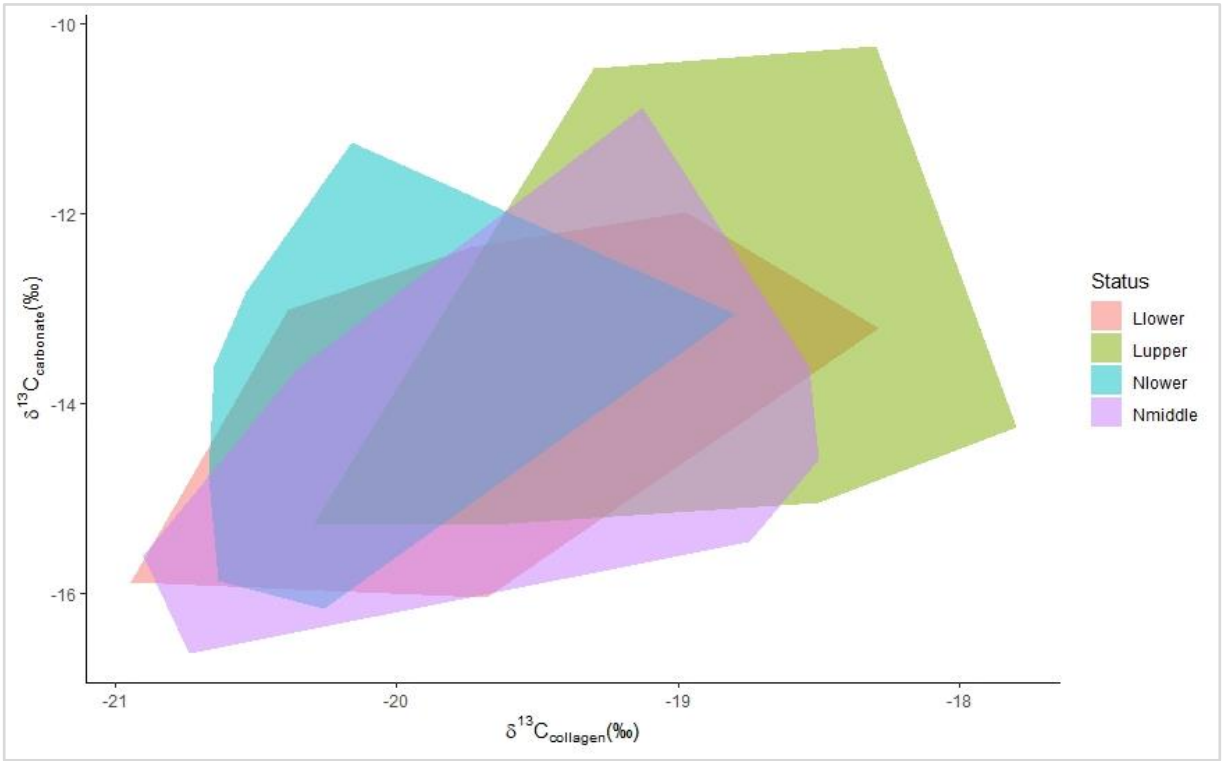


Figure 7-10: Bagplots showing comparisons of adult $\delta^{13}C_{coll}$, $\delta^{13}C_{carb}$ and $\delta^{15}N$ across different statuses between the Manufacturing Northern and London populations. Key: Llower-London lower status; Lupper-London upper status; Nlower- Northern lower status; Nmiddle- Northern middle/upper status.

7.7.4.3 Human-animal isotope offsets

In order to account for the regional variation observed in this study, it was also essential to consider the isotope values of humans in relation to local fauna. For London, the relations were assessed using animal data from Bleasdale et al. (2019). The $\delta^{13}C_{coll}$ offsets of humans against omnivores for both the northern and London populations ($\delta^{13}C_{North\text{-}omnivores}$ = 0.8‰ and $\delta^{13}C_{London\text{-}omnivores}$ = 1.8‰) are within the range of trophic level shift of 0-2‰ (Lee-Thorp et al. 1989; Bocherens and Drucker 2003), and just above the upper limit for herbivores ($\delta^{13}C_{North\text{-}herbivores}$= 2.2‰ and $\delta^{13}C_{London\text{-}herbivores}$ = 2.9‰). Regarding the $\delta^{15}N$ human-fauna offsets, the offsets between the northern populations and herbivores (5.0 ‰) and omnivores (3.4‰) fall within the expected trophic level enrichment of 3-5‰ (Schoeninger 1985; Bocherens and Drucker 2003) but those from London fall outside the upper limit of 5‰ ($\delta^{15}N_{London\text{-}herbivores}$ = 6.7‰ and $\delta^{15}N_{London\text{-}omnivores}$ = 5.5‰) reflecting the addition of other resources such as omnivores and marine/freshwater sources.

7.8 Discussion

7.8.1 Bone carbonate preservation

Results from this study indicate that most of the archaeological bones have been altered due to diagenesis. Diagenesis results in an increasingly ordered crystal structure with larger crystals and less strain, which therefore results in a measurably higher IRSF value when compared to unaltered bones (Thompson et al. 2011, 168). Higher crystallinity in diagenetically altered bone samples is a result of an increase in crystal size and atomic order due to Ostwald ripening, loss of smaller crystals, or both (Kontopoulos et al. 2018). Theoretical and empirical evidence suggests that once bone crystallites are removed from the organic matrix, they experience simultaneous dissolution and growth in a manner analogous to Ostwald ripening. Ostwald ripening is a thermodynamic process (movement of energy) whereby smaller crystals melt, and larger crystals grow at the expense of the smaller ones as a result of the difference in the chemical potential of the crystals. Due to the bones having both higher energy small crystals and lower energy larger crystals, the atoms on the small crystals will detach off the surface, diffuse and deposit on the larger crystals as the system moves from a higher to a more energetically stable lower energy state. This in turn shrinks the smaller crystals. Once a bone is removed from the biological control of an organism, the bone crystallites begin to spontaneously grow by Ostwald ripening, unless this process is constrained by the organic matrix. Therefore, as the organic matrix is broken down, bone crystallites increase in size thereby increasing the IRSF (Tuross et al. 1989; Trueman et al. 2004).

Regarding bioapatite carbonate content, the C/P ratios in both the human (Mean = 0.17±0.03) and animal (Mean = 0.16±0.02) samples in this study, are lower than those of modern bone (Mean

C/P=0.24 ± 0.003) suggesting that, generally, the samples used here had no net gain of carbonate from the burial ground. It has been reported that higher values in the C/P ratio are a result of the interchangeable carbonate ions on the bone apatite lattice (Krueger 1991). The lower C/P ratios in the current archaeological samples compared to modern samples suggest a diagenetic loss of the carbonate fraction in the bone apatite possibly due to the dissolution of the mineral phase.

Overall, the IRSF and C/P ratio display a strong inverse correlation for all the human (R^2 = 0.616) and animal (R^2 = 0.794) samples. This indicates a significant relationship between the mean C/P ratio and mean IRSF. Since the relationship is negative (Figure 7.3), there is a general trend of increasing infrared splitting factor (IRSF) values with a reduction in carbonate-to-phosphate (C/P) values reflecting the loss of carbonate with increasing crystallinity. This is consistent with previously reported work (Sillen 1989; Thompson et al. 2009; Thompson et al. 2011).

7.8.2 Animal management

Although the animals sampled in this study derive from different sites across the North of England, the herbivore (cattle and sheep) isotopic values are tightly clustered with overlapping ranges and a standard deviation of ~±0.4 within each species (Table 7.5). The herbivore $\delta^{13}C_{coll}$ results indicate that their diet was dominated by C_3 plants (Figure 7.6), conforming to the expectations that livestock were raised primarily on C_3 forage and fodder crops (Fussell 1937; Overton 1984). The omnivores (pigs and domestic fowls), on the other hand, have slightly more elevated $\delta^{13}C_{coll}$ values than herbivores which suggest a dietary source enriched in ^{13}C (Table 7.5). This is probably indicative of different management strategies for these species. Omnivores in particular would have fed on a range of resources, often being fed the scraps of human consumption. Cattle and sheep may have grazed on communal grasslands left in their natural state in summer but kept in stalls and folds respectively in winter (Fussell 1937; Anon 2001). To supplement their grazing during winter, cattle could be fed on crushed oats, clover, rapeseed residues, and cabbage whilst sheep were typically fed on oil cake made from clover, turnips, mustard, and vetches (Fussell 1937; Anon 2001). In contrast, pigs were fattened with beans, pulses, buttermilk, curds, brewers grain, and potatoes (Bowden 1990; Smith 2000) and domestic fowls were fed grain (Anon 2001) but both omnivores also consumed human food scraps since they were backyard animals (Müldner and Richards 2007; Hammond and O'Connor 2013; Bleasdale et al. 2019).

Pigs have the largest $\delta^{13}C_{coll}$ range (13.8) compared to the rest of the animals (Table 7.5), which suggests different pig husbandry feeding regimes or management in different locations. This is especially true for three pigs exhibiting strikingly high $\delta^{13}C_{coll}$ values, indicative of C_4 plant consumption. Although there is a reference to maize (a C_4 plant) being utilised as animal fodder in Britain (Holland 1919; Messer 2000; Martin 2012), it is unclear which species were specifically fed

on maize, but, from the results here, it seems that cattle and sheep were not. Some historical documents suggest that some cattle were imported from Africa where they were fed maize (Capie and Perren 1980; Plumb 2010). Pigs, however, were most likely fattened on maize, as was the case in 18th and 19th century France (Hohenberg 1977). Moreover, there are indications that during this period some pigs were imported either whole or as preserved pork from abroad , for example the Americas and Australia where they were fattened on C_4 crops such as maize (Capie and Perren 1980; Plumb 2010). The human collagen results suggest that it is most likely that the three pigs with ^{13}C enrichment in collagen were not consumed by the rest of the human population in this study.

Except for the two pigs and one cow, all the other 24 faunal remains analysed for carbonate exhibited $\delta^{13}C_{carb}$ values suggesting consumption of a C_3 based diet (Appendix A). The two pigs were those previously identified with ^{13}C enrichment in collagen. This enrichment is only evident in the collagen data for these two pigs but not for the cow which suggests that high carbohydrate C_4 foods in particular may have contributed to the diet of the cow. Nevertheless, carbonate data reveals a dietary component that is missing from the collagen data later on the discussion.

There is a large range in cattle and sheep $\delta^{15}N$ values (5.5 and 7.2 respectively) that probably reflects the geographical range from which these animals were drawn, across the country. Historical documents indicate that animals from different areas were supplied over great distances to some of the sites sampled here. For instance, animals consumed in Manchester could originate from the Welsh Borders and Scotland (Scola 1992). Other animals with elevated $\delta^{15}N$ values may have grazed in fields that had been manured, or on salt-water marsh plants (Drummond and Wilbraham 1939; Britton et al. 2008; Beaumont et al. 2013b). Elevated $\delta^{15}N$ values exhibited in some pigs and some domestic fowl, reflect a diet with a component of animal protein as to be expected in omnivores often consuming human refuse and waste (Müldner and Richards 2007; Hammond and O'Connor 2013; Halley and Rosvold 2014). Indeed, when the human and faunal sample populations are plotted together (Figure 7.7C), it is evident that some of the omnivores (pigs and domestic fowls) are quite close to the human signals, reflecting this. However, low $\delta^{15}N$ coupled with low $\delta^{13}C_{coll}$ values in some of the pigs suggest that they consumed an exclusively plant-based diet, indicating a range in husbandry strategies for these omnivores.

7.8.3 Interpreting human diet using $\delta^{13}C_{coll}$ and $\delta^{15}N$

Generally, the $\delta^{13}C$ and $\delta^{15}N$ values for all analysed individuals in this study indicate that their diet was heavily C_3-based. Although the northern diet is broadly similar, there are statistically significant differences in the mean $\delta^{13}C$ and $\delta^{15}N$ values between different sites and Hazel Grove, a lower-class population (Appendix D). However, it is worth noting that the mean $\delta^{13}C$ and $\delta^{15}N$ differences between these sites are too small (average 0.4‰ and 0.8‰ respectively) to suggest interpretable

dietary variations being the cause of these statistically significant differences. Focusing on London, the wider comparison of human collagen data from both this and other published studies supports a different dietary trend for London in comparison to other areas of England at that time (Figure 7.7D). Samples from London sites have higher mean $\delta^{15}N$ values relative to the rest, suggesting London's access to high trophic level protein, greater than other cities in England, even at lower social levels. Smith's (1864) research revealed that a lot of people from poorly paid urban occupations such as weaving and shoemaking in the north of England, consumed mostly bread and potatoes with little or no meat or milk, therefore, it is unsurprising to find low mean $\delta^{15}N$ values from individuals in the north. The two London populations analysed here (Figure 7.7D) exhibit lower $\delta^{15}N$ values that are reflective of the low socio-economic status compared to other London published populations. Smith's (1864) survey of the poor in London revealed that the London poor consumed very little meat and milk compared to the wealthier individuals (Smith 1864, 216), which would explain the two populations' low $\delta^{15}N$ values. It should also be noted that the lower social status London populations in this study still possess higher $\delta^{15}N$ values than the lower status northern populations suggesting that they were consuming a diet that was more enriched in nitrogen (Figure 7.9). Improvements in transportation and interventions from the government that lowered fish prices and increased the number of livestock brought to the city of London meant that these products were more widely available to all, more so to those of higher privilege and wealth (Drummond and Wilbraham 1939; Stead 1985), which is probably what is reflected in the isotopic values in this study. The human-animal collagen offsets for London reflect that these populations consumed more higher trophic level animal protein than those in the manufacturing towns of the north, with a greater potential input of aquatic resources. Considering collagen $\delta^{13}C$ data, all individuals, bar the outlier QCS 1123 (previously identified by Bleasdale et al. (2019) as having been born elsewhere), can be considered to have been consuming mostly C_3 plants.

7.8.4 Interpreting diet using $\delta^{13}C_{coll}$, $\delta^{15}N$, and $\delta^{13}C_{carb}$

The application of carbonate isotopic modelling techniques is increasingly becoming a significant component in palaeodietary interpretations as they provide more detailed insights into past diets than the traditional methods using bone collagen alone e.g. Somerville et al. (2013), Olsen et al. (2016) and Reitsema et al. (2017). To explore post-medieval dietary patterns further, $\delta^{13}C_{carb}$ and $\delta^{13}C_{coll}$ values from this study were plotted against regression lines of the simple carbon isotope model (Figure 7.11) developed by Froehle et al. (2010). The proximity of the values to the poles of the regression lines denotes the ratio of C_3 to C_4 foods in the diet whereas primary sources of dietary protein are indicated by the proximity of the values to the regression lines (see Chapter 5).

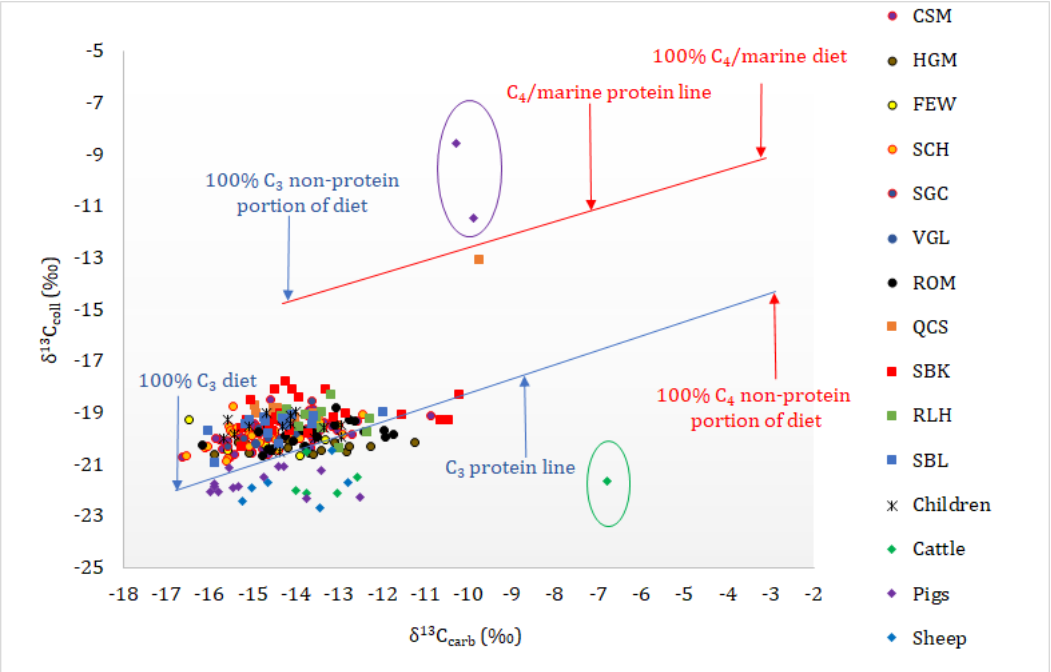


Figure 7-11: Scatter plot comparing $\delta^{13}C_{coll}$ and $\delta^{13}C_{carb}$ values on the Froehle et al. (2010) Bivariate regression model for all humans and fauna in this study.

When the human and animal data are plotted in the regression model, the majority of individuals fall near the C_3 protein line, suggesting that the primary protein contributor to their diet was from C_3 terrestrial resources (Figure 7.11). Furthermore, they fall near the 100% C_3 energy endpoint suggesting that their main energy contributor (including carbohydrates and fats) was also from C_3 terrestrial resources. Some St Barnabas individuals are shifted away from the 100% C_3 diet endpoint towards the 100% C_4 non-protein portion of the diet suggesting that it is possible that they consumed a small proportion of carbon from C_4 plants but no C_4/marine protein sources. It seems that only QCS 1123 is displaying a clear inclusion of C_4/marine protein resources in his diet. Regarding the animals, except for two pigs and a cow (circled in Figure 7.11) that are clearly separated, most animals subsisted mainly on a C_3 vegetarian diet. The cow consumed a mixture of C_3 and C_4 plants, whereas the pigs ate a mixed diet comprising both C_3 and C_4 plants and dietary protein from either C_4 or marine resources. This chimes with the respective herbivorous and omnivorous nature of these species.

The regression analysis model, however, does not differentiate the consumption of C_4 and marine resources and, therefore, to further explore the data, a multivariate isotope model (Froehle et al. 2012) which incorporates $\delta^{15}N$ collagen results using cluster and discriminant analysis and enables the plotting of data into five clusters of dietary types, was employed (Figures 7.10). Froehle et al. (2012) utilised controlled feeding experimental animal data to test the model, verifying that it could also be used for other archaeological populations. However, it should be noted that all the archaeological populations included in creating the clusters were exclusively from America.

The multivariate model produced results generally in line with the regression analysis model but the addition of the $\delta^{15}N$ values provided another dimension with which to differentiate between marine and C_4 diets.

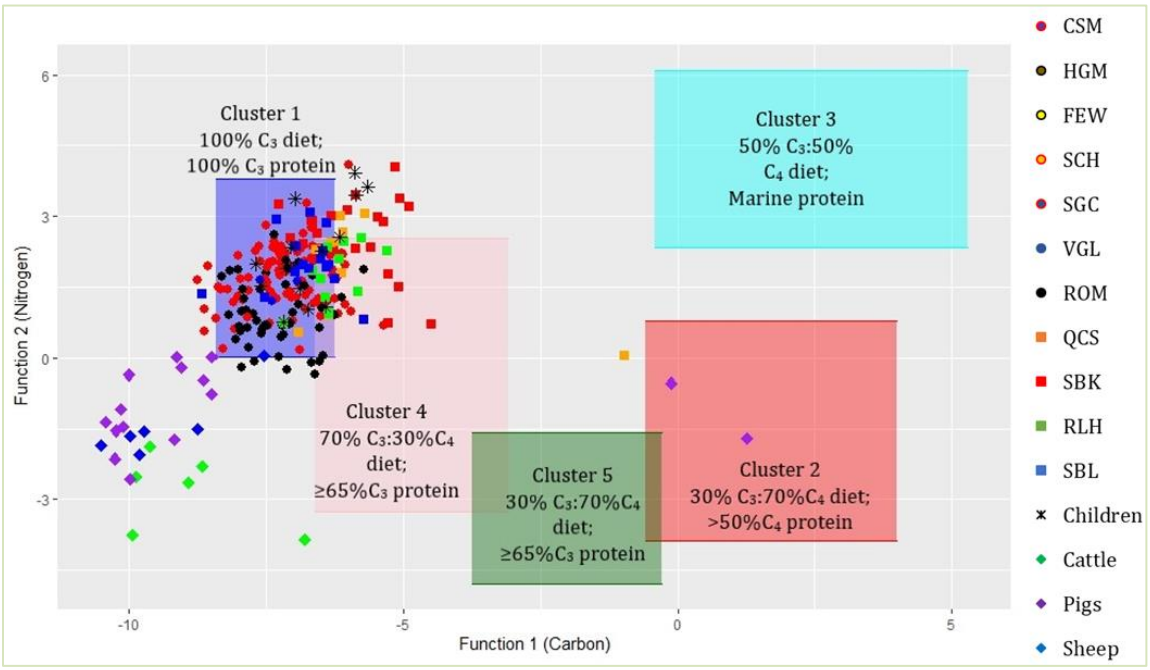


Figure 7-12: F1 and F2 discriminant function values from individuals across England plotted against previously generated dietary clusters (see Froehle et al., 2012). All children are from Manchester.

When the humans are placed within Froehle et al. (2012) clusters, ~65% of the sampled population including children plot within Cluster 1, indicating a 100% C_3 diet, (Figure 7.12). However, around 20% of the population plot within Cluster 4, which corresponds to a total diet of about 30% C_4 foods and about 35% C_4 protein. An additional ~15% plot within the area where Clusters 1 and 4 overlap, suggesting that these individuals most likely consumed some C_4 plants, although it is not clear how much. The Queens Chapel Savoy (QCS 1123) individual plots closer to Cluster 2 (30% C_3:70%C_4 diet; >50%C_4 protein, suggesting that his diet was based predominantly on C_4 sources, and not marine foods).

The multivariate model matches well with the linear regression model estimate of the C_3 herbivorous diet for all the animals except the cow and two pigs, identified as different from the rest by the linear regression model, but it however clarifies the protein consumed by the two pigs and possibly the omnivorous nature of the rest of the pigs. It seems that the two pigs that had been identified as consuming C_4 or marine protein exhibited consumption of about 70% C_4 diet and over 50% C_4 protein rather than marine protein. For the other pigs, Function 2 'nitrogen' values tentatively suggest that a few pigs consumed similar foodstuffs to some humans, as they plot very close to the Cluster 1 bounds. This supports the previously discussed notion that some pigs were consuming human food scraps during this period. Nevertheless, it is as expected that animals were mostly consuming C_3

plants because the most common plants in England during that time were C_3 plants (Barker et al. 1970; Oddy 2003). However, the three animals with a C_4 signature most likely consumed maize (Holland 1919; Martin 2012) or had been imported from areas where they were foddered on C_4 resources (Capie and Perren 1980; Plumb 2010). Therefore, consumption of C_4 foddered animals may account for some humans ^{13}C enrichment in this study.

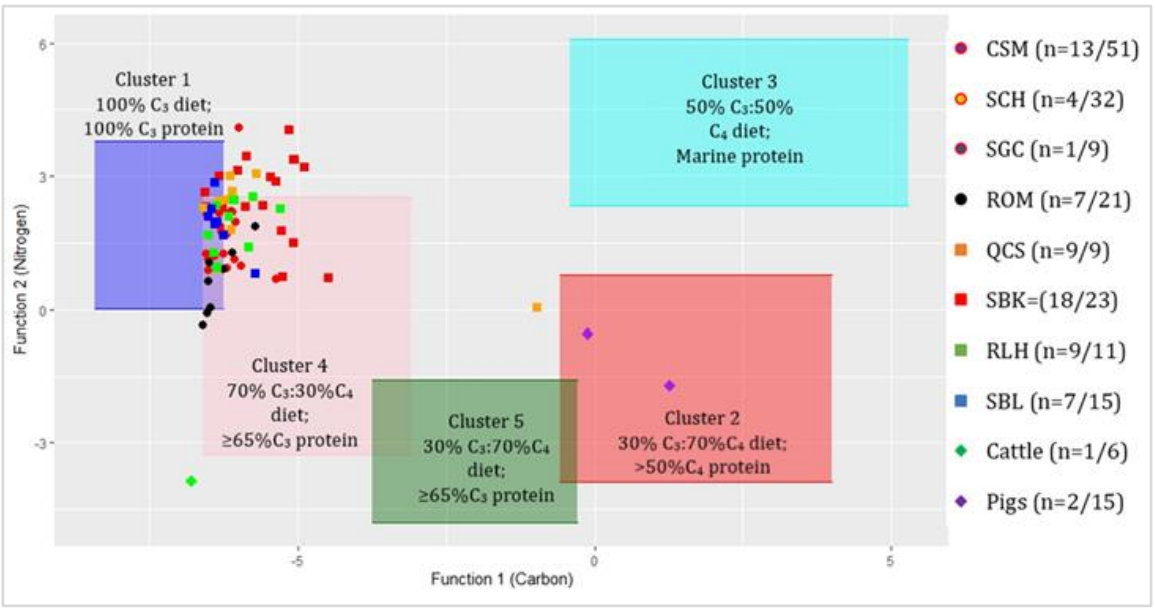


Figure 7-13: Adult individuals falling in Cluster 4 (70% C_3:30%C_4 diet ≥65%C_3 protein) and the overlap between Clusters 1 and 4 overlap at each site in the Multivariate model in Figure 7-12.

For a clearer analysis of how many people consumed C_4 resources, all adults who fell in the Cluster 4 centroid which corresponds to 30% C_4 foods and about 35% C_4 protein as well as those within the overlap between Clusters 1 and 4, were grouped together, as it still suggests some C_4 input in the diet. In cases where there are elevated $\delta^{13}C$ and $\delta^{15}N$ values (corresponding to Functions 1 and 2 respectively in this case) in children, this may be interpreted to be as a result of breastfeeding (Beaumont et al. 2015), therefore these were excluded from the overall interpretation of a C_4 diet here. A region-based comparison of these results indicates that about 18% of the northern populations exhibited a diet consisting of some C_4 foods, most likely in the form of C_4 animal protein, cane sugar, or maize. The combined northern individuals in this category were from Rotherham, Cross Street, St George's Crypt and Square Chapel populations. All except Rotherham were middle or upper social class populations, therefore it is unlikely that they consumed maize, as it was regarded as animal fodder by those classes (Holland 1919; Messer 2000; Martin 2012). Rather, it is suggested here that some C_4 signature in their diet could have derived from C_4 animal protein and cane sugar consumption. These three populations predate the 1874 Abolition of Sugar Tax when cane sugar became available to all social classes, therefore it is posited here that they must have been the wealthier members of their respective societies and could therefore better afford to buy sugar when compared to the rest of their respective communities (Mintz 1986; Walvin 2017). In contrast, the

individuals from Rotherham were poor therefore it is highly unlikely that they could have been in a position to afford to buy as much cane sugar as the middle- or upper-class populations. However, the high frequency of tooth decay observed at Rotherham Minster suggests high-sugar diets (Keefe and Holst 2011). In addition, the location of cavities in these individuals' teeth followed a pattern seen previously in individuals consuming high amounts of sugar (Corbett and Moore 1976; Keefe and Holst 2011). High consumption of sugar for these individuals is most likely a result of the cane sugar price drop between 1800 and 1850 that was a result of equalisation of cane sugar duties in Britain, which resulted in the national consumption rising from 300 million in 1800 to a billion pounds in 1852 (Mintz 1986; Walvin 2017). Once the sugar duties were equalised, the poor class individuals' consumption of cane sugar also increased (Mintz 1986, 142), making it possible for the Rotherham individuals' C_4 diet to have been from cane sugar as well as C_4 animal protein.

In London, by contrast, a larger proportion of the population (73%) had some C_4 input into their diet, including individuals of both high and low socioeconomic status. Again, there are similar sources to those of the North that most likely contributed to this C_4 signature. Unlike the northern populations, some individuals from the London sites plot above the Cluster 4 zone and these high Function 2 values (hence high $\delta^{15}N$ values) suggest consumption of a greater proportion of C_4 animal protein than the rest of the population studied. In addition, since these individuals plot between Cluster 1 (100% C_3 diet;100% C_3 protein) and Cluster 3 (50% C_3:50%C_4 diet; Marine protein), it is also possible that they also could have been consuming marine protein. However, the current model does not have a Cluster for low marine protein intake, therefore, it is impossible to determine the amount of marine protein the individuals were consuming, if any. The isotopic data here is congruent with the historical evidence that indicates that Londoners consumed more animal protein than the rest of the country during this period (Smith 1864; Barker et al. 1970; Spencer 2000; Picard 2006; Trow-Smith 2013; Metcalfe 2015). It has already been established that Londoners were receiving livestock from a wide range of areas, therefore it is possible that they also had greater access to C_4 animal protein.

Additionally, London was the commercial, financial and trading centre behind Britain's sugar colonies (Walvin 2017). From the 1650s, Londoners began using cane sugar as an additive to hot beverages such as tea, which by 1704, had become popular because of the transformation in British tea-drinking when shiploads of tea were transported to the city by the restructured United East India Company from China (Walvin 2017). By 1800, the poor in the city had also become attached to cane sugar as a sweetener in tea. This was because, by this time, there was a lot of cheap imported tea, a development helped by the reduction of tea duties. Furthermore, London employers began providing their domestic servants sugar and tea as a replacement for the ration of beer, traditionally granted to servants as part of their food allowance (Smith 1864; Graham 2008, 72). Therefore, it is possible that the poor individuals from Royal London hospital (1825-1841) displaying a C_4 signature in their diet

were consuming a significant amount of cane sugar and C_4 animal protein. It is worth noting that these individuals died before the Great Irish Famine and therefore would not have been consuming a maize diet, as described in the following paragraph.

The St Brides Lower population, on the other hand, included lodgers and inhabitants of the Bridewell workhouse. During the Great Irish Famine, many Irish people migrated to Britain where they stayed and worked in workhouses and where they also received relief maize in the form of soup or porridge (Dudley-Edwards and Williams 1956; Ó'Gráda 1989). The consumption of a short-term maize relief diet over the duration of the Famine in workhouses has also been suggested in other post-medieval studies in England (Beaumont 2013; Beaumont et al. 2013b), but it was not evident using collagen results alone. However, it is proposed here that some of the C_4 based diet in the individuals is unlikely to have been from maize. This is because the introduction of relief maize lasted for only about two years from its introduction in 1845, after the then new Prime Minister Charles Trevelyan, closed the food depots that had been selling maize and stopped its importation from America (Dudley-Edwards and Williams 1956; Ó'Gráda 1989; Swift 2002). As a consequence, the two-year consumption of maize might not have been enough to change these individuals' bone collagen isotopic signal significantly. Therefore, similar to Royal London Hospital individuals, it is also possible that their C_4 based diet was from cane sugar and C_4 animal protein. Additionally, the St Brides Lower graveyard was closed in 1849, therefore, only a few famine migrants could have been buried there.

Looking at the Queen's Chapel population, the results suggests that all individuals had access to C_4 resources. This population was made up of hospital patients, parishioners, criminals, seamen, and military personnel. The heterogeneous social structure at this site makes it challenging to draw conclusions regarding the C_4 source, but given the military connections of the site, it is highly likely that at least some of these individuals could have had access to C_4 resources while they were outside England. This is probably the case for QCS 1123 with a 70% C_4 diet indicating that he is likely to have originated from elsewhere, potentially America (Bleasdale et al. 2019).

Finally, the wealthy St Barnabas population is particularly notable for having the highest number and a high proportion (78%) of individuals (18) who consumed C_4 resources. As suggested earlier for other middle/upper social class populations in the country, these individuals are most likely to have obtained their C_4 signature from both consumption of cane sugar and C_4 animal protein.

7.9 Conclusion

This study is the first to obtain human and animal $\delta^{13}C_{carb}$ data in post-medieval England. The multi-proxy isotopic approach to a large sample of animal and human populations has provided a detailed insight into animal management and the diet of 17th to 19th century English populations. Dietary

interpretations based on traditional methods using data from the analysis of carbon and nitrogen stable isotopes only from bone collagen have been consistent and to a large extent adequate in deciphering diets dominated by C_3 plants and animal products such as the ones in England. Where C_4 diets were significant, these previous studies have only been able to make suppositions that C_4 resources were available, but it was not possible to clearly demonstrate the definitive presence of C_4 source(s) in the diet using collagen analysis only. However, with the addition of bone carbonate analysis and linear and multivariate modelling, consumption of C_4 resources has been identified more clearly than would have been the case using collagen results alone. The results demonstrate that within these post-medieval English populations, food consumption patterns were not uniform, with significant variations in animal protein as well as C_3 and C_4 plant consumption at both regional and site-specific scale. The dietary patterns at all sites indicate that individuals in London had more access to more animal protein and foods enriched in ^{13}C compared to those in the manufacturing northern towns. Intrapopulation variability was also evident, with some individuals consuming more C_4 resources than the rest of their respective populations. Differences in diet between sites discussed in this paper present striking evidence of the role played by social status in the type and quantity of food that individuals had access to during this period. In this study, social status variation investigations have revealed that individuals from the middle and upper classes had access to more animal protein and C_4 resources than their lower social class counterparts. As this is the first study to use bone carbonate data in England, no comparisons with other sites outside this study were made but future isotopic work will be useful to expand the available data beyond what has been provided here, enhancing our knowledge of the post-medieval diet in England.

Chapter 8: Isotope analysis of human dental calculus carbonate: investigating a potential new proxy for sugar consumption

8.1 Introduction

Investigations into past diets frequently draw upon direct isotopic measurements of the surviving body tissues of consumers. Bone and teeth most commonly survive in archaeological contexts but soft tissues (e.g., skin), hair and fingernails can also provide dietary information if they are preserved (Lamb 2016). Dental calculus (mineralised dental plaque) has recently received attention for its potential to reveal aspects of past diets, primarily through the analysis of biomolecules - proteins, DNA, (Warinner et al. 2014a) and micro debris trapped within the mineral matrix (Radini et al. 2017). Only a few studies have explored the potential for isotopic analysis of dental calculus but they have mainly analysed bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope compositions of the organic matrix with mixed results (Scott and Poulson 2012; Poulson et al. 2013; Salazar-Garcia et al. 2014; Eerkens et al. 2014; Price et al. 2018). One recent study targeted the inorganic fraction of calculus; however, the study was very limited in terms of the methodology as well as the small number of samples analysed (Price et al. 2018). The present study goes further to explicitly explore the potential utility of $\delta^{13}C$ in dental calculus mineral as a dietary proxy for identifying C_4 sugars. Additionally, FTIR analysis is utilised in this study to characterise the composition and crystallisation of dental calculus. A few studies have used FTIR to characterise dental calculus composition e.g., Kakei et al. (2000), Hayashizaki et al. (2008) and Satpathy et al. (2019) but there is still a paucity of detailed information. This study presents for the first-time analysis and interpretation of FTIR result in dental calculus for assessment of diagenesis in line with what has been previously undertaken for bone and enamel carbonate. It goes on to present the first comparison of dental calculus carbonate with both bone and enamel carbonate to determine whether dental calculus carbonate is a suitable substrate for identifying C_4 resource consumption. In addition, the results obtained here are used to determine if there are any intra-individual variation among tissues. This analysis is notable for analysing material from archaeological populations as well as modern individuals, the latter of which is rarely incorporated into archaeological studies.

The current understanding of diet in palaeodietary studies utilising stable isotopes ($\delta^{13}C$, $\delta^{15}N$), is mainly based on the analysis of bone and dentine collagen ($\delta^{13}C$, $\delta^{15}N$) as well as enamel and bone carbonate ($\delta^{13}C$) e.g. Ambrose and Norr (1993), Tykot et al. (1996), Lee-Thorp (2002), Beaumont and Montgomery (2016) and Beaumont et al. (2018). Bone and dentine collagen $\delta^{13}C$ represent the protein sources in the diet, but a proportion of collagen can be synthesised from lipids or

carbohydrates; bone and enamel carbonate $\delta^{13}C$ values, however, reflect whole diets - carbohydrates, lipids, and proteins (Krueger and Sullivan 1984; Ambrose and Norr 1993). Palaeodietary reconstruction using the carbon isotope values are useful in distinguishing between the consumption of C_3 (low $\delta^{13}C$) and C_4 (high $\delta^{13}C$) terrestrial resources as well as between marine (high $\delta^{13}C$) and terrestrial (low $\delta^{13}C$) foods. Nitrogen isotope values provide information on the main protein sources of the diet, for example, differentiating between plant-rich protein and animal-rich protein diets (Schwarcz and Schoeninger 2012).

Previously, C_4 cane sugar consumption has only been detected under specific circumstances, such as when sugarcane is an indigenous crop (Ambrose et al. 1997; Stone et al. 2019), or inferred where sugarcane may have been used as animal fodder, in which case the C_4 signal is acquired by the herbivores (Alexander et al. 2015). The post-medieval period between the 17th and 19th centuries saw an increase in the consumption of cane sugar in England (Walvin 2017). Nevertheless, although hypothesised in some bone collagen isotope studies of post-medieval populations in England (Trickett 2006; Nitsch et al. 2010; Nitsch et al. 2011; Bleasdale et al. 2019), the consumption of cane sugar in the English diet has been hard to detect despite it being a key element of cuisine. Although a few individuals who may have consumed it have been identified from bone carbonate isotope analysis (see Chapter 7), cane sugar has been difficult to detect most likely because it was not a dominant component of the diet. Nonetheless, in this study, it is hypothesised that dental calculus carbonate could offer a novel way of identifying cane sugar more easily than the traditional methods.

To fully assess the potential for dental calculus in palaeodietary studies, and particularly for the possibility of determining C_4 cane sugar consumption for post-medieval England, isotope analysis on dental calculus, bone, and enamel obtained from the same individuals was conducted in a broad range of populations. The Price et al.'s (2018) method was modified to grind the calculus and expose it to bleach for a longer period in an effort to ensure that only the mineral part of dental calculus was retained.

8.2 Dental Calculus and potential for isotopic analysis

Calculus is frequently preserved in archaeological contexts and has been found to survive on the teeth of late Pliocene hominins (Blumenschine et al. 2003) and Miocene apes dating up to 8 to 12 million years ago (Hershkovitz et al. 1997). Dental calculus results from the calcification of plaque biofilms that accumulate and mineralise during life, however, the mechanism and rate by which dental calculus forms are still not completely understood (Wilkins 2015). There are two types of dental calculus: supragingival and subgingival (Lieverse 1999). The present study utilises the supragingival dental calculus for all analyses.

Plaque formation around the teeth on the supragingival surface begins when microorganisms, overwhelmingly bacteria, colonise the pellicle on the surface of the teeth. These bacteria obtain their nutrients primarily from the amino acids, proteins, glycoproteins, and peptides from saliva to grow, confluence, and produce a biofilm (Mandel 1987; Hillson 1996; Lieverse 1999). During the production of the biofilm, extracellular polymer synthesis occurs resulting in glucans and fructans from sucrose (refined carbohydrate) metabolism becoming part of the plaque matrix (Lieverse 1999; Hicks et al. 2003). Plaque begins to harden after about ten days to form dental calculus which then builds up over time. Depending on an individual's hygiene, diet as well as lifestyle, the deposition of plaque begins soon after tooth eruption, and the quantity increases over time, ceasing at the death of an individual when the production of saliva stops. Mineralisation of dental plaque only occurs in the presence of saliva when an individual is alive (MacPhee and Cowley 1975; Hardy et al. 2009; Buckley et al. 2014; Warinner et al. 2014b; Hardy et al. 2016). Calculus formation is facilitated by alkaline conditions in the mouth which in turn increases precipitation of minerals from the saliva (Hillson 1979). Mineralisation also depends on the food being consumed, salivary flow, oral hygiene, and the genetics of the individual (Hardy et al. 2009). The mineral composition of calculus varies according to the concentration of calcium and phosphorus, the presence of calcification promoters such as urea, fluoride, and silicon (Jin and Yip 2002) and presumably also by the bicarbonate composition in the saliva - itself a function of the rate of carbohydrate metabolism (Hicks et al. 2003, 51).

Dental calculus is composed of about 20% organic and 80% inorganic constituents (Lieverse 1999). Calculus deposits generally contain the inorganic mineral calcium phosphate; crystalline forms of hydroxyapatite, octacalcium phosphate, and whitlockite in varying quantities, with hydroxyapatite usually being the most abundant (~58%), which has high levels of carbonate (Goldman 1986). The organic matrix contains trapped proteins, glycoproteins, plant fibers, lipids, and carbohydrates (Little and Hazen 1964; Lieverse 1999). Unlike the carbonate component of bone and enamel, which derives from blood bicarbonate (Krueger and Sullivan 1984), supragingival calculus derives its carbonate from the precipitated bicarbonate of salivary fluids (Waerhaug 1955; Dawes 1970; White 1997). Experiments have indicated that salivary fluids derive bicarbonate from two sources in the body – (i) transfer from the blood and (ii), bicarbonate that is produced in the cells of the salivary glands (Wechsler, 1959). The salivary glands bicarbonate is from the carbon dioxide (CO_2) resulting from the secretory activity of the salivary gland cell, (based on this equilibrium $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$) during the action of microbes when metabolising carbohydrates in the mouth. The concentration of bicarbonate is greatly increased during food intake and mastication (Sand 1951; Wechsler, 1959; Dawes 1969; Dawes 1974; Hicks et al. 2003).

As discussed earlier, dental calculus formation is in part, linked to the consumption of high levels of carbohydrates due to sugars that are eventually converted to carbon dioxide (Lieverse 1999; Buckley et al. 2014). Similarly, consumption of carbohydrate-containing foods has been associated with the

formation of dental caries. It has been determined that dental caries develops due to a dissolution of the enamel by the action of acids that are produced in the mouth by the fermentation of dietary carbohydrates by oral bacteria (Touger-Decker and van Loveren 2003). As is evident by the link between sugar consumption and dental caries, oral microbial communities preferentially metabolise the most bioavailable components of dietary carbohydrate (Touger-Decker and van Loveren 2003; Gupta et al. 2013; Sheiham and James 2014; Mant and Roberts 2015). Therefore, it is likely that ^{13}C enriched CO_2 generated from microbial carbohydrate metabolism in the mouth and from the blood via the salivary glands is incorporated into calculus carbonate. The hypothesis, therefore, is that calculus carbonate will reflect the consumption of C_4 cane sugar in post-medieval England, which has been difficult to identify using other isotope methods.

8.3 Experimental

8.3.1 Materials

Samples from bone, enamel, and dental calculus were collected from 57 individuals for bone collagen, bone carbonate, enamel carbonate, and calculus carbonate analysis. The three different populations sampled for this study were as follows: (i) 22 individuals from English medieval populations, Southwell Cemetery, Nottinghamshire (SCN), St Peter's Cemetery, Leicester (SPL), and Nun's Field, Chester (NFC) dating between the 7th-15th centuries, (ii) 15 English 18th to 19th-century individuals from Cross Street Manchester (CSM) and (iii) 20 modern individuals who died between 1996 and 2016, from the William M. Bass Donated Skeletal Collection housed in the Department of Anthropology, University of Tennessee, USA. The conditions under which the modern individuals were kept after death ensured minimal degradation due to the environment. When an individual dies and donates their body to the Forensic Anthropology Center (FAC), the body is placed outside at the facility to decompose naturally on the ground surface with a loose plastic covering over it to ensure anonymity. Once the body has skeletonised, the remains are recovered and cleaned of any remnant soft tissue with tap water and a toothbrush. The time between recovery and processing may be a few weeks, a year, or even longer for the individuals who died before 2012. However, since 2012, the time between recovery and curation is weeks to months. Once cleaned, the bones are air-dried, individually labelled, and then placed in an acid-free cardboard box, where they are securely stored in the William M. Bass Donated Skeletal Collection in the Department of Anthropology at the University of Tennessee.

Previous isotope analysis has revealed that English medieval populations consumed C_3 terrestrial resources (Mays 1997; Richards et al. 2002; Müldner and Richards 2005). Similarly, diet for English post-medieval individuals has been found to have been dominated by C_3 terrestrial resources,

although C_4 cane sugar or maize is known to have been part of their food mix (Richards 2006; Trickett 2006; Nitsch et al. 2010; Nitsch et al. 2011; Roberts et al. 2012; Beaumont 2013; Beaumont et al. 2013a; Beaumont et al. 2013b; Brown and Alexander 2016; Bleasdale et al. 2019). Finally, the modern population represents North American individuals, who almost certainly consumed an abundance of C_4 sugar and maize. Currently, the intake of sugar refined from C_4 plants such as corn and sugarcane makes up to 78% of the sugar consumed in the United States and forms around 16% of the total calories consumed, however, in some cases it exceeds 35% (Jahren et al. 2006; Jahren et al. 2014). The modern group, therefore, provides control for highly C_4 diets.

8.3.2 Ethical approval for research

Permission to sample and analyse modern human material was requested from the Forensic Anthropology Center (FAC), University of Tennessee, Knoxville's donated human body collection and it was granted. All body donations were anonymised to protect the identities of the donors. Furthermore, since all modern human tissue stored at the University of York must comply with the Human Tissue Act (2004) as enforced by the Human Tissue Authority (HTA), approval was sought from the NRES Committee Yorkshire & The Humber – Leeds East REC to work and store modern human tissues at the University of York. This was accepted subject to the work being carried out within the guidelines and SOPs of the York Tissue Bank under HTA license.

8.3.3 Methods

8.3.3.1 FTIR-ATR

Sample preparation and analysis of 57 bone and 52 dental calculus samples were executed according to Kontopoulos et al. (2018) method. This study followed the established bone FTIR analysis method for dental calculus samples as there is no agreed standard for the latter yet. Prior to analysis, the bones were cleaned using a sterile scalpel blade and dental calculus samples were rinsed with deionised water to remove dirt and contaminating material. Once cleaned the samples were grounded using an agate mortar and pestle. The powdered samples were then sieved through Endecotts woven stainless steel mesh sieves with an aperture size of 20μm and 50μm so that only grains between 20μm and 50μm particle size would be used. Spectral analyses were performed using OPUS software (Bruker). Spectra were collected in 144 scans, in the 4000–400 cm^{-1} wavenumber range, with a spectral resolution of 4cm^{-1} and zero-filling factor of 4. Each sample was measured in triplicate and ~ 2-3 mg of powder was pressed onto the diamond crystal and measured. The instrument's crystal and arm's tip were cleaned with a tissue paper soaked in propanol before each measurement and baseline correction and spectra normalisation was carried out using the OPUS

software as reported in Kontopoulos et al. (2018). FTIR analysis was performed before the acetic acid treatment. Two quality parameters, the infrared splitting factor (IRSF) and the carbonate-phosphate ratios (C/P), were assessed in this study. IRSF is used to evaluate the crystallinity (structural order) within the mineral component of the bone while the C/P ratio is a measure of diagenesis that reflects the changes to the carbonate in bioapatite crystals relative to the phosphate content ratio in a bone sample. The IRSF indices for bone samples were calculated following Weiner and Bar-Yosef's (1990) equation [IRSF = $[565_{ht} + 605_{ht}]/590_{ht}$] and the C/P ratio following Wright and Schwarcz's (1996) equation [C/P = $1415_{ht}/1035_{ht}$]. The mean values of modern bovine were utilised as a reference throughout. The intense peaks for dental calculus were measured at 595cm^{-1} ($\nu_4PO_4^{3-}$), 600 cm^{-1} ($\nu_4PO_4^{3-}$), 595cm^{-1} ($\nu_3PO_4^{3-}$) and 870 cm^{-1} ($\nu_3CO_3^{2-}$) following Hayashizaki et al.'s (2008) measurements. The C/P ratio and the IRSF were then calculated as follows [C/P = $870_{ht}/595_{ht}$] and [IRSF = $[580_{ht} + 600_{ht}]/590_{ht}$].

8.3.3.2 Lipid removal from bone

Prior to collagen and carbonate analysis on modern material, lipids were removed following Colonese et al. (2017). Briefly, samples were rinsed six times with a 2:1 dichloromethane:methanol solvent solution (DCM:MeOH; 3 × 2 mL). Each sample was ultrasonicated for 15 min and then centrifuged at 850 x g for 10 min. Samples were then rinsed with deionized water and dried at room temperature.

8.3.3.3 Bone collagen extraction

Collagen extraction from the 57 bone samples followed the standard operating procedure for BioArCh at the University of York which is based on the Longin (1971) method modified by Brown et al. (1988). Each bone sample was cleaned using a scalpel, to remove contaminants of the outer layer of bone. Following this, bone chunks of about 300-500 mg were demineralised in 8ml of 0.6M hydrochloric acid (HCl), agitated twice daily. The acid was changed every two days until demineralisation was complete. After that, the supernatant was removed, and the samples were rinsed thrice using deionised water and then gelatinised using pH3 HCl on hot blocks at 80°C for 48 hours. Next, the supernatant liquid containing the collagen was filtered using Ezee™ filters to remove unwanted particulate matter from the collagen solution and was then frozen for a minimum of 12 hours at -20°C before being freeze-dried for 48 hours. Collagen yields were estimated through dividing the collagen mass after filtration by the original bone mass after cleaning.

8.3.3.4 Bone carbonate extraction

Carbonate extraction of bone samples followed the standard operating procedure used at the University of York adapted from Snoeck and Pellegrini (2015) and Pellegrini and Snoeck (2016). Firstly, the bones were cleaned using a sterile scalpel blade to remove dirt and contaminating

material. The cleaned bones were crushed using an agate mortar and pestle. Approximately 7.5 mg of bone powder was required for each sample. To remove secondary minerals from the samples, the weighed samples were dissolved in 15 ml of calcium acetate [$(CH_3COO)_2Ca$] buffered 1 M solution (ratio 1:2) and placed on a roller rocker for about 30 minutes. After treatment, the samples were rinsed six times with deionised water, placed in the freezer for 24 hours, and freeze-dried for 24 hours to remove all the water and isolate the apatite. The 15 ml centrifuge tubes containing the treated samples were reweighed and the mass loss generated by the treatment was measured by subtracting the original weight of the tube.

8.3.3.5 Enamel carbonate extraction

A section of enamel, approximately 3 mm wide that spanned from the cervical margin to the cusp was separated from the tooth crown. In order to minimise contamination, all tools were cleaned prior to use and between samples. All surfaces of the enamel samples were lightly drilled with an abrasive drill bit, set as low as possible to avoid unnecessary heating. Any evident cracks as well as cut surfaces were lightly drilled. Each enamel chip was placed in a 2ml Eppendorf vial with deionised water and sonicated for 3min to remove any fine powder. In cases where water remained cloudy after sonication, repeat washes were performed. Enamel was then finely ground by using an agate mortar and pestle to a particle size of less than 50μm. Organic components of the enamel were removed by soaking it in a solution of 1.5% sodium hypochlorite over 24 hours, after which the enamel was rinsed with deionised water three times. Possible diagenetic contaminants were removed by soaking the enamel powder in 0.1ml of 0.1 M acetic acid per 1 mg of enamel and agitated for 10min. The acid was removed by repeated rinsing in distilled water five times centrifuging for 2 min at 13,700 x g in between rinses. Small sheets of parafilm were placed over each Eppendorf tube and a small hole was made using a sharp object to enable the sample to dry appropriately. The samples were placed in the freezer for 24 hours and freeze-dried for 24 hours to remove all the water and isolate apatite. The tubes containing the treated samples were reweighed and the mass loss generated by the treatment was measured by subtracting the original weight of the tube.

8.3.3.6 Dental calculus carbonate extraction

The extraction of mineral calculus followed the Price et al. (2018) protocol for mineral calculus analysis but with modifications from the Crisp et al. (2013) protocol on ostrich eggshell. Before carbonate analysis, the optimal time for the calculus to be submerged in NaOCl (bleach) was checked using Crisp et al. (2013) protocol. The bleach was expected to oxidise amino acids rendering them unavailable for analysis. The Crisp et al. (2013) study noted that ostrich eggshells coarse grain (500 - 1000 μm) free amino acid samples took a long time to demineralise, probably due to the reduced surface area to volume ratio, therefore they decided not to use powdered particles >500 μm in size in further experiments. They isolated a stable intracrystalline fraction within 72 hrs when using ostrich

eggshell powders of <1000 μm, but 120-hour exposure to bleach was required when using ostrich eggshell >1000 μm (fragment samples); therefore, subsequent experiments used ostrich eggshell powdered to <500 μm.

In light of the preceding recommendations by Crisp et al. (2013), for this study, the dental calculus samples were first washed in deionised water four times in order to remove any soil loosely adhering to it. The clean calculus was left to dry at room temperature and then powdered with an agate pestle and mortar to <500 μm which is a different approach from the one taken by Price et al. (2018), who utilised fragment samples. Since dental calculus is much richer in organics than shells (Brooks et al. 1990; Mackie et al. 2017), considering that for powdered shells, after 72 hrs, all amino acids accessible by 50 ml of 12% (w/v) NaOCl had been removed, the powdered samples in this study were submerged in 50 ml of 12% (w/v) NaOCl per mg of sample for 96 hrs. The samples were agitated every 24 hrs to ensure that the whole calculus matrix was exposed to the bleach. The bleach was removed by pipette and spotted onto coloured tissue paper to test if it was still active. The dental calculus was then washed five times in distilled water, with a sixth wash with HPLC-grade methanol to reduce any leftover bleach, and then air-dried overnight. To extract the mineral in dental calculus, ~1.5 mg of each bleached sample was reacted with 0.2 ml per 5mg of calcium acetate [$(CH_3COO)_2Ca$] buffered 1 M solution (ratio 1:2) (ph ~4.5) and placed on a roller rocker for about 40 minutes to remove exogenous carbonates. The samples were cleaned with distilled water four times and dried at 40°C.

8.3.3.7 Analytical measurement

All $\delta^{13}C$ and $\delta^{15}N$ ratios are expressed using the delta notation (δ) in parts per thousand (‰) relative to the international standards, VPDB for $\delta^{13}C$ and atmospheric N_2 (AIR) for $\delta^{15}N$, using the following equation: [δ (‰) = ($R_{sample}/R_{standard}$ -1) x 1000] (McKinney et al. 1950; Craig 1957).

8.3.3.7.1 Bone collagen

Approximately 0.5 mg of freeze-dried retentate was weighed out into 4x3.2 mm tin capsules and combusted alongside international standards in an Elemental Analyser/Isotope Ratio Mass Spectrometer (EA/IRMS): a Sercon 20-22 mass spectrometer coupled with a Sercon GSL Sample Preparation System module at BioArch, University of York. The collagen samples within the tin foil were completely combusted in an oxygen-rich atmosphere in a quartz reactor maintained at 1000°C in the elemental analyser to produce N_2, CO_2, NO_x, and H_2O. The resultant gases were transported in a helium stream through the reactor containing CuO and Cr_2O_3 oxidation catalysts and silver wool to bind unwanted sulphur and halogen products. The gaseous products were then swept in the reduction reactor with high purity copper where excess oxygen was removed, and NO_x was reduced to N_2 at 600°C. Water was then removed via a water trap containing magnesium perchlorate

($Mg(ClO_4)_2$). Nitrogen and carbon dioxide were separated by their molecular weight via a column packed with a stationary phase held at 70°C. Finally, they were then delivered sequentially to the mass spectrometer, where they underwent ionisation.

All samples were analysed in duplicate. Accuracy for bone collagen was determined at the University of York by measurements of international standard reference materials within each analytical run. These were IAEA 600 $\delta^{13}C_{raw}$ = -27.65 ±0.09 ‰, $\delta^{13}C_{true}$ = -27.77 ±0.043 ‰, $\delta^{15}N_{raw}$ = 0.92 ±0.21 ‰, $\delta^{15}N_{true}$ = 1 ±0.2 ‰; IAEA N2 $\delta^{15}N_{raw}$ = 20.35 ±0.13 ‰, $\delta^{15}N_{true}$ = 20.3 ±0.2 ‰; IA Cane, $\delta^{13}C_{raw}$ = -11.77 ±0.09 ‰; $\delta^{13}C_{true}$ = -11.64 ±0.03 ‰. The overall uncertainties on the measurements of each sample were calculated based on the method of Kragten (1994) by combining uncertainties in the values of the international reference materials and those determined from repeated measurements of samples and reference materials. These are expressed as one standard deviation. The maximum uncertainty for all samples across all runs was <0.2 ‰ for both $\delta^{13}C$ and $\delta^{15}N$. In addition, a homogenised bovine bone extracted and analysed within the same batch as the samples produced the following average values; $\delta^{13}C$ = -23.01 ±0.13 ‰; $\delta^{15}N$ = 6.21 ±0.44 ‰. This was comparable to the overall mean value from 50 separate extracts of this bone sample, which produced values of $\delta^{13}C$ = -22.97 ±0.19 ‰ and $\delta^{15}N$ = 6.19±0.30 ‰.

8.3.3.7.2 Bone, enamel, and dental calculus carbonates

All carbonate analysis was carried out using an IsoPrime 100 dual inlet mass spectrometer plus Multiprep device at the Natural Environment Research Council Isotope Geosciences Laboratory (Keyworth, Nottingham, England). Approximately 50–100 micrograms of each sample were loaded into glass vials and sealed with septa. The vials were evacuated, and anhydrous phosphoric acid was delivered to the carbonate at 90 °C. The evolved CO_2 was collected for 15 minutes, cryogenically cleaned, and passed to the mass spectrometer. Accuracy for bone, enamel, and dental calculus carbonates was determined by measurements of the Keyworth Carrera Marble (KCM), the laboratory's carbonate reference material (average KCM $\delta^{13}C$ = 2.00±0.02 ‰), calibrated to NBS19 certified reference material. In addition, three homogenised bovine bones extracted and analysed within the same batch as the samples produced the $\delta^{13}C$ average values of -16.01 ±0.08 ‰. To determine the overall reproducibility of the carbonate analysis method, duplicate analysis was carried out on 4 bone, 4 calculus, and 7 enamel samples. The duplicate pairs did not vary by >0.2‰ for all paired sample $\delta^{13}C$ values.

8.3.3.8 Suess effect correction

All the modern tissue $\delta^{13}C$ values were corrected for the Suess effect (Appendix A2) which is defined as the global decrease of ^{14}C and ^{13}C relative to ^{12}C in atmospheric CO_2 which occurred primarily due to fossil fuel burning since the Industrial Revolution (Graven et al. 2017). A time-depended correction

per year to each sample was applied according to Graven et al (2017), normalised up to 2015. For all individuals who died after 2015, the 2015 normalisation was utilised.

8.3.3.9 Statistical analysis

Statistical analysis was carried out using R statistics, PAST statistics, and IBM SPSS statistics version 26 (Hammer et al. 2001; IBM 2019; R Core Team 2020). Non-parametric statistics were used to compare isotope values among the population groups because of the non-normal distribution of some data as indicated by Kolmogorov-Smirnov and Shapiro-Wilk tests. The tests were executed using the non-parametric equivalent of a one-way analysis of variance (ANOVA) and the non-parametric equivalent of the independent T-test, the Kruskal Wallis Test and the Mann Whitney test respectively.

8.4 Results and Discussion

In total, 57 samples: modern (n=20); medieval (n=22), and post-medieval (n=15), were subjected to bone collagen, bone carbonate, enamel carbonate, and calculus carbonate isotope analysis. FTIR-ATR analysis was performed on 57 bones and 52 dental calculus samples. Tooth enamel is generally assumed to be resistant to diagenetic alteration because of its structure (Koch et al., 1997; LeeThorp, 2000), and since there was not enough material for FTIR analysis as well as isotope analysis, FTIR-ATR analysis on enamel samples was not performed. Raw isotope data and information for all remains are presented in Appendix A.

8.4.1 Bone and dental calculus FTIR-ATR analysis

Diagenesis in bones and dental calculus was assessed using FTIR-ATR but dental calculus was insufficient to use for both FTIR and isotope analysis in five of the individuals studied. FTIR data from all sites in this study are provided in Table 8.1 and plotted in Figures 8.1 and 8.2. Analysis and interpretation of FTIR results in dental calculus for assessment of diagenesis before isotope analysis is still in the exploratory stage. Only Price et al. (2018) have previously measured IRSF values in archaeological dental calculus before isotope analysis. The values for C/P ratio and IRSF that indicate that a bone is unaltered are not expected to be relevant to the understanding of the preservation quality of dental calculus. Further work is still required to study the FTIR-ATR spectra in dental calculus and to identify the exact phosphates and carbonate spectra that can be used specifically for dental calculus diagenesis analysis. Therefore, dental calculus parameters are provided only for building a database for dental calculus parameters that can be used to further analyse calculus structure and its diagenetic alteration (Table 8.1). The parameters found in this study, however, cannot be compared to Price et al. (2018) study parameters because that study was not utilising the same FTIR-ATR method used in the present study.

Table 8-1: Summary of bone and dental calculus FTIR-ATR data displaying the average of data measured in triplicate - IRSF: Infrared splitting factor; C/P: carbonate-to-phosphate ratio.

Sample	*Bone IRSF*	*Bone C/P*	*Calculus IRSF*	*Calculus C/P*
FAC 01	3.21 ± 0.06	0.32 ± 0.02	3.59 ± 0.05	0.08 ± 0.00
FAC 02	3.36 ± 0.03	0.24 ± 0.01	3.65 ± 0.01	0.10 ± 0.00
FAC 03	3.19 ± 0.03	0.33 ± 0.00	3.77 ± 0.09	0.05 ± 0.00
FAC 04	3.22 ± 0.10	0.32 ± 0.00	3.75 ± 0.06	0.07 ± 0.00
FAC 05	3.38 ± 0.11	0.21 ± 0.01	3.59 ± 0.00	0.08 ± 0.00
FAC 06	3.16 ± 0.06	0.34 ± 0.01	3.58 ± 0.03	0.06 ± 0.01
FAC 07	3.35 ± 0.01	0.25 ± 0.01	3.63 ± 0.07	0.08 ± 0.00
FAC 08	3.28 ± 0.01	0.31 ± 0.00	3.73 ± 0.05	0.07 ± 0.01
FAC 09	3.37 ± 0.02	0.23 ± 0.01	3.33 ± 0.03	0.09 ± 0.00
FAC 10	3.35 ± 0.01	0.24 ± 0.01	3.78 ± 0.02	0.07 ± 0.00
FAC 11	3.16 ± 0.02	0.33 ± 0.00	3.72 ± 0.04	0.07 ± 0.00
FAC 12	3.32 ± 0.04	0.29 ± 0.02	3.91 ± 0.07	0.03 ± 0.00
FAC 13	3.34 ± 0.01	0.26 ± 0.01	3.95 ± 0.01	0.04 ± 0.00
FAC 14	3.33 ± 0.06	0.28 ± 0.01	3.71 ± 0.05	0.07 ± 0.00
FAC 15	3.04 ± 0.04	0.36 ± 0.00	3.73 ± 0.12	0.05 ± 0.00
FAC 16	3.35 ± 0.03	0.25 ± 0.00	-	-
FAC 17	3.33 ± 0.12	0.27 ± 0.00	3.28 ± 0.02	0.11 ± 0.00
FAC 18	3.34 ± 0.05	0.26 ± 0.01	-	-
FAC 19	3.28 ± 0.04	0.30 ± 0.00	3.84 ± 0.06	0.05 ± 0.00
FAC 20	3.31 ± 0.03	0.29 ± 0.00	3.28 ± 0.03	0.13 ± 0.01
SPL 226	4.01 ± 0.04	0.12 ± 0.00	3.70 ± 0.05	0.12 ± 0.00
SPL 552	3.82 ± 0.10	0.17 ± 0.02	3.90 ± 0.16	0.09 ± 0.00
SPL 1063	3.59 ± 0.01	0.19 ± 0.00	3.71 ± 0.05	0.12 ± 0.00
SPL 1069	3.76 ± 0.00	0.17 ± 0.00	4.02 ± 0.01	0.09 ± 0.00
SPL 1248	3.74 ± 0.01	0.15 ± 0.00	3.97 ± 0.07	0.09 ± 0.00
SPL 1384	3.64 ± 0.01	0.19 ± 0.00	3.97 ± 0.03	0.09 ± 0.00
SCN 20	3.67 ± 0.10	0.22 ± 0.00	3.26 ± 0.03	0.17 ± 0.00
SCN 27	3.59 ± 0.01	0.17 ± 0.00	3.36 ± 0.02	0.14 ± 0.00
SCN 29	3.52 ± 0.02	0.16 ± 0.00	3.35 ± 0.07	0.15 ± 0.01
SCN 33	3.68 ± 0.05	0.20 ± 0.00	-	-
SCN 35	3.87 ± 0.02	0.20 ± 0.00	3.28 ± 0.05	0.17 ± 0.00
NFC 13	3.89 ± 0.02	0.15 ± 0.00	3.93 ± 0.04	0.09 ± 0.00
NFC 19	3.85 ± 0.01	0.15 ± 0.00	3.83 ± 0.04	0.10 ± 0.00
NFC 39	3.75 ± 0.05	0.13 ± 0.00	3.87 ± 0.04	0.10 ± 0.00
NFC 60	3.71 ± 0.02	0.15 ± 0.00	3.69 ± 0.07	0.11 ± 0.02
NFC 68	3.72 ± 0.07	0.15 ± 0.00	3.81 ± 0.06	0.10 ± 0.00
NFC 72	3.67 ± 0.00	0.20 ± 0.00	3.86 ± 0.03	0.10 ± 0.00
NFC 74	3.86 ± 0.01	0.15 ± 0.00	3.87 ± 0.09	0.10 ± 0.00
NFC 80	3.66 ± 0.01	0.20 ± 0.00	-	-
NFC 81	3.64 ± 0.06	0.16 ± 0.00	3.49 ± 0.09	0.12 ± 0.01
NFC 86	3.70 ± 0.00	0.18 ± 0.00	3.56 ± 0.02	0.12 ± 0.00
NFC 91	3.92 ± 0.01	0.15 ± 0.00	-	-
CSM 2.15	3.68 ± 0.06	0.18 ± 0.01	3.46 ± 0.04	0.09 ± 0.00

Sample	*Bone IRSF*	*Bone C/P*	*Calculus IRSF*	*Calculus C/P*
CSM 2.18	3.80 ± 0.02	0.17 ± 0.00	3.46 ± 0.01	0.09 ± 0.00
CSM 2.31	3.58 ± 0.17	0.20 ± 0.03	3.48 ± 0.02	0.10 ± 0.01
CSM 2.34	4.05 ± 0.09	0.15 ± 0.01	3.31 ± 0.13	0.13 ± 0.00
CSM 2.36	4.01 ± 0.08	0.14 ± 0.00	3.35 ± 0.09	0.12 ± 0.00
CSM 2.37	4.01 ± 0.04	0.14 ± 0.00	3.14 ± 0.10	0.16 ± 0.00
CSM 2.49	3.87 ± 0.03	0.16 ± 0.00	3.55 ± 0.08	0.10 ± 0.01
CSM 4.12	3.81 ± 0.02	0.16 ± 0.01	3.56 ± 0.05	0.10 ± 0.01
CSM 4.24	4.02 ± 0.02	0.15 ± 0.00	3.23 ± 0.06	0.15 ± 0.00
CSM 5.09	3.60 ± 0.01	0.18 ± 0.00	3.86 ± 0.01	0.08 ± 0.00
CSM 5.16	3.80 ± 0.02	0.18 ± 0.01	3.68 ± 0.01	0.09 ± 0.00
CSM 12	3.87 ± 0.04	0.16 ± 0.00	3.82 ± 0.02	0.04 ± 0.00
CSM 37	3.50 ± 0.04	0.21 ± 0.02	3.72 ± 0.02	0.05 ± 0.00
CSM 41	3.80 ± 0.00	0.18 ± 0.00	3.79 ± 0.01	0.05 ± 0.00
CSM 62.02	3.86 ± 0.01	0.16 ± 0.00	3.83 ± 0.02	0.05 ± 0.00

8.4.1.1 Infrared splitting factor (Crystallinity)

The crystallinity (IRSF) values in modern bones ranged from 3.04 to 3.38 (Mean = 3.28±0.09; see Table 8.1; Figure 8.1A), slightly lower than the value (IRSF=3.357 ± 0.007) that was obtained for the modern bones in Kontopoulos et al. (2019a, 145). The crystallinity in all the archaeological samples in this project range from 3.50 to 4.05 (Medieval mean = 3.74±0.12; post-medieval mean= 3.82±0.17), higher than both the samples in Kontopoulos et al. (2019a) and the modern bones from this study. Alteration induced by age-related diagenesis is demonstrated for any bone if its crystallinity is higher than that of the modern samples. The study by Kontopoulos et al. (2020) revealed that archaeological samples with IRSF values greater than 4.2 did not preserve DNA, therefore, all archaeological bone samples with values higher than 4.2 were excluded from this study on the assumption that they may have poor bioapatite preservation as well. The crystallinity values for modern dental calculus samples ranged from 3.28 to 3.95 (Mean = 3.66 ± 0.19) and archaeological samples ranged from 3.14 to 4.02 (Medieval mean = 3.71±0.24; post-medieval mean= 3.55±0.23). The mean IRSF values for all groups in this study are higher than the value (mean = 3.2 ± 0.2) observed by Price et al. (2018), but this could be due to the small sample size (n=7) out of the 28 samples they analysed in their study. Additionally, Price et al. (2018) used a different FTIR methodology therefore the values may not be entirely comparable. For the present study, only grains between 20μm and 50μm particle size were used and were sampled using the Attenuated Total Reflectance (ATR) technique on the FTIR. On the other hand, Price et al. (2018) study did not use the ATR nor did they consider the effect of sample particle size on FTIR measurements as observed previously in bones (Kontopoulos et al. 2018).

There seems to be no clear relationship or a constant offset between each individual's bone and calculus IRSF values (Figure 8.1A).

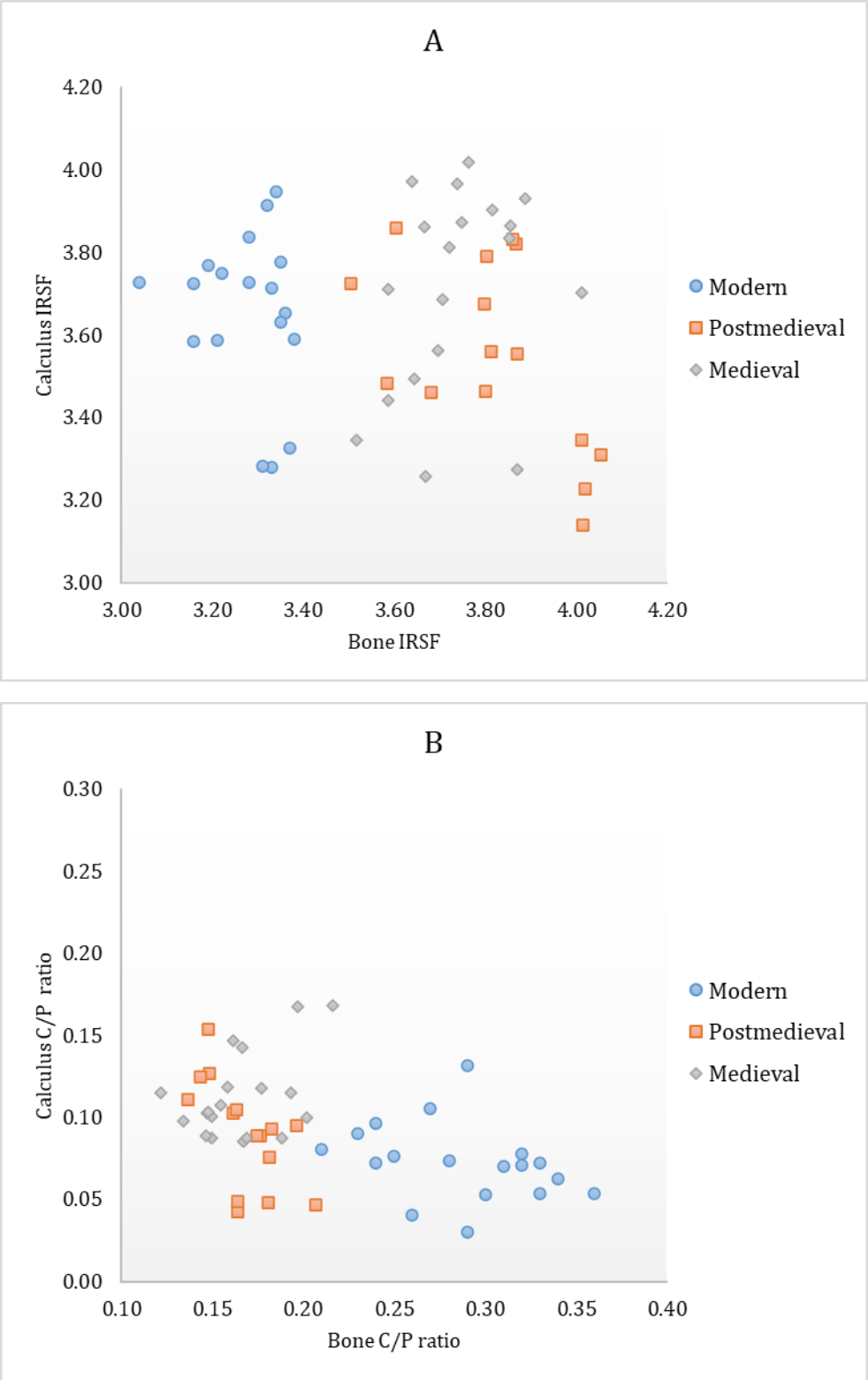


Figure 8-1: Comparisons between A. Bone IRSF and Dental calculus IRSF B. Bone C/P ratio and Calculus C/P ratio.

8.4.1.2 Carbonate-phosphate ratio

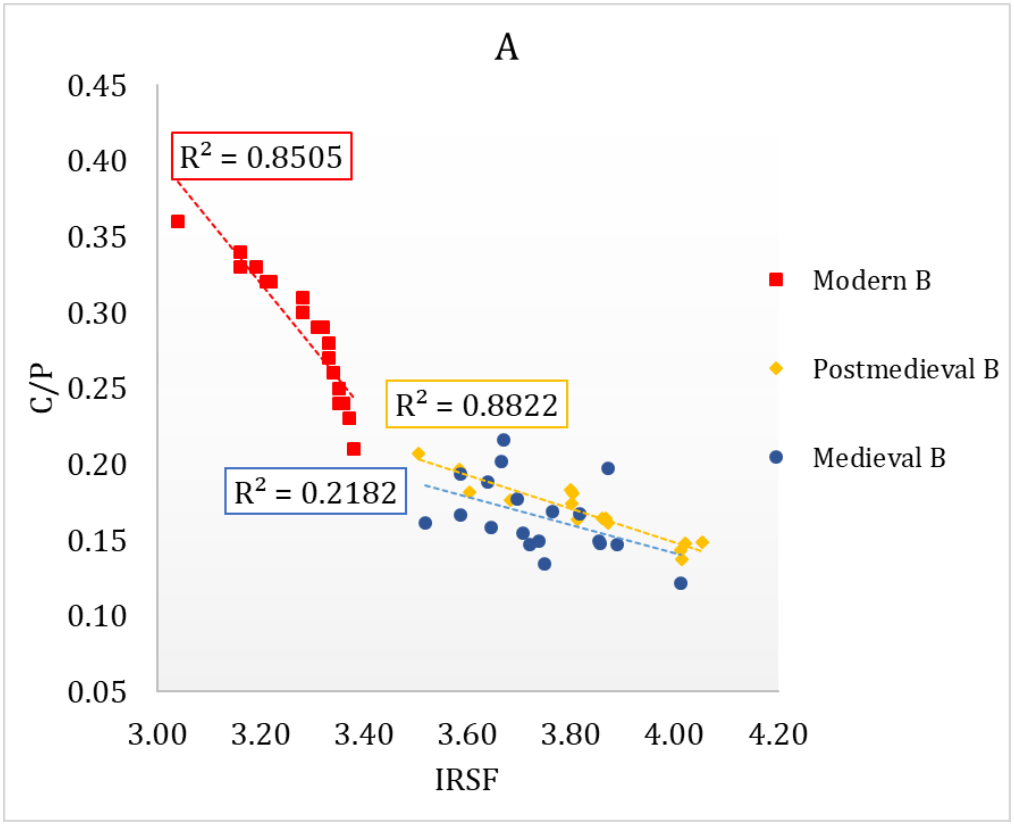
The modern bone CO_3/PO_4 absorbance ratios range from 0.21 to 0.36 (Mean = 0.28±0.04) and the archaeological samples range from 0.12 to 0.22 (medieval mean = 0.17±0.03; post-medieval mean= 0.17±0.02) indicating remarkable similarity between the two archaeological sets of samples (Table 8.1; Figure 8.1B). The C/P values for all of the archaeological bones fell below the mean C/P values that were obtained from modern bones analysed in this study as well as those that have been previously obtained in modern unaltered bone (mean C/P=0.24 ± 0.003) (Kontopoulos et al. 2019a) indicating a loss of the carbonate fraction from the bone apatite.

The C/P ratio for modern dental calculus samples ranged from 0.03 to 0.13 (Mean = 0.07 ± 0.02) and archaeological samples ranged from 0.04 to 0.17 (medieval mean = 0.11±0.03; post-medieval mean= 0.09±0.03) (Table 8.1). Similarly, there seems to be no relationship between the individual bone and calculus C/P values (Figure 8.1B). Hayashizaki et al. (2008, 172) study revealed that the carbonate content in dental calculus was higher when compared to other biological apatites such as bone, enamel, and dentine, therefore, the C/P ratios in dental calculus were expected to be higher in this study. However, the C/P ratios of dental calculus in this study are lower than that of bone, suggesting that there could be other contributing factors. It has been established that dental calculus contains non-apatitic calcium phosphates (Hayashizaki et al. 2008, 173) which are not present in other normal mineralised tissues (Laskus and Kolmas 2017, 4). Dental calculus may therefore have an inherent higher phosphate content since, in addition to non-apatitic calcium phosphates, it also has hydroxyapatite leading to a potentially lower C/P ratio relative to bone. The mean value of specific phosphorus concentration in human rib bone weights has been found to be 8.42 ± 2.14% of dry bone weight (Tzaphlidou and Zaichick 2003) whereas that of dental calculus has been found to be 19% (Nasution and Amatanesia 2018). This strongly suggests that phosphates may be the cause of the lower C/P values in dental calculus when compared to bone.

Moreover, it was also observed that unlike in bone, the most recent calculus samples have the lowest C/P ratio followed by the post-medieval samples and then finally, the medieval samples. This pattern seems to indicate that the C/P ratio of dental calculus increases with the age of the deposit. On the other hand, Hayashizaki et al. (2008) revealed that carbonate content in dental calculus depended on its location in the mouth such that the lower anterior teeth have higher carbonate content compared to the upper posterior teeth. All the calculus in this study was collected from posterior teeth, but randomly from either the mandible or maxilla, therefore, if the differences in carbonate content can occur due to the location of where calculus was formed as shown in Hayashizaki et al. (2008) study, it is, therefore, possible that the apparent trend in C/P ratios observed in this study could be a function of where each sample was formed (Figure 8.1 B).

8.4.1.3 IRSF and C/P relationship

Overall, the bone IRSF and C/P ratios display a very strong inverse correlation for modern and post-medieval bone samples (Figure 8.2A). The weaker correlation in medieval samples may relate to the varied ages of the burials (7th to 16th century) as well as the different environments from which they were recovered. Environmental factors that degrade samples differ from site to site and, even in the same setting, the type of burial can influence how environmental factors interact with archaeological remains. Moreover, the longer the remains are buried for, the less preserved they become (Nielsen-Marsh and Hedges 2000; Nielsen-Marsh et al. 2000). Alteration of the carbonate content in skeletal material has also been shown to be site-specific (Kohn et al. 1999; Trueman et al. 2008). Both the modern and post-medieval populations were each obtained from a single site while the medieval samples were collected from three sites. On the other hand, there is a strong inverse correlation for dental calculus samples in all periods (Figure 8.2B). Since the relationship is negative for both bones and dental calculus (Figure 8.2), there is a general trend of increasing infrared splitting factor (IRSF) values with a reduction in carbonate/ phosphate (C/P) values reflecting the loss of carbonate with increasing crystallinity. This is in keeping with the previously reported work on bones (Sillen 1989; Thompson et al. 2009; Thompson et al. 2011).



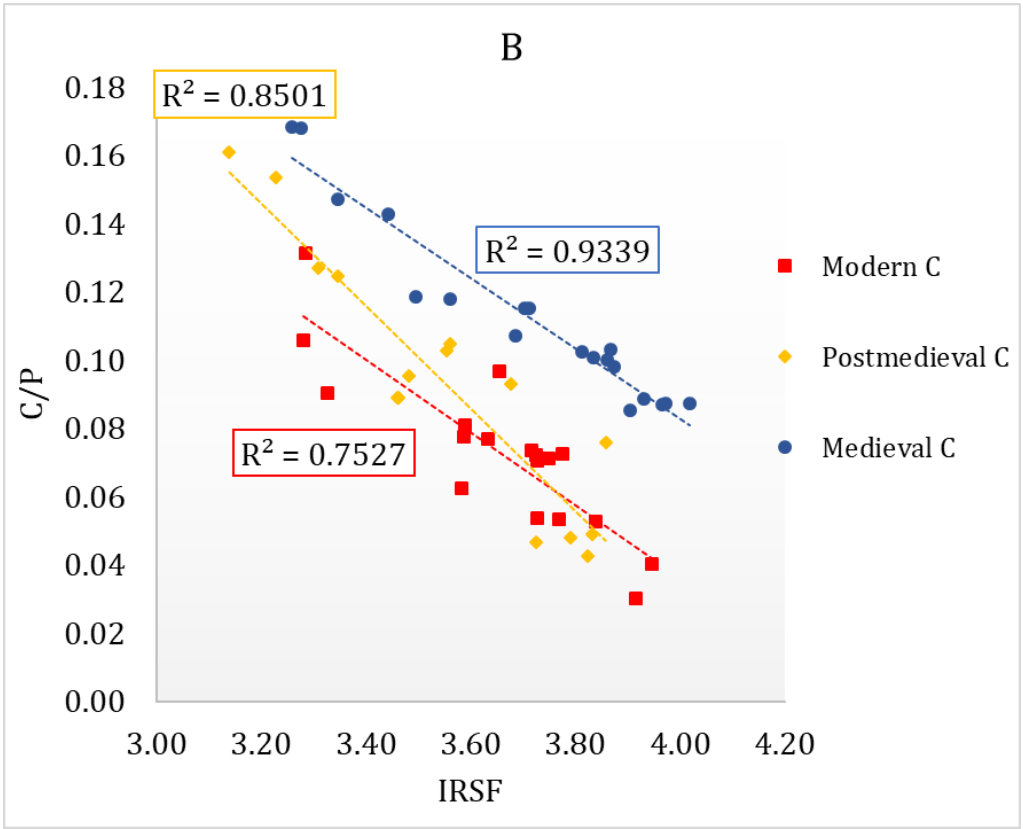


Figure 8-2: Plots of A. Bone IRSF vs Bone C/P ratio B. Calculus IRSF vs Calculus C/P ratio.

8.4.2 Isotope results

Collagen quality was assessed using the established collagen quality criteria (Ambrose 1990; van Klinken 1999). All samples in this study produced sufficient collagen for mass spectrometry (Appendix A), with collagen yields ranging between 2.7% and 24.0% (mean = 14.5%). The atomic C:N values ranged from 3.1 to 3.4 and are within the range of C:N 2.9 to 3.6 that DeNiro (1985) specified as acceptable. Furthermore, the percentage of carbon and nitrogen in all the collagen samples from bone fell within the elemental percentages reported for modern mammalian bone collagen by Ambrose (1990, 441) that ranged between 15.3% - 47% and 5.5% - 17.3% by weight for carbon and nitrogen, respectively.

The mean bone collagen $\delta^{13}C$ values for medieval, post-medieval, and modern populations are -19.7 ± 0.4‰, -19.8 ± 0.5‰, and -14.0 ± 0.6‰ while their mean $\delta^{15}N$ values are 11.7 ± 0.9‰, 11.8 ± 1.1‰, and 10.8 ± 0.6‰ respectively. In addition, the mean bone carbonate $\delta^{13}C$ values for the same populations are -13.4 ± 0.8‰, -14.3 ± 1.0‰ and -9.3 ± 1.1‰ while their mean enamel carbonate $\delta^{13}C$ values are -13.6 ± 1.0‰, -13.3 ± 1.0‰ and -6.8 ± 1.1‰ respectively (Table 8.2).

Table 8-2: Descriptive statistics for all $\delta^{13}C$ (‰) isotopes being investigated in this study.

		N	*Minimum*	*Maximum*	*Range*	*Mean*	*Std. Deviation*
Bone collagen $\delta^{13}C$ (‰)	Modern	20	-15.3	-12.4	2.9	-14.0	0.6
Medieval	Medieval	22	-20.9	-18.9	1.9	-19.7	0.4
Post-medieval	Post-medieval	15	-20.7	-19.1	1.6	-19.8	0.5
Bone collagen $\delta^{15}N$ (‰)	Modern	20	9.6	12.0	2.4	10.8	0.6
Medieval	Medieval	22	10.1	13.7	3.6	11.7	0.9
Post-medieval	Post-medieval	15	9.5	13.0	3.5	11.8	1.1
Bone carbonate $\delta^{13}C$ (‰)	Modern	20	-11.0	-7.1	3.9	-9.3	1.1
Medieval	Medieval	22	-14.8	-12.1	2.7	-13.4	0.8
Post-medieval	Post-medieval	15	-15.7	-12.6	3.1	-14.3	1.0
Enamel carbonate $\delta^{13}C$ (‰)	Modern	20	-9.2	-4.6	4.6	-6.8	1.1
Medieval	Medieval	22	-15.2	-12.1	3.1	-13.6	1.0
Post-medieval	Post-medieval	15	-14.8	-11.9	2.8	-13.3	1.0
Calculus carbonate $\delta^{13}C$ (‰)	Modern	20	-7.0	-1.2	5.8	-4.1	1.8
Medieval	Medieval	22	-12.7	-8.0	4.7	-9.7	1.2
Post-medieval	Post-medieval	15	-11.4	-6.1	5.3	-9.2	1.4

There are statistically significant differences in both $\delta^{13}C$ and $\delta^{15}N$ values between populations as determined by the Kruskal-Wallis H test [bone collagen $\delta^{13}C$ - $X^2(2) = 38.295$, $p = 0.000$, $\delta^{15}N$ - $X^2(2) = 14.730$, $p = 0.001$; bone carbonate $\delta^{13}C$ - $X^2(2) = 41.149$, $p = 0.000$; calculus carbonate $\delta^{13}C$ - $X^2(2) = 37.615$, $p = 0.000$ and enamel carbonate $\delta^{13}C$ - $X^2(2) = 38.489$, $p = 0.000$]. The posthoc comparisons from the Kruskal-Wallis Test revealed that for all tissue $\delta^{13}C$ and bone collagen $\delta^{15}N$ values there were

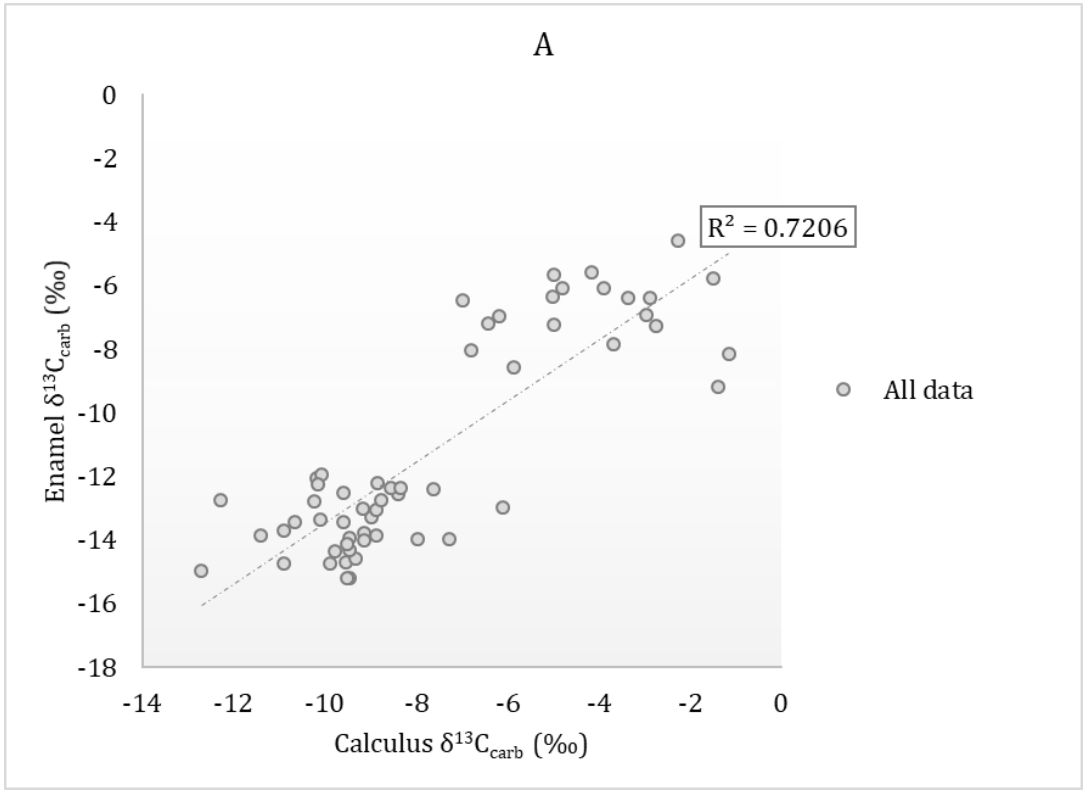
significant differences between the modern population and both the medieval and post-medieval populations. There was, however, no statistically significant difference between the medieval and the post-medieval populations (Table 8.3).

Table 8-3: Post hoc results for all populations. The significance level is 0 .05 and the significance values have been adjusted by the Bonferroni correction for multiple tests.

Sample 1-Sample 2	*Bone collagen $\delta^{13}C$*	*Bone collagen $\delta^{15}N$*	*Bone carbonate $\delta^{13}C$*	*Calculus carbonate $\delta^{13}C$*	*Enamel carbonate $\delta^{13}C$*
Modern-Post-medieval	0.000	0.03	0.000	0.000	0.000
Modern-Medieval	0.000	0.03	0.000	0.000	0.000
Post-medieval-Medieval	1.000	1.000	0.270	1.000	1.000

8.4.2.1 Correlations between tissues

When considered as a collective dataset (the medieval, post-medieval, and modern populations together), there is a strong correlation between bone carbonate $\delta^{13}C$ and calculus carbonate $\delta^{13}C$ and between enamel $\delta^{13}C$ and calculus $\delta^{13}C$ (enamel $\delta^{13}C$ and calculus $\delta^{13}C$ -R^2= 0.7206; bone $\delta^{13}C$ and calculus $\delta^{13}C$- R^2= 0.7159; see Figure 8.3A and 8.3B). Strong correlations are expected when people are consuming different diets (Eerkens et al. 2014). In this study, there are two groups consuming completely different diets - the diet of the modern population will include high fructose corn syrup (C_4) (Frary et al. 2004; Slining and Popkin 2013; Bailey et al. 2018) whereas that of archaeological populations will have had substantially less C_4 input (Dyer 1989; Oddy 2000; Woolgar et al. 2006). The collective correlations are, however, misleading as these populations are completely different. In contrast, there is no correlation between either calculus carbonate $\delta^{13}C$ and bone carbonate $\delta^{13}C$ or calculus carbonate $\delta^{13}C$ and enamel carbonate $\delta^{13}C$ when data is examined within specific time periods (Figure 8.4A and 8.4B). Weaker correlations at sites were also observed in Eerkens et al. (2014). The authors suggested that correlations between bones and calculus at individual sites are weaker than across sites because of a limited range of isotopic values that are found when individuals are consuming similar diets, and this study agrees with that conclusion.



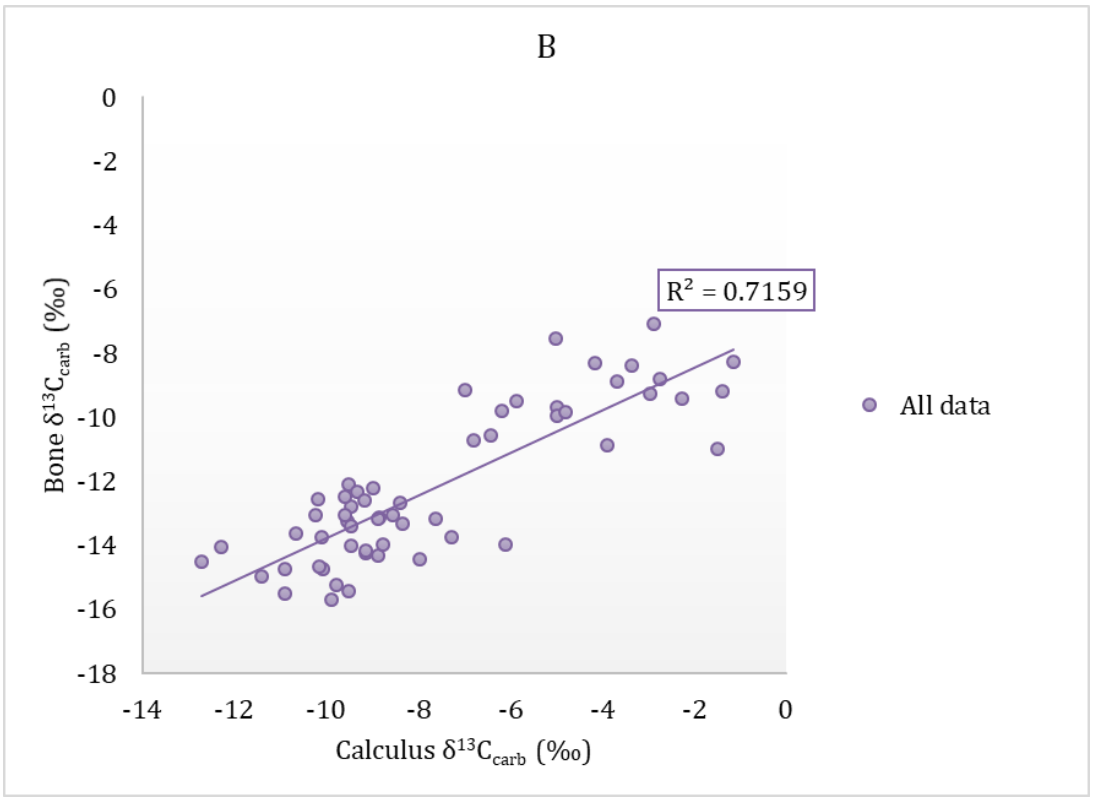


Figure 8-3: Graphs showing $\delta^{13}C$ correlations between A. calculus carbonate and enamel carbonate B. calculus carbonate and bone carbonate for combined modern, medieval, and post-medieval individuals.

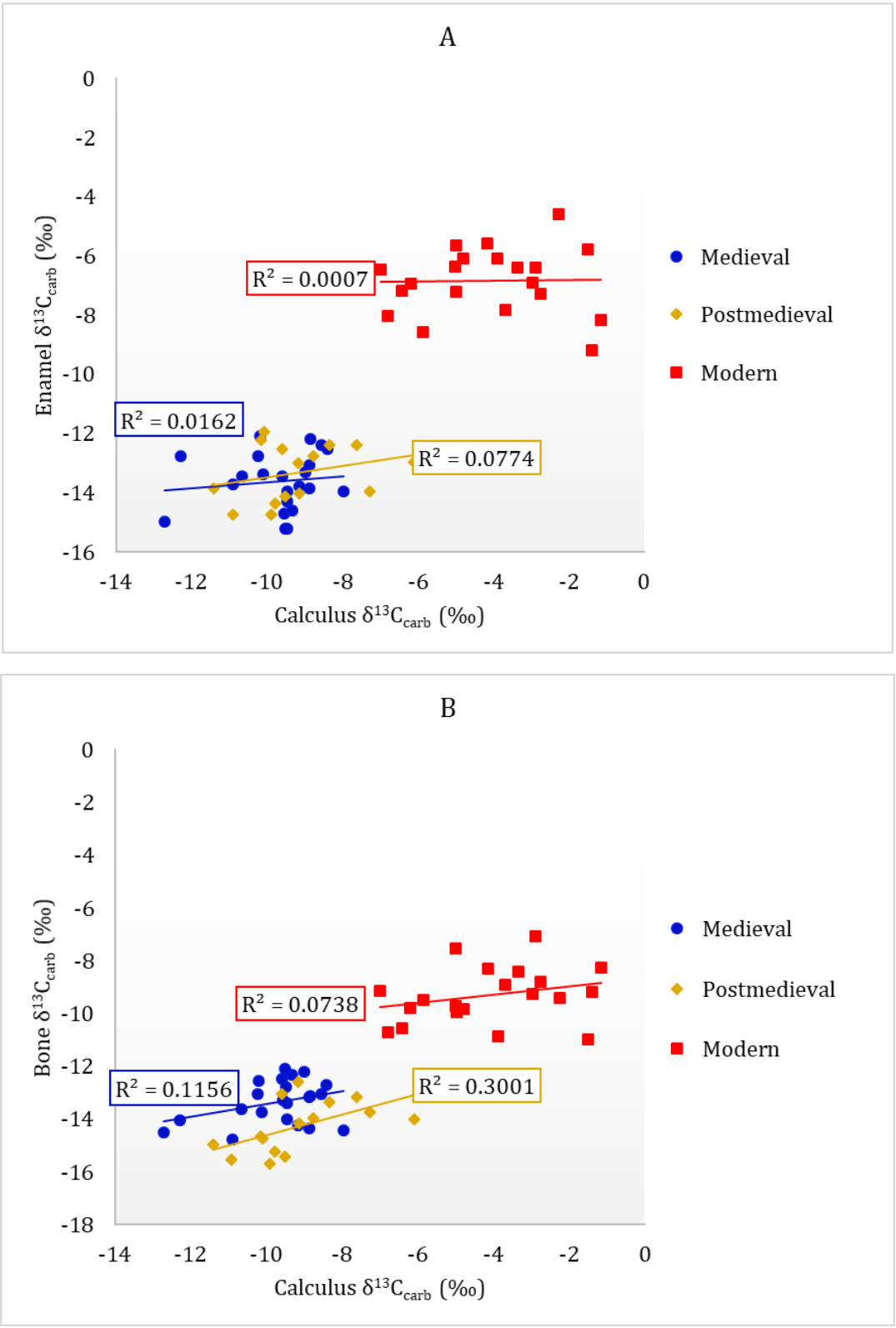


Figure 8-4: Graphs showing $\delta^{13}C$ correlations between A. calculus carbonate and enamel carbonate B. calculus carbonate and bone carbonate for separate modern, medieval, and post-medieval population groups.

8.4.2.2 Carbonate $\delta^{13}C$ tissue offsets

As expected, the modern population shows higher $\delta^{13}C$ values in all three tissue carbonates compared to both the medieval and post-medieval populations. The offset in dental calculus and either bone and

enamel carbonate $\delta^{13}C$ values are large and consistent in direction i.e., in all populations, dental calculus $\delta^{13}C$ is always significantly higher (Figure 8.5). The modern, medieval, and post-medieval carbonate $\triangle^{13}C_{calculus\text{-}enamel}$ and $\triangle^{13}C_{calculus\text{-}bone}$ spacing are listed in Table 8.4. However, there is no consistent offset between the $\delta^{13}C$ values for the three tissues per individual (Table 8.4, Figure 8.5). No constant offsets were observed in the one study that has also examined calculus mineral and bone carbonate (Price et al. 2018). Despite this, the authors suggested that their average calculus-bone offset of 3.1 was probably due to the isotopic exchange between saliva and atmospheric carbon dioxide (CO_2).

Table 8-4: Individual $\delta^{13}C$ isotope offsets in this study.

Sample	*Calculus $\delta^{13}C$ (‰)*	*Enamel $\delta^{13}C$ (‰)*	*Bone $\delta^{13}C$ (‰)*	*$\Delta^{13}C_{calculus\text{-}enamel}$*	*$\Delta^{13}C_{calculus\text{-}bone}$*	*$\Delta^{13}C_{enamel\text{-}bone}$*
FAC 01	-3.7	-7.8	-8.9	4.2	5.2	1.1
FAC 02	-5.9	-8.6	-9.5	2.7	3.6	0.9
FAC 03	-1.5	-5.8	-11.0	4.3	9.5	5.2
FAC 04	-6.4	-7.2	-10.6	0.8	4.1	3.4
FAC 05	-1.2	-8.2	-8.3	7.0	7.1	0.1
FAC 06	-5.0	-6.4	-7.5	1.3	2.5	1.2
FAC 07	-3.9	-6.1	-10.9	2.2	7.0	4.8
FAC 08	-6.8	-8.0	-10.7	1.2	3.9	2.7
FAC 09	-2.3	-4.6	-9.4	2.4	7.2	4.8
FAC 10	-6.2	-7.0	-9.8	0.8	3.6	2.8
FAC 11	-2.8	-7.3	-8.8	4.5	6.1	1.5
FAC 12	-3.4	-6.4	-8.4	3.1	5.1	2.0
FAC 13	-5.0	-5.7	-9.7	0.7	4.7	4.0
FAC 14	-5.0	-7.2	-10.0	2.3	5.0	2.7
FAC 15	-3.0	-6.9	-9.3	4.0	6.3	2.3
FAC 16	-1.4	-9.2	-9.2	7.8	7.8	0.0
FAC 17	-4.8	-6.1	-9.8	1.3	5.0	3.7
FAC 18	-2.9	-6.4	-7.1	3.5	4.2	0.7
FAC 19	-4.2	-5.6	-8.3	1.4	4.2	2.7
FAC 20	-7.0	-6.5	-9.2	-0.5	2.2	2.7
SPL 226	-9.6	-13.4	-12.5	3.8	2.9	-1.0
SPL 552	-8.8	-12.2	-13.1	3.4	4.3	0.9
SPL 1063	-9.3	-14.6	-12.3	5.3	3.0	-2.3
SPL 1069	-10.2	-12.8	-13.0	2.6	2.8	0.3
SPL 1248	-9.0	-13.3	-12.2	4.3	3.2	-1.1
SPL 1384	-10.2	-12.1	-12.6	1.9	2.4	0.5
SCN 20	-8.9	-13.9	-14.3	5.0	5.5	0.5
SCN 27	-9.5	-15.2	-12.8	5.7	3.3	-2.4
SCN 29	-9.5	-15.2	-12.1	5.7	2.6	-3.1
SCN 33	-8.4	-12.5	-12.7	4.1	4.3	0.2
SCN 35	-9.6	-14.7	-13.3	5.2	3.7	-1.5
NFC 13	-8.0	-14.0	-14.4	6.0	6.5	0.5
NFC 19	-10.9	-13.7	-14.8	2.8	3.9	1.1
NFC 39	-9.5	-13.9	-14.0	4.5	4.6	0.1

Sample	*Calculus* $\delta^{13}C$ (‰)	*Enamel* $\delta^{13}C$ (‰)	*Bone* $\delta^{13}C$ (‰)	$\Delta^{13}C_{calculus\text{-}enamel}$	$\Delta^{13}C_{calculus\text{-}bone}$	$\Delta^{13}C_{enamel\text{-}bone}$
NFC 60	-9.5	-14.3	-13.4	4.9	3.9	-0.9
NFC 68	-10.1	-13.4	-13.8	3.3	3.6	0.4
NFC 72	-12.7	-15.0	-14.5	2.3	1.8	-0.5
NFC 74	-10.7	-13.4	-13.6	2.8	3.0	0.2
NFC 80	-12.3	-12.8	-14.1	0.5	1.8	1.3
NFC 81	-9.2	-13.8	-14.2	4.6	5.1	0.5
NFC 86	-8.9	-13.1	-13.2	4.2	4.3	0.1
NFC 91	-8.5	-12.4	-13.0	3.8	4.5	0.7
CSM 2.15	-10.9	-14.8	-15.5	3.8	4.6	0.8
CSM 2.18	-9.9	-14.8	-15.7	4.9	5.8	0.9
CSM 2.31	-9.6	-12.5	-13.1	2.9	3.5	0.5
CSM 2.34	-7.6	-12.4	-13.2	4.8	5.5	0.8
CSM 2.36	-9.5	-14.1	-15.4	4.6	5.9	1.3
CSM 2.37	-8.8	-12.8	-14.0	4.0	5.2	1.2
CSM 2.49	-10.1	-11.9	-14.7	1.9	4.7	2.8
CSM 4.12	-10.2	-12.2	-14.7	2.1	4.5	2.4
CSM 4.24	-9.1	-14.0	-14.2	4.9	5.0	0.2
CSM 5.09	-9.2	-13.0	-12.6	3.9	3.4	-0.4
CSM 5.16	-8.3	-12.4	-13.3	4.0	5.0	1.0
CSM 12	-9.8	-14.4	-15.2	4.6	5.5	0.9
CSM 37	-6.1	-13.0	-14.0	6.9	7.9	1.0
CSM 41	-7.3	-14.0	-13.7	6.7	6.5	-0.2
CSM 62.02	-11.4	-13.9	-15.0	2.5	3.6	1.1

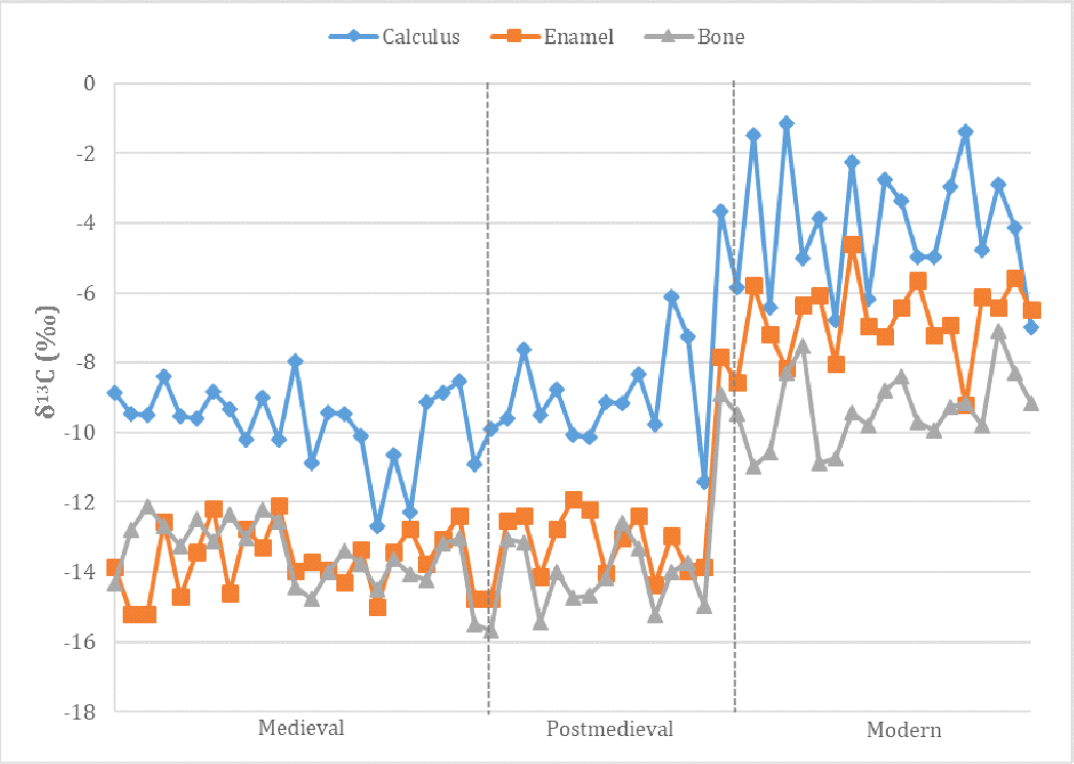


Figure 8-5: Carbon by individual data trends for all individuals in this study.

It is, however, suggested here that a direct comparison between different tissues' isotopic composition is not strictly possible as there may be a difference between the isotopic composition of carbon preserved in bone, teeth, and dental calculus. The isotopic composition of carbon in bone carbonate and enamel carbonate has been investigated elsewhere (Warinner and Tuross 2009). Comparisons were made between bone and enamel carbonate on pigs raised on controlled diets containing either raw maize or nixtamalized maize for 13 weeks. The results revealed a significant difference between bone and enamel carbonate in the animals with the $\delta^{13}C$ in enamel being higher by 2.2‰ and 2.3‰ in the nixtamalized and raw diets respectively. Simultaneously forming bone and enamel carbonate were used and therefore, the offset found was not attributed to "preferential or differential digestion since the carbonate in both the enamel and bone apatite was deposited from dissolved blood bicarbonate during the same experiment" (Warinner and Tuross 2009). Considering that enamel once formed, cannot be remodelled whereas bone undergoes remodelling once formed, the authors suggested that the enamel carbonate and bone carbonate of adult animals, therefore, represented 'temporally segregated isotopic deposition events'. Therefore, in this study, it is proposed that the inconsistent offsets between enamel and bone were most likely to be due to each individual's different childhood and adult diets.

Additionally, it is proposed here that the isotopic fractionation in dental calculus may not be associated simply with metabolism and food source, unlike bone and enamel. In the case of dental calculus, which is a living biofilm, it is envisaged here that the isotopic fractionation would be greater in the thicker calculus biofilm, similar to what has been observed in other biofilms (Staal et al. 2007), therefore, different from bone and enamel. However, it is also notable that in modern individuals the extent of fractionation is lower than in medieval and post-medieval populations. Although this study does not have the details on the thickness of the dental calculus biofilm, it is proposed here that greater oral hygiene may lead to less well-established calcified biofilms. Therefore, it is suggested that since the mechanisms involved in the formation of dental calculus, fractionation effects, and turnover time in this tissue are different from that of enamel and bone carbonate, a constant offset is not expected between dental calculus carbonate and either bone carbonate or enamel carbonate.

Secondly, this study disagrees with the suggestion by Price et al. (2018, 98) that the calculus carbonate $\delta^{13}C$ values were consistently higher than that of bone carbonate due to the exchange with atmospheric CO_2 breathed in (which has a $\delta^{13}C$ value of -7‰) and saliva. Although this study agrees that it is impossible to exclude atmospheric CO_2 from the sample being analysed, other studies have established that $\delta^{13}C_{breath}$ is reflective of the food ingested (Ayliffe et al. 2004; Passey et al. 2005). Therefore, it is argued here that since the $\delta^{13}C_{breath}$ reflects the isotopic signature of the food ingested and if the calculus $\delta^{13}C$ values are affected by breath CO_2 then they will reflect the isotope composition of the food consumed. This is, of course, in addition to the bicarbonate derived from saliva that derives

carbonate from the blood and the action of microbes when metabolising carbohydrates in the mouth (Sand 1951; Wechsler, 1959; Dawes 1969; Dawes 1974; Hicks et al. 2003). .

8.4.3 Dietary interpretation from the three tissues

Generally, the carbon isotope values for the modern North American population are extremely high for all tissue types and consistently higher than those of the medieval and post-medieval populations. Unlike the archaeological populations from England, the modern individuals are influenced by the dominant consumption of C_4 foods like fructose and corn syrup (Frary et al. 2004; Slining and Popkin 2013; Bailey et al. 2018). The medieval and post-medieval diet, on the other hand, was dominated by C_3 resources (Dyer 1989; Oddy 2000; Woolgar et al. 2006).

Overall, the mean collagen results for medieval and post-medieval individuals suggest a diet dominated by C_3 terrestrial resources while that of the modern individuals is dominated by C_4 resources as expected. The relatively low $\delta^{15}N$ values in all populations including the modern ones suggest that the diets were most likely to have been dominated by terrestrial rather than marine foods. When the archaeological bone data was examined using the Froehle et al. (2012) multivariate isotope model which incorporates $\delta^{13}C_{collagen}$, $\delta^{15}N_{collagen}$ and $\delta^{13}C_{carbonate}$ data to reconstruct diet, the model suggests that there are some individuals who consumed some C_4 based food in addition to their C_3 foods and the C_4 component in these individuals was originating from C_4 plants instead of marine resources (Figure 8.6). This finding is supported by the collagen $\delta^{15}N$ values that do not suggest marine resources were consumed by any of the populations.

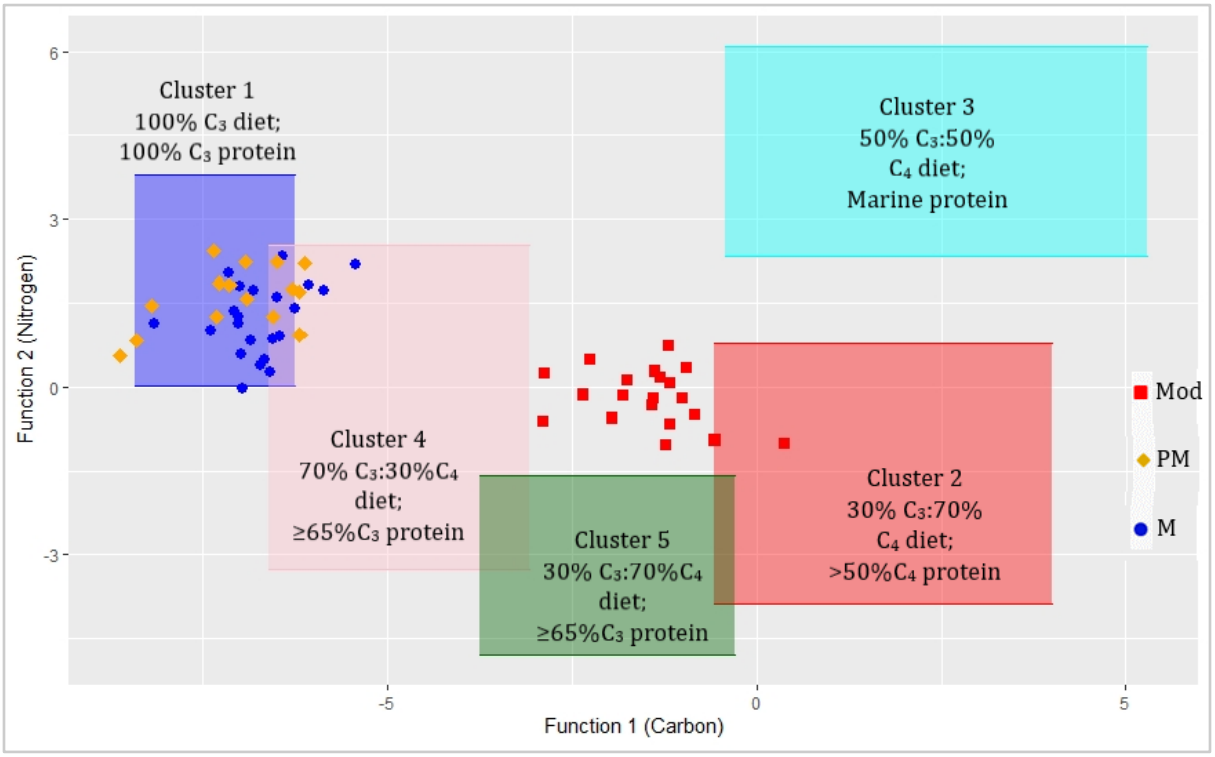


Figure 8-6: F1 and F2 discriminant function values from bone archaeological individuals in England plotted against previously generated dietary clusters (see Froehle et al., 2012); (Key-Mod-Modern; PM-Post-medieval; M-Medieval).

In contrast, based on the carbon isotope values of enamel, a distinctive C_4 signature is observed in the modern enamel as expected, however, this is not the case with both the medieval and post-medieval populations. The average $\delta^{13}C$ values of -13.6 ± 1.0‰ and -13.3 ± 1.0‰ for medieval and post-medieval samples respectively are consistent with uptake of C_3 resources. Tooth $\delta^{13}C_{enamel}$ values are expected to fall between − 17‰ and -13‰ for a pure C_3-based diet (Koch et al. 1998; Krigbaum 2003). The average $\delta^{13}C_{enamel}$ values for both the medieval and post-medieval populations are therefore consistent with the typical spectrum of a terrestrial diet in England characterised by high consumption of C_3 resources as suggested in historical sources (Dyer 1989; Oddy 2000; Woolgar et al. 2006).

Although there are some suggestions of C_4 food consumption using the Froehle et al. (2012) multivariate isotope model, the use of carbon isotope ratios on dental calculus carbonate should be able to determine if the post-medieval populations were consuming C_4 resources (maize or cane sugar). The mean calculus carbonate $\delta^{13}C$ values for medieval, post-medieval, and modern populations are -9.7 ± 1.2‰, -9.2 ± 1.4‰, and -4.1 ± 1.8‰. If these $\delta^{13}C$ values are interpreted as akin to bone carbonate and enamel carbonate values for individuals who consumed an abundance of C_4 resources (bone carbonate modern samples for this study = -9.3 ± 1.1‰; enamel carbonate modern samples for this study = -6.8 ± 1.1‰) as well as those found from other communities consuming C_4 resources e.g. Tykot et al. (1996) whose values are C_4 bone apatite =-9.8±1.0‰; C_4

tooth enamel =-8.7±2.3‰, the archaeological populations' calculus $\delta^{13}C$ values would suggest high inclusion of C_4 terrestrial resources in their diet.

8.4.3.1 Causes of diet differences between bone and enamel

The modern individuals' enamel carbonate $\delta^{13}C$ values for this study are consistently ^{13}C -enriched relative to bone carbonate whereas those for medieval and post-medieval individuals are broadly similar. Throughout an individual's life bone is constantly remodelled and reflects diet ingested over a number of years prior to death, depending on the skeletal element (Cox and Sealy 1997; Hedges et al. 2007; Meier-Augenstein 2017). In contrast, enamel in teeth forms over a relatively short time with the materials used in the present study (M1, M2, and M3) completing formation at age 2.5-3 years, 7-8 years, and 12-16 years respectively (Schuurs 2013). Consequently, the differences that are being observed between these two tissues in modern individuals would be heavily influenced by dietary changes in diet due to age. The enamel carbonate $\delta^{13}C$ values for the rest of the population are consistent with the well-documented consumption of sugary foods among children and adolescents (Frary et al. 2004; Slining and Popkin 2013; Bailey et al. 2018). The total daily energy from added sugars for the American adolescent and teenage population group has been found to be higher than that of adults - approximately 13.1% to 17.5% among children compared to 11.2% to 14.5% among adults (Ervin et al. 2012; Ervin and Ogden 2013). Therefore, Americans consuming more sugar during childhood would create significant offsets between bone and tooth $\delta^{13}C$ values.

In the case of the medieval and the post-medieval individuals, the enamel and the bone $\delta^{13}C$ values are the same because not only are the bones and enamel tapping from the same pool of the bicarbonates in the blood during formation but also the children were not exposed to sugary foods, unlike their modern counterparts. Unlike modern individuals, the archaeological children's diet did not differ substantially from their adult diet, at least in terms of consumption of C_4 resources.

8.4.3.2 High calculus $\delta^{13}C$ values

In all populations, the calculus carbonate $\delta^{13}C$ values are consistently higher than in enamel and bone. Firstly, this is most likely because of the different sources of the carbonate between dental calculus and the two other tissues. The source for both bone and tooth carbonate is blood bicarbonate which is sourced from CO_2 produced via oxidation of the whole diet in the human cells which initially passes through the stomach where it gets broken via microbial activity, followed by the diffusion of ^{13}C enriched CO_2 from the stomach to the bloodstream (Hedges 2003; Passey et al. 2005). In contrast, calculus derives its bicarbonate from two sources, (i) blood, which is the same source as the other two tissues and (ii) the action of the microbes during the metabolism of carbohydrates in the mouth (Sand 1951; Dawes 1969; Dawes 1974; Hicks et al. 2003). In addition, dental calculus forms as a biofilm and it is known from other environments that biofilm thickness can be a factor in ^{13}C isotope

fractionation and ultimately the $\delta^{13}C$ values (Staal et al. 2007). It is therefore proposed here that there is a possibility that the complex activities of bacteria in the mouth during the formation of plaque and calculus could be resulting in calculus becoming more enriched in ^{13}C as the ^{12}C isotope is preferentially used by bacteria and is eventually exhaled. On the other hand, for the bones and tooth carbonate, the consumed carbohydrates are all metabolised and breathed out as CO_2 in a less complex process resulting in a lower level of carbon fractionation than in calculus.

Overall, the results indicate that the North American individuals that consume a diet with a high proportion of sugar have high $\delta^{13}C$ values across all three tissues, but with much higher values in dental calculus. It is therefore argued here that, in modern individuals, all the sugar-based foods get incorporated into the calculus in a different way compared to enamel and bone because of different carbon fractionation and different timelines represented by the tissues. Moreover, this suggests that dental calculus samples are more sensitive to sugar consumption than both bone and enamel tissues. For both the medieval and post-medieval individuals, although calculus is also more enriched in ^{13}C compared to the other tissues, the values are less than those found in modern individuals who in this case are known to have consumed C_4 resources. There are, however, slightly higher $\delta^{13}C$ values in post-medieval dental calculus that suggest consumption of a more enriched ^{13}C diet like cane sugar. Nevertheless, it should be noted that the calculus $\delta^{13}C$ values may not necessarily only represent fractionated diet alone as inorganic debris, consisting of soil, mineral grit, and even pigments, inhaled or accidentally ingested during the performance of daily activities have been previously observed in dental calculus microscopically (Blatt et al. 2011; Hardy et al. 2012; Radini et al. 2016a; Radini et al. 2016b; Bleasdale et al. 2019; Radini et al. 2019). Therefore, the presence of these inorganic materials on the dental calculus can potentially affect the isotope ratios. The present study did not control for the inclusion of such material.

8.5 Conclusions

In this study, stable isotope analysis of dental calculus was performed alongside bone and enamel from archaeological samples from England and modern samples from the William M. Bass Donated Skeletal Collection in the USA. Diagenesis of dental calculus samples was also assessed, and the calculus diagenetic parameters were compared with those of bone. In general, the availability of modern unaltered calculus enabled this study to introduce FTIR parameters that can be used to investigate diagenesis in dental calculus. It is accepted that the diagenesis process of dental calculus is not fully understood, therefore, it is still impossible to show whether the archaeological dental samples used here were diagenetically altered or not, however, this study has provided a large body of data to the dental calculus FTIR dataset. Nevertheless, it is believed that the pretreatments that were performed before analysis removed the majority of any diagenetically altered carbonate

material. In addition, the unaltered dental calculus revealed a similar pattern of higher calculus carbonate $\delta^{13}C$ values compared to their bone and enamel values.

Previous studies on isotope analysis of dental calculus to investigate diet have mixed reviews, with some suggesting the usefulness of the tissue in the analysis of diet while others disagree. This study argues that with additional studies, calculus could prove to be a potential biomaterial to use in the identification of C_4 resources through isotope analysis. This study effectively produced results that show differences in diet between different populations whereby higher $\delta^{13}C$ values found in modern calculus samples confirmed that those people were consuming more C_4 foods than the English archaeological populations. Additionally, it has been established that the calculus $\delta^{13}C$ will most certainly be high due to the sugars that are being metabolised in the mouth as well as the effect of more fractionation in this much more poorly biologically controlled environment compared to that of bone and enamel. Using information from previous breath studies, this study has also demonstrated that it is highly unlikely that atmospheric CO_2 is causing higher $\delta^{13}C$ values as suggested by a previous study (Price et al. 2018) as $\delta^{13}C_{breath}$ is reflective of the whole diet ingested. It is therefore argued here that calculus carbonate $\delta^{13}C$ values are highly likely to represent the carbohydrates consumed. In spite of these conclusions, there is an awareness that the formation process and composition of dental calculus are highly variable between individuals therefore there are still a number of gaps in knowledge that need to be addressed before its full potential as a viable tool for the identification of C_4 resources in diet can be realised. For instance, the potential issue of the presence of particles (inorganic debris) in dental calculus is still outstanding, and more information is required to understand how the micro debris affects the overall isotope values found in dental calculus. Additionally, it is suggested that a controlled animal feeding study should be conducted in order to clearly observe the effect of different carbohydrates on dental calculus carbon isotope ratios.

Chapter 9: Conclusions

9.1 Introduction

This thesis presented a comprehensive analysis of diet using a combination of bone collagen and bone carbonate analysis on multiple 17th to 19th century populations in England, with a focus on populations in the northern manufacturing towns, an area little studied using these methods until now. The research also introduced the first in-depth analysis of diet in Manchester, using bone collagen isotope analysis. Finally, the use of dental calculus carbonate in identifying cane sugar consumption in these post-medieval individuals was explored. These analyses have produced a large body of novel results and have a range of interesting implications for future post-medieval palaeodietary studies in England.

9.2 Major Research Findings

9.2.1 Manchester diet

Chapter 6 was the first research-based chapter presenting the first-ever study that uses bone collagen isotope analysis to study the diet of post-medieval human populations in the Greater Manchester area in England. This study had the opportunity to explore the diversity of the populations, for instance, two populations of different social status (low-class Chapel Street, Hazel Grove population; middle/upper-class Cross Street Unitarian Chapel population), with possible African or mixed ancestry of some of the individuals as well as different sex and age groups within and between the two populations.

In line with findings in other palaeodietary post-medieval studies in England, bone collagen isotope analysis revealed a diet predominantly based on plants and animal protein from C_3 terrestrial environment for both populations. Moreover, the results supported suggestions that individuals from London had access to more varied foodstuffs compared to the rest of the country. In addition, the results here suggested that, unlike some of the populations in the south of England, part of dietary protein consumed by Manchester individuals derived from terrestrial and/or freshwater animal sources but not marine sources. In addition, the hypothesis that the diet of middle/upper classes was different from that of lower classes in the post-medieval period, as drawn from the historical evidence in Chapter 3, was also supported in the higher $\delta^{15}N$ and $\delta^{13}C$ values obtained in the middle/upper-class Cross Street population compared to those from the lower-class Hazel Grove population.

Furthermore, the results showed that in some cases, the diet was influenced by age and sex. Food access was similar across all age groups at Cross Street whereas the adults consumed more animal

protein than non-adults at Hazel Grove. This was as expected as adults in lower socioeconomic classes during that time, especially men, consumed more meat for them to get the energy to work (Rowntree 1901). In terms of sex, there were no significant differences in diet between men and women at the Cross Street site, but this was regarded as likely due to the existing flexible gender roles for middle/upper-class populations during the post-medieval period which allowed everyone to get access to the same food despite their gender. The surprising finding, however, was that no apparent differences in diet could be detected between the males and females at the Hazel Grove site, despite their differing roles in society as discussed in Chapter 3. It, however, should be highlighted that the Hazel Grove population had fewer sexed individuals so could not be compared statistically. While this patterning could be the result of no differences in resource distribution between sexes in both populations, larger sample sizes of individuals from both sites of known sex are required to investigate this more fully. Finally, in terms of ethnic differences, no differences in diet were observed between the four individuals with possible African or mixed ancestry from Hazel Grove and the rest of the population. However, one of the four had a slightly higher $\delta^{13}C$ value than the rest of the population suggesting she was non-local. Since she is an adult, it cannot be ruled out that she may have originated from a place where C_4 crops dominated the diet, and her bone collagen may have shifted towards the C_3 local diet later in life. Again, it should be noted that the number of individuals with possible African or mixed ancestry is too low to make definitive conclusions on whether they were consuming a similar diet to the rest of the population.

9.2.2 Post-medieval diet through bone carbonate analysis

Chapter 7 presented the first application of carbon isotope analysis of bone carbonate ($\delta^{13}C_{carb}$) to post-medieval English skeletal material alongside the more traditional collagen-based isotopic analysis ($\delta^{13}C_{coll}$, $\delta^{15}N$). This study provided a new large multi-isotope dataset deriving from several post-medieval sites representing major regional centres in England to consider diets by geographical location and socioeconomic status. In addition, animal remains analysed here added to the animal dietary baseline for post-medieval England and provided an insight into animal management during this time.

The research was proposed from the need to identify C_4 resources (cane sugar/maize) in the English diet through isotope analysis. In addition, most individuals sampled in this thesis had poor dental health, with high levels of tooth decay and antemortem tooth loss, consistent with the consumption of a diet high in refined sugars and processed carbohydrates (Zero 1999; Bradshaw and Lynch 2013; Mant and Roberts 2015). Although C_4 food consumption has been alluded to in previous post-medieval palaeodietary studies in England, its consumption was not observed clearly using the traditional methods of isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). This is most likely because when isotope analysis is carried out in collagen, which itself is derived mainly from dietary

protein, the contribution of either cane sugar or maize (carbohydrates) is more likely to be masked by the protein contributions, hence their expression is less apparent. This thesis utilised bone carbonate isotope analysis because of its potential to augment bone collagen stable isotopic analyses as it offers information on whole diets (carbohydrates, lipids, and proteins) in palaeodietary interpretation, therefore, has the ability to identify C_4 energy sources in the diet (Krueger and Sullivan 1984; Ambrose and Norr 1993; Tykot et al. 1996). The carbohydrates (e.g., cane sugar or maize) contributions are more likely to be observed in the data with this technique when compared to results of bone collagen isotope analysis alone.

The traditional methods involving the assessment of stable carbon and nitrogen isotope data from bone collagen results only to interpret post-medieval diet in this study were consistent with a diet that incorporated C_3 plants and animal products, supporting previously identified results from other post-medieval studies. The most significant finding from this study after the addition of bone carbonate analysis and linear and multivariate modelling was a clearer identification of C_4 resource consumption from some individuals from post-medieval populations in England. The study revealed that about 35% of all individuals analysed consumed about 30% C_4 diet and about 35% C_4 protein. Additionally, for animals, the stable isotope results identified a few animals that consumed some C_4 diet, most likely to have been maize, as well as about 50% C_4 protein, indicating some availability of C_4 animal protein for post-medieval populations in the country.

As previously stated, before this study, post-medieval palaeodietary studies had mainly been focused on the south of Englad, utilising bone collagen isotope studies only, therefore, there was sparse stable isotope evidence to support suggestions that Londoners had access to a wider range of food resources compared to the rest of the country during this period. This study was able to reveal the differences between the diets of populations from London and the northern manufacturing towns diets in England in more detail using isotope analysis of eleven populations across the country. The results of the combined bone collagen and bone carbonate analysis confirmed suggestions from the historical data that Londoners had more access to animal and marine protein as well as foods enriched in ^{13}C compared to the rest of the country. Furthermore, investigations revealed that access to these resources within individual populations also varied. Social status investigations also agreed with the historical evidence (Chapter 3), that access to food varied according to status. The present study showed that the middle and upper classes had more access to animal protein as well as C_4 resources compared to the lower classes. Another notable finding was that the middle and upper classes from the northern manufacturing towns did not have similar access to food resources as the Londoners despite their similar social status. It has also been observed that that the differences between London and the northern manufacturing towns are not temporal as the populations studied were broadly contemporaneous.

9.2.3 Dental calculus as a biomaterial in isotope studies

Chapter 8 details stable isotope analysis carried out on dental calculus, bone, and enamel obtained from the same individuals in a broader range of populations that included medieval and post-medieval individuals from England as well as modern individuals from the Forensic Anthropology Center, University of Tennessee, USA. The study was developed because the prior investigations to determine the consumption of C_4 cane sugar using bone carbonate isotope analysis had been successful at identifying the cane sugar but not the quantities expected. In those studies, cane sugar was difficult to detect because although it was a key element of cuisine, it was not a dominant component of the post-medieval diet. However, due to the location of dental calculus in the mouth, and it being hydroxyapatite containing high levels of carbonate, it was hypothesised that calculus carbonate could offer a direct link to ingested simple carbohydrates and therefore, be the more sensitive indicator of the consumption of cane sugar and a viable substitute for bone carbonates.

The stable isotope results demonstrated that dental calculus carbonate $\delta^{13}C$ values are consistently significantly higher than those of bone and enamel. Due to the different sources of the carbonate between dental calculus and the bone and enamel, dental calculus was not expected to produce similar stable isotope results to the other two tissues. Despite that caveat, this study established that, due to its location in the mouth, there is likely to be greater carbon fractionation which in turn would result in consistently high $\delta^{13}C$ values in dental calculus compared to the other two tissues. It has also been demonstrated that the atmospheric CO_2 breathed in did not result in these high values as has been suggested previously in Price et al. (2018) study, since $\delta^{13}C_{breath}$ is reflective of the whole diet ingested. The study has also posited that the complex nature and formation of dental calculus as a biofilm may be an important factor in the enriched ^{13}C levels noted in dental calculus relative to enamel and bone further supporting the conclusion. Still, clear differences in $\delta^{13}C$ values were observed between the modern individuals who clearly had consumed a high sugar diet and the archaeological populations from England who had not. Furthermore, between the archaeological populations themselves, the post-medieval population had slightly higher calculus $\delta^{13}C$ values than the medieval population suggesting that the former consumed a more sugary diet than the latter population, thereby supporting the historical evidence that cane sugar consumption increased in the post-medieval period. However, there is an awareness that the formation process and composition of dental calculus as well as the inclusion of non-dietary particles (e.g., inorganic debris) within dental calculus may affect the $\delta^{13}C$ values. These results, although they strongly suggest the potential of dental calculus as a new biomaterial suitable for palaeodietary isotope studies, cannot be used at this stage to ascertain whether individuals are consuming cane sugar as there seems to be more that is causing the higher $\delta^{13}C$ values than is currently understood. Therefore, it is suggested here that additional work will be necessary to find solutions for these potential problems.

9.3 Future Research

Considering all these findings, this study has highlighted several recommendations for future research based on the following questions:

1. Is it worth undertaking bone carbonate isotope analysis in areas where C_4 resources are not a major part of the diet?
2. How might the potential for micro-debris' impact on calculus carbonate $\delta^{13}C$ be incorporated into future work?
3. What implications does micro-debris have for pre-treatment of calculus and how can it be tested?
4. How can the fractionation processes in dental calculus be identified?

Although bone carbonate isotope analysis has revealed C_4 resources that would not have otherwise been identified, it was discovered that it was still challenging to identify since cane sugar or maize were not a major part of the diet in post-medieval populations in England. However, the limitation of this project was probably the unavailability of more populations after 1850, when there was a large increase in the uptake of cane sugar per capita in the United Kingdom. It is believed that had there been more populations available from this time period, bone carbonate analysis would probably have produced better results in the analysis of diet in post-medieval populations. In addition, another problem encountered was that dating post-medieval individuals is based on the entire cemetery unless there are named individuals with known birth dates. As a result, even for populations dating after 1874 for example Hazel Grove (1794-1910) and St George's Crypt (1840-1911), it was difficult to establish when each individual was buried. Therefore, for future research using bone carbonate isotope analysis, it is recommended that bone carbonate isotope analysis should be utilised mostly in cases where burial dating is clear. Additionally, data from populations in this study do not map well onto the north America categories presented in Froehle (2012) perhaps due to the effects of climatic differences, therefore, it is recommended here that European studies are carried out to create clusters that are more in line with Europeans populations. Furthermore, compound-specific amino acid isotope analysis can be added to the analysis to enable the detection of freshwater resource consumption among post-medieval populations.

For dental calculus studies, the potential for inorganic micro-debris to have an impact on $\delta^{13}C$ in calculus needs to be explored further. It is recommended that during the pretreatment of dental calculus, an additional step to separate the inorganic micro-debris from dental calculus should be added. The Mays et al. (2018) supplementary report provides a methodology to achieve this. Following this the carbon isotope ratios for both the micro-debris and dental calculus should be measured and compared, to establish if the values in dental calculus are affected by those in the

micro-debris. Additionally, it is suggested that a controlled animal feeding study should be conducted in order to clearly observe the effect of different carbohydrates on carbon isotope ratios. This will also allow a better understanding of the fractionation processes in dental calculus.

9.4 Concluding remarks

In conclusion, considering the research questions, it has been established that bone carbonate isotope analysis in conjunction with bone collagen isotope analysis offer good complementary evidence for C_4 diet in Post-Medieval England. Additionally, using both isotope techniques, variations in diet can be observed between and within different populations by geographical location, age, socioeconomic status, and sex. The utilisation of FTIR-ATR to exclude samples that were significantly altered by diagenesis ensured that the samples used would provide acceptable results. Furthermore, ensuring that all bone carbonate isotope analysis samples were handled and prepared using the same methodology provide assurance that the variations observed between or within populations can only be attributed to dietary differences. Finally, it has been established that dental calculus carbonate has the potential to be utilised in palaeodietary studies in the future, but it is not yet a feasible substitute for bone carbonate isotope analysis and cannot yet be utilised to ascertain C_4 sugar consumption. More studies are still required to realise those objectives.

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Appendix A.

A.1 Collagen quality indicators, Collagen and Carbonate stable isotope values for all post-medieval humans analysed in this study

Sex category: U =Sex not determined, F = female, F? = probably female, M =Male, M? = probably male
Age category: U = age not determined, m= months,
Data: -= No data available

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	$\delta^{13}C_{coll}$ *(‰)*	$\delta^{15}N$ *(‰)*	$\delta^{13}C_{carb}$ *(‰)*	$\delta^{18}O_{carb}$ *(‰)*
Cross Street Manchester	CSM 2.03	L.metatarsal	M	46+	11.1	42.1	15.1	3.3	-19.4	12.4	-	-
	CSM 2.05	Fibula	U	13-17	10.7	41.7	15.3	3.2	-18.9	11.5	-13.6	-3.76
	CSM 2.07	L. arm	F	18+	8.2	43.8	16.0	3.2	-20.4	10.8	-13.7	-4.29
	CSM 2.1	L.Arm	U	46+	10.5	43.2	15.9	3.2	-20.4	10.7	-	-
	CSM 2.11	Rib	U	A18+	14.2	41.7	15.3	3.2	-20.0	11.5	-	-
	CSM 2.12	Rib	M	46+	16.4	41.1	14.7	3.3	-19.6	11.3	-13.6	-3.39
	CSM 2.14	Rib	U	7-12	10.7	39.2	14.0	3.3	-20.2	10.5	-	-
	CSM 2.15	Ribs	F	46+	5.9	41.8	15.3	3.2	-20.7	9.5	-15.5	-4.89
	CSM 2.16	Fibula	M	46+	14.1	42.3	15.5	3.2	-20.4	10.0	-14.8	-4.61
	CSM 2.18	Clavical	M	18+	12.6	40.2	14.8	3.2	-20.4	9.8	-15.7	-4.48
	CSM 2.20	L.ulna	F	46+	8.2	42.3	15.3	3.2	-20.7	10.6	-16.6	-2.79
	CSM 2.21	Rib	F	46+	18.8	42.5	15.4	3.2	-19.4	12.3	-14.4	-3.28
	CSM 2.23	Tibia	U	7-12	19.7	43.1	16.0	3.1	-20.2	12.3	-	-
	CSM 2.24	Fibula	U	1-6	9.1	43.0	15.3	3.3	-20.2	11.2	-	-
	CSM 2.25	Rib	F	46+	17.8	43.7	16.1	3.2	-19.7	11.5	-15.0	-5.48
	CSM 2.28	Femur	U	1-6	5.0	40.8	14.7	3.2	-20.0	11.3	-	-
	CSM 2.29	Long Bone	F	18+	1.6	41.3	15.0	3.2	-20.5	11.0	-15.0	-5.02

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	CSM 2.3	Rib	U	7-12	21.3	46.1	16.7	3.2	-19.7	12.6	-	-
	CSM 2.31	Rib	F	46+	15.0	42.4	15.4	3.2	-19.7	12.0	-13.1	-5.15
	CSM 2.32	Rib	F	18+	8.7	46.1	16.7	3.2	-19.7	12.6	-14.2	-4.09
	CSM 2.34	Rib	F	36-45	17.1	43.4	15.8	3.2	-19.4	12.6	-13.2	-4.13
	CSM 2.35	L.Arm	F	18+	2.2	26.5	9.5	3.3	-19.5	11.7	-13.2	-5.18
	CSM 2.36	Rib	M	18+	7.6	41.5	15.0	3.2	-20.6	11.0	-15.4	-3.75
	CSM 2.37	2nd Metacarpal	M	46+	12.9	43.7	15.9	3.2	-19.8	11.9	-14.0	-5.76
	CSM 2.38	Tibia	M?	36-45	14.4	41.9	15.2	3.2	-19.8	10.5	-	-
	CSM 2.39	Femur	U	1-6	15.9	42.0	15.5	3.2	-20.4	10.6	-	-
	CSM 2.40	Femur	U	6.0	5.6	42.5	15.5	3.2	-19.1	12.3	-10.9	-3.62
	CSM 2.41	Rib	F	26-35	8.1	43.2	15.6	3.2	-19.6	12.1	-14.6	-4.35
	CSM 2.43	Arm bone	M	26-35	11.1	41.9	15.0	3.3	-20.1	12.9	-15.8	-4.98
	CSM 2.47	Rib	M	18+	8.1	40.6	15.1	3.1	-20.1	12.1	-	-
	CSM 2.49	Rib	M	18-25	11.3	41.6	15.3	3.2	-19.6	12.5	-14.7	-5.43
	CSM 2.5	Tibia	U	10-12 m	17.9	46.5	17.0	3.2	-20.3	10.8	-	-
	CSM 2.51	Rib	M	46+	15.3	43.9	16.0	3.2	-19.7	12.0	-13.5	-5.61
	CSM 2.52	Rib	F	46+	21.5	45.2	16.1	3.3	-19.3	12.3	-14.6	-5.22
	CSM 2.53	Rib	F	36-45	13.3	43.7	15.8	3.2	-19.6	12.7	-	-
	CSM 2.54	Rib	M	46+	5.5	43.3	15.2	3.3	-19.5	12.9	-15.0	-4.05
	CSM 3.00	Cranium	U	10m-1	9.6	47.5	17.2	3.2	-19.0	15.0	-14.0	-3.42
	CSM 3.1	Rib	M	36-45	5.2	44.1	15.8	3.3	-18.7	12.5	-	-
	CSM 3.14	Rib	U	7-12	13.3	45.3	16.7	3.2	-20.2	11.6	-	-
	CSM 3.25	Ulna	F	18+	4.0	43.9	15.7	3.3	-19.9	11.8	-15.3	-4.90
	CSM 3.33	Rib	U	13-17	9.0	41.8	15.1	3.2	-19.7	13.1	-14.1	-4.87
	CSM 3.34	Rib	M	36-45	15.5	45.3	16.3	3.2	-20.6	9.5	-14.6	-4.64
	CSM 3.36	Rib	U	1-6	17.3	45.3	16.4	3.2	-19.1	14.7	-14.1	-6.17
	CSM 3.37	Fibula	U	7-12	5.5	42.3	15.3	3.2	-20.7	9.5	-	
	CSM 3.38	Femur	U	1-6	13.0	42.9	15.4	3.3	-20.2	10.8	-	
	CSM 3.43	Rib	U	13-17	12.1	43.7	15.6	3.3	-20.1	11.7	-13.6	-3.78

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	CSM 3.45	Fibula	F	18+	4.0	41.9	15.3	3.2	-20.1	12.2	-15.6	-3.62
	CSM 3.48	Rib	M	36-45	17.2	44.2	15.9	3.2	-20.0	11.9	-15.8	-3.70
	CSM 3.51	Rib	U	1-6	4.0	38.6	13.5	3.4	-19.7	11.7	-	-
	CSM 3.52	Rib	M	26-35	17.7	44.0	16.0	3.2	-19.9	10.4	-	-
	CSM 3.53	Rib	F	36-45	19.5	44.2	16.1	3.2	-19.9	10.5	-12.7	-4.24
	CSM 4.05	Rib	F	36-45	2.7	30.6	10.8	3.3	-19.9	11.7	-13.7	-4.08
	CSM 4.11	Rib	M	18-25	14.2	42.1	15.5	3.2	-20.1	11.2	-15.6	-4.16
	CSM 4.12	Rib	F	26-35	31.5	41.5	15.2	3.2	-19.8	11.9	-14.7	-4.29
	CSM 4.2	Fibula	U	1-6	22.6	44.1	15.6	3.3	-20.0	10.9	-	-
	CSM 4.24	Rib	M	26-35	18.4	44.6	16.5	3.2	-20.1	11.3	-14.2	-4.16
	CSM 4.28	Rib	M	36-45	13.4	42.9	15.6	3.2	-18.5	12.3	-14.6	-5.79
	CSM 4.3	Ulna	F	13-17	16.9	43.7	16.0	3.2	-20.1	11.6	-	-
	CSM 4.38	Rib	F	46+	20.1	43.7	15.3	3.3	-19.5	12.5	-15.2	-4.58
	CSM 4.39	Radius	U	1-6	1.2	33.8	12.0	3.3	-20.0	11.2	-	-
	CSM 4.4	Rib	U	1-6	18.0	44.6	16.4	3.2	-19.6	11.8	-	-
	CSM 4.53	Rib	F?	18+	10.3	41.3	14.9	3.2	-20.1	10.9	-15.3	-3.69
	CSM 5.05	Rib	M	46+	19.7	42.1	15.2	3.2	-18.9	12.5	-13.7	-4.06
	CSM 5.07	Ulna	F	46+	2.8	27.8	10.0	3.2	-20.6	10.8	-15.0	-3.71
	CSM 5.09	Rib	M	18+	8.0	42.2	15.4	3.2	-19.3	11.7	-12.6	-4.39
	CSM 5.1	Clavical	U	7-12	8.7	38.2	13.8	3.2	-19.8	11.5	-	-
	CSM 5.16	Rib	M	46+	12.6	43.5	15.9	3.2	-19.4	12.5	-13.3	-4.68
	CSM 5.23	Clavical	F	18+	16.1	42.2	15.3	3.2	-19.5	11.8	-13.1	-2.68
	CSM 5.36	Humerus	U	1-6	5.1	36.8	13.2	3.3	-19.5	11.7	-12.9	-4.05
	CSM 7	Rib	M	36-45	1.8	30.9	11.0	3.3	-19.8	10.9	-13.5	-3.36
	CSM 10	Rib	U	1-6	8.5	42.4	15.6	3.2	-20.6	10.1	-	-
	CSM 12	Rib	M	36-45	4.4	41.0	14.9	3.2	-19.6	11.5	-15.2	-3.63
	CSM 24	Rib	U	1-6	15.8	41.5	15.2	3.2	-19.5	12.6	-	-
	CSM 25	Rib	U	7-12	19.1	43.0	15.7	3.2	-19.8	11.2	-	-
	CSM 27	Skull frag	F	18+	6.5	41.6	15.3	3.2	-20.2	10.6	-15.3	-4.30

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	CSM 28	Rib	U	13-17	11.2	42.0	15.2	3.2	-19.9	10.8	-	-
	CSM 29	Femur	U	1-6	10.3	41.0	15.0	3.2	-20.4	12.2	-	-
	CSM 31	Rib	U	1-6	17.3	43.0	15.7	3.2	-20.4	10.9	-	-
	CSM 32	Tibia	U	10-12 m	21.4	43.2	15.7	3.2	-19.4	12.8	-	-
	CSM 35	Rib	U	13-17	18.9	42.4	15.6	3.2	-19.2	11.6	-	-
	CSM 37	Rib	F	46+	19.5	42.0	15.1	3.2	-19.5	12.9	-14.0	-4.42
	CSM 38	Rib	U	1-6	17.3	42.9	15.7	3.2	-20.0	12.3	-	-
	CSM 39	Rib	U	7-12	12.2	39.9	14.8	3.2	-19.9	11.9	-	-
	CSM 41	Rib	M	46+	3.1	36.7	13.3	3.2	-19.1	13.0	-13.7	-5.78
	CSM 44	Rib	F	46+	19.7	40.1	14.7	3.2	-18.1	11.9	-	-
	CSM 61.03	Metacarpal	U	36-45	17.8	43.7	15.9	3.2	-19.2	12.7	-14.1	-4.64
	CSM 61.04	Femur	U	1-6	12.5	41.8	15.3	3.2	-19.0	15.1	-14.7	-4.53
	CSM 62.02	Rib	F	26-35	19.9	43.0	15.8	3.2	-20.2	12.8	-15.0	-6.12
	CSM 66.01	Humerus	U	1-6	11.2	41.5	15.1	3.2	-19.7	11.2	-	-
	CSM 67.01	Femur	U	1-6	9.7	41.3	14.4	3.3	-19.9	14.4	-	-
	CSM 4021	Tibia	U	U	8.2	43.3	15.9	3.2	-20.3	9.9	-	-
	CSM 5052	Rib	U	U		39.6	13.8	3.3	-18.4	11.9	-	-
Hazel Grove Manchester	HGM 1	Fibula	M?	26-35	9.8	45.9	16.9	3.2	-20.4	10.2	-14.4	-5.12
	HGM 2	Tibia	U	0	8.5	45.9	16.5	3.2	-20	11.6	-15.7	-4.76
	HGM 3	Rib	U	6-8	13.6	46.4	17.1	3.2	-20.1	10.2	-14.2	-4.61
	HGM 4	Rib	U	12-14	9.3	46.3	16.9	3.2	-20	10.2	-14.3	-4.59
	HGM 5	Rib	U	1-2	16.1	47.2	17.1	3.2	-19.8	13.9	-15.4	-4.74
	HGM 6	Rib	F	46+	13.3	45.8	16.8	3.2	-20.4	11.5	-14.5	-5.64
	HGM 7	Rib	U	1-3	14.6	46.2	16.78	3.21	-19.76	13.39	-	-
	HGM 8	Tibia	U	6m -2	5.4	46.4	16.6	3.3	-19.4	12.9	-14.1	-4.26
	HGM 9	Femur	U	0-1	11.4	43.3	15.7	3.2	-19.6	13.9	-13.4	-4.33
	HGM 10	Rib	F	36-45	14.9	46.8	17.3	3.2	-20.2	10.3	-15.2	-5.30
	HGM 11	Femur	U	0-3m	13.6	46.3	16.8	3.2	-19.5	11.7	-15.1	-4.48
	HGM 12	Humerus	M?	18+	8.1	43.3	15.7	3.2	-20.5	9.8	-14.5	-5.15

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	HGM 13	Petrous	U	0m	3	41.8	14	3.5	-20.5	11.8	-14.4	-5.95
	HGM 14	Rib	F?	26-45	10.2	42.4	15.5	3.2	-19.2	11.4	-	-
	HGM 15	Rib	M	36-45	6.4	44.5	16	3.2	-20.2	11.2	-13.1	-4.91
	HGM 16	Tibia	U	18+	8.5	44.4	15.9	3.3	-20.6	10.3	-15.1	-3.92
	HGM 17	Tibia	U	4-6	9.5	45.3	16.1	3.3	-20.6	9.4	-13.7	-3.85
	HGM 19	Rib	U	8-10	7.7	43.7	15.8	3.2	-20.7	10.2	-13.6	-4.69
	HGM 20	Tibia	M?	18+	9.8	41.1	15.1	3.2	-20.4	12.1	-	-
	HGM 21	Rib	M	46+	9.4	42.8	15.4	3.2	-20.5	11.4	-12.8	-4.74
	HGM 22	Rib	F	36-45	6.2	42.9	15.6	3.2	-20.4	10.7	-14.2	-4.53
	HGM 23	Tibia	U	7-9m	9.8	42.4	15.3	3.2	-19.5	10.4	-14.3	-4.83
	HGM 24	Rib	U	2-4	12.7	44	16	3.2	-19.9	11.7	-12.9	-5.64
	HGM 26	Rib	F	46+	9.4	43.6	16	3.2	-20.2	10.6	-11.3	-5.22
	HGM 27	Tibia	U	18+	4.9	42.7	15.4	3.2	-19.7	11.1	-13.8	-5.22
	HGM 28	Rib	U	5-7	11.1	43.2	15.8	3.2	-20.5	9.8	-13.4	-6.06
	HGM 29	Radius	U	18+	6.6	45.8	16.5	3.2	-20.6	11.1	-15.9	-2.21
	HGM 30	Fibula	U	18+	7	44.4	16.2	3.2	-20.1	10.6	-15.3	-5.37
	HGM 31	Femur	U	2-4	6.7	42.9	15.5	3.2	-20	12.1	-13.6	-4.23
	HGM 32	Rib	M	46+	10.3	43.3	15.7	3.2	-20.3	10.3	-12.8	-3.76
	HGM 33	Rib	F	36-45	9	47.2	17.3	3.2	-20.3	10.2	-12.3	-5.42
	HGM 34	Tibia	U	18+	7.8	42.2	15.2	3.2	-19.8	11.4	-13.6	-5.13
	HGM 35	Fibula	U	18+	5.3	44.4	16	3.2	-20.5	10.8	-14.6	-4.73
	HGM 36	Rib	F	60+	4.9	42.3	15.5	3.2	-20.3	10.7	-14.4	-4.08
Fewston, Harrogate	FEW 53	Rib	F	46+	-	-	-	-	-20.1	10.7	-13.3	-4.36
	FEW 77	Rib	M	17-20	-	-	-	-	-20.7	10.7	-13.9	-4.85
	FEW 130	Rib	M	66	-	-	-	-	-19.3	11.9	-16.5	-5.34
	FEW 156	Rib	F?	66?	-	-	-	-	-20.3	10.7	-13.8	-5.16
	FEW 177	Rib	F	36-35	-	-	-	-	-19.8	12.2	-14.5	-5.92
	FEW 238	Rib	F	49	-	-	-	-	-20.5	11.5	-15.6	-4.37
	FEW 241	Rib	M	46+?	-	-	-	-	-20.3	11.9	-14.7	-5.75

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
Square Chapel Halifax	SCH 35	Rib	M	46+	16.1	43.1	15.7	3.2	-19.6	12.2	-15.6	-6.79
	SCH 39	Rib	F	46+	13.1	45.5	16.7	3.2	-19.7	13.5	-14.5	-4.89
	SCH 59	Rib	F	46+	15.2	46	17.1	3.1	-18.7	14.8	-15.5	-6.64
	SCH 80	Rib	U	3	12.1	42.9	15.9	3.2	-19.3	12.1	-15.6	-5.22
	SCH 88	Ulna	M	46+	7.6	44.5	16.4	3.2	-19.7	12.1	-15.4	-5.80
	SCH 92	Rib	F	17-22	10.7	45.3	16.5	3.2	-19.7	11.7	-15.5	-5.76
	SCH 98	Humerus	M	18+	13.4	42	15.2	3.2	-19.7	13.9	-15.1	-5.42
	SCH 119	Fibula	M	18+	9.4	45.3	16.4	3.2	-19.6	11.9	-15.4	-6.87
	SCH 140	Femur	F	36-45	11.3	44.8	16.4	3.2	-19.8	12.1	-14.6	-8.25
	SCH 191	Rib	M	18+	5.9	44.1	16.1	3.2	-19.8	12.4	-14.9	-5.49
	SCH 1144	Left medial clavicle	F	18+	4.3	40.7	15	3.2	-19.8	11.9	-12.9	-5.07
	SCH 1146	1st right rib	F	26-35	9.6	41.2	15.2	3.2	-19.5	11.4	-13	-5.24
	SCH 1171	Left proximal radius	M	36-45	5.9	41.6	15.4	3.2	-20.5	10.6	-14.4	-5.45
	SCH 1202	2nd right rib	M	45+	5	41.6	15.1	3.2	-19.3	11.5	-14.6	-5.05
	SCH 1232	1st right rib	M	36-45	8.7	41.3	15.2	3.2	-20	12.2	-14.6	-5.64
	SCH 1247	Right distal radius	M	26-35	1.5	37.9	13.7	3.2	-20.3	12.1	-15.1	-5.15
	SCH 1268	1st left rib	M	U	9.7	41.8	15.3	3.2	-19.1	11.8	-12.5	-4.60
	SCH 1329	2nd left rib	M	36-45	2.1	41.1	15	3.2	-19.4	12.6	-13.5	-5.02
	SCH 1347	Left medial clavicle	F?	18+	6.5	42.2	15.4	3.2	-19.9	11.3	-15.1	-5.33
	SCH 1357	2nd right rib	F	26-35	11.6	41.8	15.4	3.2	-20.1	11.4	-14.4	-5.43
	SCH 1377	2nd left rib	F	18-25	11.5	44.6	16.3	3.2	-19.8	11.6	-14.1	-5.72
	SCH 1381	Left ulna midshaft	M	18+	3.5	40.3	14.8	3.2	-20.3	10.5	-16.1	-5.16
	SCH 1384	2nd left rib	M	18-25	16.2	43.6	15.9	3.2	-20	11.3	-14.5	-4.91
	SCH 1415	Left radius midshaft	M	18+	7.5	42.7	15.1	3.3	-20.7	11.1	-16.5	-5.41
	SCH 1470	11th left rib	M	36-45	16.2	42.3	15.4	3.2	-19.8	11.5	-15.5	-5.28
	SCH 1525	1st right rib	F	18-25	12.4	42	15.3	3.2	-20.1	11.6	-14.8	-5.71
	SCH 1546	2nd right rib	M	18-25	11.5	41.2	15.1	3.2	-20.8	11	-15.6	-5.64

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	SCH 1159	Humerus	U	18+	13.5	36.8	13.4	3.2	-19.9	11.2	-15	-5.21
	SCH 1337	Ulna	U	18+	19	39.9	14.3	3.3	-20.9	10.3	-15.6	-5.10
	SCH 1452	Metatarsal	U	U	22.7	38.5	14.1	3.2	-20.1	10.6	-14	-6.63
	SCH 1482	Rib	U	18+	24.4	39.8	14.5	3.2	-20	11.6	-13.9	-5.59
	SCH 1513	Rib	U	18+	15.2	41.3	14.8	3.3	-20.3	11.7	-16	-6.50
St. George's Crypt, Leeds	SGC 1003	Metatarsal	U	18+	19.5	43.2	15.8	3.2	-20	12.4	-15.2	-5.73
	SGC 1006	Metatarsal	U	18+	19.4	43.6	15.9	3.2	-18.5	11.1	-13.6	-4.40
	SGC 1010	Metatarsal	U	18+	22.5	44.1	16	3.2	-20	12.4	-14.4	-5.70
	SGC 1014	Hand Phalanx	F?	18-25	20.2	43.6	16	3.2	-19.6	11.6	-13.7	-5.71
	SGC 1017	Metatarsal	M?	36-45	21.3	43.3	15.8	3.2	-19.6	11.9	-14.9	-4.95
	SGC 1020	Hand Phalanx	M	36-45	17.3	44.4	16.1	3.2	-19.8	11.9	-14.8	-5.10
	SGC 1024	Hand Phalanx	M	18-25	19.7	43.1	15.8	3.2	-19.5	11.8	-14.3	-4.71
	SGC 1029	Hand Phalanx	M	46+	19.7	42.8	15.6	3.2	-20.3	11.2	-15.6	-4.33
	SGC 5003	Hand Phalanx	F	46+	20.4	41.9	15.2	3.2	-20	12.2	-14.6	-6.65
Victoria Gate, Leeds	VGL 12	Rib	F?	16-19	-	42.9	15.7	3.2	-19.8	12	-14	-6.26
	VGL 2	Rib	U	5-6	-	43	15.6	3.2	-20.4	11.6	-13.8	-4.42
	VGL6	Rib	U	7-8	-	43.4	16	3.2	-20.2	11.6	-14.9	-4.16
Rotherham Minster, Rotherham	ROM 3	Tibia shaft	U	18+	10.4	44.5	16.1	3.2	-20.7	11.4	-14.8	-4.79
	ROM 7	Left rib	F	18-20	10.6	43	15.5	3.2	-20.3	11.1	-16.2	-5.53
	ROM 9	Right rib	M	18+	9.2	43.4	15.4	3.3	-20.4	10.2	-14.7	-4.65
	ROM 11	Right phalanx	M?	46+	1.4	42.3	15.2	3.3	-20.1	10.5	-14.1	-4.38
	ROM 13	Rib	M	36-45	6.3	42.5	15	3.3	-19.5	12.2	-14.7	-4.99
	ROM 17	Right temporal	F?	18+	8.4	42.9	15.5	3.2	-20	10.4	-13.5	-5.44
	ROM 23	Ulna	F	18-25	5.6	43.3	15.5	3.2	-19.8	11.4	-12.4	-5.02
	ROM 24	Radius	F	46+	19.5	43.2	15.8	3.2	-19.3	12.1	-12.8	-4.44
	ROM 25	Humerus	M	26-35	20.2	42.6	15.6	3.2	-18.8	12.8	-13.1	-4.63
	ROM 28	Rib	F	26-35	26.8	42.7	15.6	3.2	-19.7	10.7	-12	-4.49
	ROM 31	Rib	F?	46+	10.4	42.7	15.4	3.2	-19.8	10.8	-13.6	-5.02
	ROM 35	Humerus	M?	18+		42.5	15.6	3.2	-19.9	10.7	-11.7	-4.87

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	$\delta^{13}C_{coll}$ *(‰)*	$\delta^{15}N$ *(‰)*	$\delta^{13}C_{carb}$ *(‰)*	$\delta^{18}O_{carb}$ *(‰)*
	ROM 37	Rib	F	46+	14.3	43.6	15.6	3.3	-20.3	12.7	-13.8	-5.75
	ROM 44	Femur	F	46+	11.5	43.1	15.4	3.3	-19.8	12.1	-14.9	-5.16
	ROM 47	Rib	M	36+	15	42.3	15.5	3.2	-20.4	10.1	-14.6	-5.32
	ROM 51	Right distal tibia	F	46+	4.5	42.4	14.7	3.4	-19.9	11.7	-14.2	-5.96
	ROM 55	Rib	M	46+	9	43.1	15.7	3.2	-19.7	11.9	-13.8	-4.05
	ROM 56	Radius	M	18-21	5	42	15.4	3.2	-19.4	11.6	-12.7	-4.72
	ROM 57	Radius	F	46+	23.7	43.7	15.8	3.2	-19.5	12.2	-14	-7.26
	ROM 58	Radius	M	36-45	15.1	42.3	15.5	3.2	-19.5	11.6	-13.1	-4.48
	ROM 60	Rib	M	26-35	23	42.6	15.6	3.2	-19.9	10.6	-11.9	-5.11
Queen's Chapel Savoy, London	QCS 122	Rib	F	18-30	13.3	41.2	15.3	3.1	-19.2	10.1	-14	-4.16
	QCS 124	Rib	F?	45+	11	41.5	15.4	3.2	-18.7	12.5	-15	-4.09
	QCS 163	Rib	F	18+	2.2	36	12.9	3.3	-19.2	11.3	-13.1	-6.66
	QCS 534	Rib	F	45+	5.9	42.9	15.7	3.2	-19.1	12.4	-13.5	-4.22
	QCS 589	Rib	M	45+	5.9	44.4	16.2	3.2	-19	13.7	-14.5	-5.44
	QCS 1123	Mandible	M	31-45	15.1	43.8	16.2	3.1	-13.1	10.7	-9.8	-3.77
	QCS 1804	Rib	F	31-45	15.7	43.7	16.1	3.2	-18.8	13.2	-14.4	-4.93
	QCS 1810	Rib	F	45+	5.7	43.2	15.9	3.2	-19	12.3	-14.9	-4.55
	QCS 1817	Rib	F	45+	13.3	43.3	15.9	3.2	-18.8	12.8	-14.5	-4.13
	QCS 1998	Rib	M?	31-45	13	45.5	16.7	3.2	-19	14.4	-13.6	-4.16
St. Barnabas Kensington, London	SBK 7	Metacarpal	F	81	7.5	38	13.9	3.2	-19.6	12.6	-15.3	-2.86
	SBK 8	Mandible	M	71	1.3	42	15.3	3.2	-19.8	13.3	-13.7	-4.84
	SBK 9	Metacarpal	F	57	9.4	41.5	15	3.3	-19.3	12.7	-10.5	-3.59
	SBK 10	Metacarpal	M	42	2.5	43.3	14.6	3.3	-19	14	-14.4	-4.15
	SBK 11	Metacarpal	M	64	2.2	43.5	15.4	3.3	-19.2	13.5	-13.1	-4.38
	SBK 12	Metacarpal	M	71	5.3	43.7	15.2	3.3	-19.3	13.2	-15.2	-4.99
	SBK 15	Metacarpal	M	48	10.8	43.6	15.4	3.3	-19.3	13.8	-14.5	-4.10
	SBK 17	Metacarpal	M	81	8	45.6	16.5	3.3	-18.3	12.6	-10.2	-3.15
	SBK 18	Metacarpal	M	55	8.3	48.2	17	3.3	-18.1	14.6	-13.3	-2.97
	SBK 21	Rib	M	73	11.3	44.6	15.9	3.3	-19.3	13.8	-10.6	-3.71

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	$\delta^{13}C_{coll}$ *(‰)*	$\delta^{15}N$ *(‰)*	$\delta^{13}C_{carb}$ *(‰)*	$\delta^{18}O_{carb}$ *(‰)*
	SBK 26	Metacarpal	F	43	9.4	43.5	15.8	3.3	-19.2	12.5	-14.5	-4.87
	SBK 30	Rib	M	18	14.7	44.6	16	3.3	-19	13.7	-12.9	-2.15
	SBK 34	Metacarpal	M	83	11.9	42.7	15.4	3.3	-18.4	13.9	-13.9	-4.32
	SBK 36	Metacarpal	M	18+	9.4	43.1	15.9	3.3	-17.8	14.2	-14.3	-5.75
	SBK 43	Rib	F	46	11.1	42.8	15.5	3.3	-19.1	13.6	-11.6	-4.04
	SBK 44	Metacarpal	F	74	12.4	44.8	15.8	3.3	-18.1	15.2	-14.5	-3.77
	SBK 45	Metacarpal	F	29	11.7	45.9	16.4	3.3	-19.6	13.4	-13.4	-3.61
	SBK 46	Metacarpal	F	44	5.8	43.1	15.8	3.2	-19.6	13.5	-14.6	-8.35
	SBK 48	Metacarpal	F	72	8.9	40.9	14.9	3.2	-18.1	13.6	-14.1	-4.47
	SBK 53	Metacarpal	M	41	12	42.9	15.6	3.3	-19.7	12.9	-14.5	-4.33
	SBK 54	Rib	F	40	9.2	44.7	16.2	3.3	-19.7	13.5	-14.2	-4.92
	SBK 57	Metacarpal	F	76	10.1	43.7	14.9	3.4	-20.3	13.9	-15.3	-3.93
	SBK 58	Metacarpal	M	51	2.6	41.2	14.8	3.2	-18.5	14	-15	-3.68
Royal London Hospital, London	RLH 103	Rib	F	26-35	10	38.8	14.2	3.2	-19.2	12.6	-12.3	-5.24
	RLH 135	Rib	M	36-45	6.1	35.1	12.7	3.2	-19.6	12.4	-13.4	-4.23
	RLH 208	Rib	M?	36-45	7.7	38.2	14	3.2	-19.1	12.7	-13.8	-4.47
	RLH 340	Rib	M?	36-45	4.7	38.5	13.7	3.3	-18.3	13.2	-13.2	-5.09
	RLH 349	Rib	M	36-45	17.1	39.8	14.6	3.2	-18.9	13	-14.2	-5.13
	RLH 356	Rib	M?	18+	6.8	35.9	12.9	3.2	-19.7	11.9	-12.4	-4.94
	RLH 367	Rib	M	36-45	2.6	41.5	15.1	3.2	-19.1	12.8	-14.3	-5.19
	RLH 386	Rib	F?	36-45	5.7	37	13.4	3.2	-19.2	11.6	-13.5	-4.44
	RLH 397	Rib	F	36-45	15.6	32.7	11.9	3.2	-19	13.6	-13.4	-4.61
	RLH 421	Rib	M	36-45	7	36	12.9	3.3	-20.4	11.3	-13	-4.47
	RLH 572	Rib	M	36-45	4.3	43	15.7	3.2	-19.6	12.3	-13.9	-4.52
St Brides Lower, London	SBL 1203	Rib	F	46+	18.5	43.6	15.9	3.2	-19.1	12.3	-14.1	-6.41
	SBL 1207	Rib	F	26-35	18.8	41.5	15.3	3.2	-19.3	12.8	-14.2	-4.00
	SBL 1215	Rib	F?	36-45	11.1	41.8	15.3	3.2	-20.2	11.3	-14.4	-4.29
	SBL 1244.1	Rib	M	18+	19.6	43.4	16	3.2	-19.5	12.2	-14.2	-4.40
	SBL 1526	Rib	M	26-35	17.8	42.2	15.6	3.2	-19.4	13.5	-15.2	-5.40

Site & Location	*Sample No.*	*Element*	*Sex*	*Age (years)*	*Collagen Yield (%)*	*%C*	*%N*	*C:N ratio*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	SBL 1558	Rib	M	36-45	10.9	40.3	14.8	3.2	-19.2	12.4	-14.3	-5.97
	SBL 1641	Rib	F	36-45	17	43	15.8	3.2	-19.3	11.5	-15.1	-6.25
	SBL 1653	Rib	F	26-35	16.8	43	15.9	3.2	-19.3	12	-14.7	-4.64
	SBL 1785	Rib	M	46+	9.2	41.2	14.9	3.2	-19.1	12.2	-13.6	-4.50
	SBL 1799	Rib	F	36-45	13.6	42.4	15.7	3.1	-19.2	13.4	-14.8	-4.63
	SBL 1872	Rib	M	36-45	16.7	42.2	15.6	3.1	-19.4	12.6	-13.6	-4.65
	SBL 1932	Rib	M	36-45	18.1	43.8	16.1	3.2	-19.7	12.8	-16	-5.14
	SBL 2049	Rib	F	36-45	18.4	43.7	15.9	3.2	-20.9	10.6	-15.9	-4.68
	SBL 2134	Rib	F	26-35	12.8	42.6	15.4	3.2	-19.8	12.8	-14.7	-4.78
	SBL 2296	Rib	M	46+	19.4	42.3	15.7	3.2	-19	11.8	-12	-4.77

A.2 Modern carbon isotope data with required adjustments/corrections.

							$\delta^{13}C$ ‰ values with fossil fuel correction as appropriate[2]			
Sample	***Year of Death***	$\delta^{13}C_{coll}$ ‰	$\delta^{13}C_{carb}$ ‰	$\delta^{13}C_{calc}$ ‰	$\delta^{13}C_{ena}$ ‰	***Fossil fuel correction to 1860***	$\delta^{13}C_{coll}$ ‰	$\delta^{13}C_{carb}$ ‰	$\delta^{13}C_{calc}$ ‰	$\delta^{13}C_{ena}$ ‰
FAC 1	2013	-15.41	-10.68	-5.46	-9.61	1.78	-13.63	-8.90	-3.68	-7.8
FAC 2	2013	-16.22	-11.26	-7.64	-10.35	1.78	-14.44	-9.48	-5.86	-8.6
FAC 3	2012	-15.99	-12.72	-3.24	-7.53	1.75	-14.24	-10.97	-1.49	-5.8
FAC 4	2014	-16.86	-12.37	-8.23	-8.99	1.81	-15.05	-10.56	-6.42	-7.2
FAC 5	2006	-15.69	-9.91	-2.78	-9.80	1.63	-14.06	-8.28	-1.15	-8.2
FAC 6	2008	-15.19	-9.20	-6.68	-8.02	1.67	-13.52	-7.53	-5.01	-6.4
FAC 7	2005	-16.85	-12.48	-5.48	-7.69	1.6	-15.25	-10.88	-3.88	-6.1
FAC 8	2001	-16.04	-12.19	-8.25	-9.48	1.46	-14.58	-10.73	-6.79	-8.0
FAC 9	2001	-15.95	-10.89	-3.71	-6.06	1.46	-14.49	-9.43	-2.25	-4.6
FAC 10	2002	-15.28	-11.29	-7.69	-8.45	1.5	-13.78	-9.79	-6.19	-7.0
FAC 11	2015	-15.9	-10.64	-4.58	-9.10	1.83	-14.07	-8.81	-2.75	-7.3
FAC 12	2013	-15.35	-10.18	-5.13	-8.19	1.78	-13.57	-8.40	-3.35	-6.4
FAC 13	2011	-15.01	-11.42	-6.71	-7.38	1.72	-13.29	-9.70	-4.99	-5.7
FAC 14	2011	-15.89	-11.67	-6.69	-8.94	1.72	-14.17	-9.95	-4.97	-7.2
FAC 15	2016	-15.76	-11.09	-4.79	-8.75	1.83	-13.93	-9.26	-2.96	-6.9
FAC 16	2010	-15.71	-10.88	-3.09	-10.89	1.7	-14.01	-9.18	-1.39	-9.2
FAC 17	2000	-15.09	-11.27	-6.23	-7.55	1.45	-13.64	-9.82	-4.78	-6.1

							δ¹³C ‰ values with fossil fuel correction as appropriate[2]			
Sample	***Year of Death***	***$\delta^{13}C_{coll}$ ‰***	***$\delta^{13}C_{carb}$ ‰***	***$\delta^{13}C_{calc}$ ‰***	***$\delta^{13}C_{ena}$ ‰***	***Fossil fuel correction to 1860***	***$\delta^{13}C_{coll}$ ‰***	***$\delta^{13}C_{carb}$ ‰***	***$\delta^{13}C_{calc}$ ‰***	***$\delta^{13}C_{ena}$ ‰***
FAC 18	1999	-13.85	-8.54	-4.33	-7.85	1.45	-12.4	-7.09	-2.88	-6.4
FAC 19	1996	-15.26	-9.65	-5.49	-6.91	1.34	-13.92	-8.31	-4.15	-5.6
FAC 20	1997	-15.62	-10.53	-8.36	-7.85	1.37	-14.25	-9.16	-6.99	-6.5

A.3 Enamel carbonate, calculus carbonate, bone collagen and bone carbonate isotope data as well as collagen quality indicators for all samples analysed in Chapter 8.

Sex category: U =Sex not determined, F = female, M =Male
Age category: All adults
Bone Element: Man=Mandible; Clav=Clavicle; MC=Metacarpal; PP=Pedal phalanx; HP=Hand phalanx

Location	*Period*	*Sample*	*Sex*	*Tooth element*	$\delta^{13}C_{calc}$ (‰)	$\delta^{13}C_{ena}$ (‰)	*Bone element*	*%Coll yield*	*%C*	*%N*	*C:N (ratio)*	$\delta^{13}C_{coll}$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C_{carb}$ (‰)
Southwell Cemetery Nottinghamshire	7th - 9th c.	SCN 20	M	M2	-8.9	-13.9	Man	7.6	43.9	16.3	3.1	-20.1	12.5	-14.3
	7th - 9th c.	SCN 27	F	M1	-9.5	-15.2	Man	6.4	42.9	15.8	3.2	-19.8	10.1	-12.8
	7th - 9th c	SCN 29	M	M1	-9.5	-15.2	Man	3.1	42.8	15.5	3.2	-20.1	11.4	-12.1
	7th - 9th c	SCN 33	M	M2	-8.4	-12.5	Man	5.2	42.6	15.6	3.2	-19.8	11.6	-12.7
	7th - 9th c	SCN 35	M	M1	-9.6	-14.7	Man	5.3	44.0	16.3	3.2	-19.8	11.2	-13.3
St Peter's Cemetery Leicester	10th - 16th c.	SPL 226	M	M2	-9.6	-13.4	Man	5.7	40.9	14.8	3.2	-18.9	13.7	-12.5
	10th - 16th c.	SPL 552	F	M1	-8.8	-12.2	Skull	10.5	41.0	15.0	3.2	-19.3	12.1	-13.1
	10th - 16th c.	SPL 1063	F	M1	-9.3	-14.6	Man	14.7	41.9	15.3	3.2	-19.9	11.9	-12.3
	10th - 16th c.	SPL 1069	U	M1	-10.2	-12.8	Man	10.3	41.7	15.3	3.2	-19.5	10.5	-13.0
	10th - 16th c.	SPL 1248	U	M2	-9.0	-13.3	Man	2.7	38.5	13.6	3.3	-19.8	11.0	-12.2
	10th - 16th c.	SPL 1384	U	M2	-10.2	-12.1	Man	11.5	39.7	14.5	3.2	-19.3	12.9	-12.6
Nun's Field Chester	12th - 16th c.	NFC 13	U	M2	-8.0	-14.0	Rib	19.0	41.9	15.3	3.2	-19.1	12.7	-14.4
	12th - 16th c.	NFC 19	F	M2	-10.9	-13.7	Rib	18.9	40.4	14.8	3.2	-20.9	11.0	-14.8
	12th - 16th c.	NFC 39	F	M2	-9.5	-13.9	Rib	9.8	41.8	15.2	3.2	-19.7	12.1	-14.0
	12th - 16th c.	NFC 60	F	M2	-9.5	-14.3	Rib	7.0	42.0	15.0	3.3	-19.6	12.3	-13.4
	12th - 16th c.	NFC 68	F	M2	-10.1	-13.4	Rib	13.5	38.9	14.4	3.2	-20.1	11.8	-13.8
	12th - 16th c.	NFC 72	F	M1	-12.7	-15.0	Rib	5.7	30.2	11.4	3.1	-19.4	11.0	-14.5
	12th - 16th c.	NFC 74	F	M2	-10.7	-13.4	Rib	7.3	30.7	11.5	3.1	-19.6	10.5	-13.6
	12th - 16th c.	NFC 80	F	M2	-12.3	-12.8	Rib	8.4	31.2	11.4	3.2	-20.1	11.0	-14.1
	12th - 16th c.	NFC 81	F	M2	-9.2	-13.8	Rib	13.6	38.0	14.0	3.2	-19.8	12.1	-14.2

Location	*Period*	*Sample*	*Sex*	*Tooth element*	$\delta^{13}C_{calc}$ (‰)	$\delta^{13}C_{ena}$ (‰)	*Bone element*	*%Coll yield*	*%C*	*%N*	*C:N (ratio)*	$\delta^{13}C_{coll}$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C_{carb}$ (‰)
	12th - 16th c.	NFC 86	M	M2	-8.9	-13.1	Rib	9.2	36.6	12.6	3.4	-20.3	11.8	-13.2
	12th - 16th c.	NFC 91	M	M2	-8.5	-12.4	Rib	14.6	35.1	13.0	3.2	-19.3	12.8	-13.0
Cross Street Manchester	18th - 19th c.	CSM 2.15	F	M2	-10.9	-14.8	Ribs	5.9	41.8	15.3	3.2	-20.7	9.5	-15.5
	18th - 19th c.	CSM 2.18	M	M2	-9.9	-14.8	Clav	12.6	40.2	14.8	3.2	-20.4	9.8	-15.7
	18th - 19th c.	CSM 2.31	F	M2	-9.6	-12.5	Rib	15.0	42.4	15.4	3.2	-19.7	12.0	-13.1
	18th - 19th c.	CSM 2.34	F	M2	-7.6	-12.4	Rib	17.1	43.4	15.8	3.2	-19.4	12.6	-13.2
	18th - 19th c.	CSM 2.36	M	M2	-9.5	-14.1	Rib	7.6	41.5	15.0	3.2	-20.6	11.0	-15.4
	18th - 19th c.	CSM 2.37	M	M2	-8.8	-12.8	MC	12.9	43.7	15.9	3.2	-19.8	11.9	-14.0
	18th - 19th c.	CSM 2.49	M	M2	-10.1	-11.9	Rib	11.3	41.6	15.3	3.2	-19.6	12.5	-14.7
	18th - 19th c.	CSM 4.12	F	M2	-10.2	-12.2	Rib	31.5	41.5	15.2	3.2	-19.8	11.9	-14.7
	18th - 19th c.	CSM 4.24	M	M2	-9.1	-14.0	Rib	18.4	44.6	16.5	3.2	-20.1	11.3	-14.2
	18th - 19th c.	CSM 5.09	M	M2	-9.2	-13.0	Rib	8.0	42.2	15.5	3.2	-19.3	11.7	-12.6
	18th - 19th c.	CSM 5.16	M	M2	-8.3	-12.4	Rib	12.6	43.5	15.9	3.2	-19.4	12.5	-13.3
	18th - 19th c.	CSM 12	M	M2	-9.8	-14.4	Rib	4.4	41.0	14.9	3.2	-19.6	11.6	-15.2
	18th - 19th c.	CSM 37	F	M2	-6.1	-13.0	Rib	19.5	42.0	15.1	3.2	-19.5	12.9	-14.0
	18th - 19th c.	CSM 41	M	M2	-7.3	-14.0	Rib	3.1	36.7	13.3	3.2	-19.1	13.0	-13.7
	18th - 19th c.	CSM 62.02	F	M2	-11.4	-13.9	Rib	19.9	43.0	15.8	3.2	-20.2	12.8	-15.0
Forensic Anthropology Center Tennessee, USA	2013	FAC 1	F	M2	-3.7	-7.8	PP	23.0	42.2	15.5	3.2	-13.6	11.0	-8.9
	2013	FAC 2	F	M3	-5.9	-8.6	PP	21.3	42.8	15.7	3.2	-14.4	10.9	-9.5
	2012	FAC 3	F	M2	-1.5	-5.8	PP	22.5	42.4	15.6	3.2	-14.2	9.9	-11.0
	2014	FAC 4	F	M2	-6.4	-7.2	Rib	23.5	42.1	15.6	3.2	-15.1	9.6	-10.6
	2006	FAC 5	F	M2	-1.2	-8.2	PP	21.0	43.4	16.1	3.2	-14.1	10.7	-8.3
	2008	FAC 6	F	M1	-5.0	-6.4	PP	24.0	43.0	14.8	3.4	-13.5	10.6	-7.5
	2005	FAC 7	F	M2/M3	-3.9	-6.1	PP	21.6	41.8	15.1	3.2	-15.3	10.8	-10.9
	2001	FAC 8	F	M2	-6.8	-8.0	PP	20.7	42.6	15.7	3.2	-14.6	11.1	-10.7
	2001	FAC 9	F	M1	-2.3	-4.6	PP	21.6	42.9	15.2	3.3	-14.5	10.3	-9.4
	2002	FAC 10	F	M2	-6.2	-7.0	PP	22.1	42.4	15.3	3.2	-13.8	12.0	-9.8
	2015	FAC 11	M	M2	-2.8	-7.3	PP	20.2	41.9	15.3	3.2	-14.1	11.6	-8.8

Location	Period	Sample	Sex	Tooth element	$\delta^{13}C_{calc}$ (‰)	$\delta^{13}C_{ena}$ (‰)	Bone element	%Coll yield	%C	%N	C:N (ratio)	$\delta^{13}C_{coll}$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C_{carb}$ (‰)
	2013	FAC 12	M	M2	-3.4	-6.4	PP	22.3	42.8	16.0	3.1	-13.6	10.8	-8.4
	2011	FAC 13	M	M2	-5.0	-5.7	PP	22.5	42.9	15.5	3.2	-13.3	11.3	-9.7
	2011	FAC 14	M	M2	-5.0	-7.2	PP	21.4	42.5	15.6	3.2	-14.2	10.9	-10.0
	2016	FAC 15	M	M3	-3.0	-6.9	HP	21.4	43.6	16.1	3.2	-13.9	10.6	-9.3
	2010	FAC 16	M	M2	-1.4	-9.2	PP	21.3	43.2	16.1	3.1	-14.0	10.9	-9.2
	2000	FAC 17	M	M2	-4.8	-6.1	Rib	22.1	41.7	15.7	3.1	-13.6	11.0	-9.8
	1999	FAC 18	M	M3	-2.9	-6.4	PP	22.9	42.7	15.7	3.2	-12.4	10.6	-7.1
	1996	FAC 19	M	M2	-4.2	-5.6	PP	22.2	42.9	15.6	3.2	-13.9	10.1	-8.3
	1997	FAC 20	M	M2	-7.0	-6.5	PP	22.0	42.1	15.1	3.3	-14.3	11.7	-9.2

Appendix B

B.1 Collagen quality indicators, Collagen and Carbonate stable isotope values for all faunal remains in this study

Data: -= No data available

Site	*Period*	*Sample*	*Taxon*	*Element sampled*	*Coll Yield (%)*	*%C*	*%N*	*C:N (ratio)*	$\delta^{13}C_{coll}$ *(‰)*	$\delta^{15}N$ *(‰)*	$\delta^{13}C_{carb}$ *(‰)*	$\delta^{18}O_{carb}$ *(‰)*
Cross Street, Manchester	17th -19thC	CSM 2030	Cattle	Rib	17.4	39.2	14.6	3.1	-22.2	7.0	-	-
	17th -19thC	CSM 2050	Mallard	Femur	17.0	38.3	14.1	3.2	-21.9	10.9	-	-
	17th -19thC	CSM 2051-2	Cattle	Rib	16.0	38.1	14.4	3.1	-22.7	6.4	-	-
	17th -19thC	CSM 2056	Sheep	Tibia	6.9	40.7	14.9	3.2	-21.9	6.5	-15.0	-4.81
	17th -19thC	CSM 2056-1	Sheep	Phalynx	7.8	34.9	13.0	3.1	-21.9	4.5	-	-
	17th -19thC	CSM 2061	Cattle	Rib		45.1	16.7	3.2	-20.5	6.0	-13.8	-4.42
	17th -19thC	CSM 2170	Sheep	Phalynx	22.5	42.4	15.6	3.2	-21.7	5.1	-	-
	17th -19thC	CSM 3006	Pig	Tibia	14.8	37.9	14.0	3.2	-22.1	6.5	-16.0	-5.18
	17th -19thC	CSM 3006-1	Cattle	Scapula	7.4	35.9	12.8	3.3	-21.1	6.0	-	-
	17th -19thC	CSM 3006-2	Cattle	Tibia	17.6	38.0	14.2	3.1	-22.3	6.7	-	-
	17th -19thC	CSM 3007	Sheep	Metacarpal	11.9	35.5	13.0	3.2	-22.4	6.4	-	-
	17th -19thC	CSM 3008	Sheep	Radius	4.4	21.7	8.2	3.1	-22.4	5.3	-	-
	17th -19thC	CSM 3008-1	Pig	Tibia	10.5	36.9	13.7	3.2	-21.3	8.8	-13.4	-4.76
	17th -19thC	CSM 3009-1	Cattle	Tibia	3.7	35.7	13.3	3.1	-22.4	7.5	-	-
	17th -19thC	CSM 3009-2	Cattle	Tibia	6.1	32.9	12.2	3.2	-22.2	6.5	-	-
	17th -19thC	CSM 3011	Sheep	Tibia	24.1	39.6	14.6	3.2	-21.4	5.0	-	-
	17th -19thC	CSM 3011-1	Sheep	Metacarpal	17.1	39.7	14.7	3.2	-21.7	3.3	-	-
	17th -19thC	CSM 3011-2	Sheep	Tibia	13.8	41.1	15.2	3.2	-21.4	4.5	-	-
	17th -19thC	CSM 4021 -3	Sheep	Radius	25.3	40.2	14.8	3.2	-22.2	5.9	-	-
	17th -19thC	CSM 4021-1	Cattle	Tibia		41.8	15.3	3.2	-21.9	6.1	-	-

Site	*Period*	*Sample*	*Taxon*	*Element sampled*	*Coll Yield (%)*	*%C*	*%N*	*C:N (ratio)*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	17th -19thC	CSM 4050	Cattle	Metatarsal		43.0	15.9	3.2	-22.0	5.8	-14.0	-4.85
	17th -19thC	CSM 4054	Cattle	Scapula	17.8	39.3	14.5	3.2	-22.1	7.0	-13.8	-4.95
	17th -19thC	CSM 4055	Pig	Tibia	14.9	39.5	14.4	3.2	-21.9	5.5	-15.3	-4.46
	17th -19thC	CSM 4062	Cattle	Femur		43.1	16.0	3.1	-21.5	6.4	-12.6	-4.33
	17th -19thC	CSM 4238	Sheep	Radius	7.7	40.0	14.2	3.3	-21.7	6.1	-14.7	-5.23
	17th -19thC	CSM 5027	Sheep	Femur	8.8	36.1	13.2	3.2	-22.4	6.2	-	-
	17th -19thC	CSM 5028	Mallard	Rib	6.8	33.1	11.9	3.2	-19.8	11.7	-	-
	17th -19thC	CSM 5029	Cattle	Radius	18.8	41.2	15.3	3.2	-22.5	5.7	-	-
	17th -19thC	CSM 5030	Pig	Radius	14.8	39.4	14.5	3.2	-21.8	6.1	-15.9	-5.99
	17th -19thC	CSM 6134	Pig	Metapodial	11.9	38.8	14.4	3.1	-22.3	6.0	-13.7	-4.10
	17th -19thC	CSM 6217	Pig	Tibia	16.6	43.7	16.2	3.1	-16.2	6.4	-	-
	17th -19thC	CSM 6217-1	Sheep	Metapodial	10.4	39.5	14.6	3.2	-22.2	4.5	-	-
	17th -19thC	CSM 6323	Pig	Tibia	11.8	35.7	13.2	3.2	-21.9	6.6	-15.5	-5.01
	17th -19thC	CSM 6602	Cattle	Tibia		43.8	15.8	3.2	-22.1	5.4	-	-
	17th -19thC	CSM 6832	Cattle	Tibia		43.2	15.9	3.2	-22.1	5.8	-	-
	17th -19thC	CSM 6838	Pig	Tibia		43.0	15.5	3.2	-20.2	8.8	-	-
	17th -19thC	CSM 6925	Sheep	Tibia		44.4	16.2	3.2	-22.1	6.8	-	-
Norton Priory, Chester	16th-18thC	NPBT 1	Cattle	Tibia	18.1	39.7	14.5	3.2	-22.4	5.2	-	-
	16th-18thC	NPBT 2	Cattle	Tibia	12.0	40.0	14.5	3.2	-22.2	7.1	-	-
	16th-18thC	NPBT 3	Cattle	Tibia	14.8	42.3	15.5	3.2	-22.4	5.9	-	-
	16th-18thC	NPBT 4	Cattle	Tibia	14.1	37.8	13.7	3.2	-22.2	5.9	-	-
	18th-20thC	NPBT 5	Cattle	Mandible	16.8	37.6	13.9	3.2	-22.1	5.8	-	-
	18th-20thC	NPBT 6	Cattle	Metatarsal	14.1	38.9	13.9	3.3	-22.2	6.0	-	-
	16th-18thC	NPBT 7	Cattle	Humerus	6.9	36.2	13.3	3.2	-21.9	4.9	-	-
	16th-18thC	NPBT 8	Cattle	Radius	10.1	39.3	14.4	3.2	-22.1	5.8	-	-
	16th-18thC	NPBT 9	Cattle	Radius	10.0	40.7	14.9	3.2	-22.1	6.9	-	-
	16th-18thC	NPBT 10	Cattle	Radius	14.4	40.8	14.8	3.2	-22.2	7.2	-	-
	16th-18thC	NPBT 11	Cattle	Radius	9.4	41.0	15.1	3.2	-22.4	6.1	-	

Site	*Period*	*Sample*	*Taxon*	*Element sampled*	*Coll Yield (%)*	*%C*	*%N*	*C:N (ratio)*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	16th-18thC	NPBT 12	Cattle	Astragalus	9.5	38.7	13.4	3.4	-22.8	7.1	-	-
	16th-18thC	NPBT 13	Cattle	Astragalus	7.5	41.2	15.0	3.2	-22.4	5.4	-	-
	18th-20thC	NPBT 14	Cattle	Metatarsal	4.3	39.5	14.4	3.2	-22.1	6.1	-	-
	18th-20thC	NPBT 15	Cattle	Metatarsal	9.0	39.9	14.7	3.2	-22.1	7.0	-	-
	18th-20thC	NPBT 16	Cattle	Metatarsal	11.3	40.9	14.9	3.2	-22.2	7.3	-	-
	18th-20thC	NPBT 17	Cattle	Tibia	8.3	39.6	14.6	3.2	-22.4	6.1	-	-
	18th-20thC	NPBT 18	Cattle	Humerus	5.0	36.8	13.5	3.2	-21.8	5.0	-	-
	16th-18thC	NPBT 19	Cattle	Femur	16.0	38.3	14.1	3.2	-21.5	7.3	-	-
	16th-18thC	NPBT 20	Cattle	Tibia	10.0	40.1	14.6	3.2	-22.1	6.3	-	-
	16th-18thC	NPDF 1	Chicken	Metatarsus	13.7	42.4	15.4	3.2	-20.5	8.1	-	-
	16th-18thC	NPDF 2	Chicken	Metatarsus	17.9	43.5	15.8	3.2	-20.9	10.2	-	-
	16th-18thC	NPDF 3	Chicken	Metatarsus	17.5	46.5	16.8	3.2	-20.6	8.4	-	-
	16th-18thC	NPDF 4	Chicken	Metatarsus	14.4	45.9	16.7	3.2	-20.8	8.3	-	-
	16th-18thC	NPDF 5	Chicken	Metatarsus	18.6	42.1	15.2	3.2	-20.7	9.1	-	-
	16th-18thC	NPDF 6	Chicken	Humerus	13.3	39.8	13.9	3.4	-21.0	9.6	-	-
	16th-18thC	NPDF 7	Chicken	Humerus	13.6	41.2	15.0	3.2	-21.4	9.0	-	-
	16th-18thC	NPDF 8	Chicken	Femur	17.2	37.6	13.7	3.2	-21.2	10.1	-	-
	16th-18thC	NPDF 9	Chicken	Tibiotarsus	15.4	43.1	15.5	3.2	-21.8	8.4	-	-
	16th-18thC	NPDF 10	Chicken	Femur	20.4	41.2	15.0	3.2	-21.1	8.7	-	-
	16th-18thC	NPDF 11	Chicken	Tarsometatarsus	14.9	42.7	15.2	3.3	-20.5	11.6	-	-
	16th-18thC	NPDF 12	Chicken	Tarsometatarsus	18.9	44.2	15.9	3.2	-21.4	10.9	-	-
	16th-18thC	NPSG 1	Sheep	Radius	15.7	41.9	15.4	3.2	-22.3	7.8	-	-
	16th-18thC	NPSG 2	Sheep	Scapula	15.6	42.5	15.6	3.2	-22.2	9.0	-	-
	16th-18thC	NPSG 3	Sheep	Humerus	15.6	42.9	15.7	3.2	-21.8	9.2	-	-
	16th-18thC	NPSG 4	Sheep	Humerus	14.5	43.5	15.9	3.2	-22.7	9.8	-	-
	16th-18thC	NPSG 5	Sheep	Tibia	15.2	39.1	14.5	3.1	-22.2	9.3	-	-
	16th-18thC	NPSG 5B	Sheep	Humerus	16.2	40.2	14.8	3.2	-21.3	7.3	-	-
	18th-20thC	NPSG 6	Sheep	Scapula	6.6	37.9	14.0	3.2	-22.2	7.3	-	-
	18th-20thC	NPSG 7	Sheep	Astragalus	6.8	42.2	15.4	3.2	-22.3	8.0	-	-

Site	*Period*	*Sample*	*Taxon*	*Element sampled*	*Coll Yield (%)*	*%C*	*%N*	*C:N (ratio)*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	18th-20thC	NPSG 8	Sheep	Metacarpal	9.9	40.5	14.6	3.2	-22.0	7.4	-	-
	18th-20thC	NPSG 9	Sheep	Tibia	13.5	43.9	15.8	3.2	-22.6	8.6	-	-
	18th-20thC	NPSG 10	Sheep	Metatarsal	18.6	43.6	16.3	3.1	-21.8	8.1	-	-
	18th-20thC	NPSG 11	Sheep	Tibia	18.0	44.0	16.1	3.2	-22.2	8.5	-	-
	18th-20thC	NPSG 12	Sheep	Scapula	18.0	44.0	16.3	3.2	-21.7	4.6	-	-
	18th-20thC	NPSG 13	Sheep	Tibia	13.3	40.3	14.6	3.2	-22.2	6.0	-	-
	16th-18thC	NPSG 14	Sheep	Tibia	9.9	41.6	15.1	3.2	-22.1	6.7	-	-
	18th-20thC	NPSG 14B	Sheep	Ulna	14.3	37.5	13.9	3.2	-22.2	7.2	-	-
	16th-18thC	NPSG 15	Sheep	Ulna	16.3	40.6	14.9	3.2	-22.3	5.9	-	-
	16th-18thC	NPSG 16	Sheep	Humerus	15.0	40.7	14.7	3.2	-22.0	7.2	-	-
	16th-18thC	NPSG 17	Sheep	Scapula	15.8	42.4	15.5	3.2	-22.5	8.9	-	-
	16th-18thC	NPSG 18	Sheep	Scapula	15.4	42.1	15.5	3.2	-22.3	10.5	-	-
	16th-18thC	NPSG 19	Sheep	Humerus	19.2	42.0	15.2	3.2	-22.4	9.1	-	-
	16th-18thC	NPSG 20	Sheep	Humerus	8.8	40.2	14.6	3.2	-22.0	7.2	-	-
	16th-18thC	NPSG 21	Sheep	Humerus	4.8	41.5	15.2	3.2	-21.3	7.2	-	-
	16th-18thC	NPSS 1	Pig	Humerus	4.9	38.1	13.9	3.2	-21.3	9.3	-	-
	16th-18thC	NPSS 2	Pig	Metapodial	11.0	37.4	13.6	3.2	-21.3	9.1	-	-
	18th-20thC	NPSS 3	Pig	Mandible	7.1	43.6	16.0	3.2	-21.2	8.4	-	-
	18th-20thC	NPSS 4	Pig	Mandible	9.5	40.5	14.6	3.2	-21.3	8.5	-	-
	16th-18thC	NPSS 5	Pig	Tibia	5.0	43.1	15.8	3.2	-21.3	8.5	-	-
	16th-18thC	NPSS 6	Pig	Scapula	16.8	43.4	15.6	3.2	-21.9	5.1	-	-
	16th-18thC	NPSS 7	Pig	Humerus	18.8	34.0	12.5	3.2	-21.5	6.8	-	-
	16th-18thC	NPSS 8	Pig	Radius	23.5	39.0	14.4	3.2	-21.3	6.4	-	-
	16th-18thC	NPSS 9	Pig	Radius	14.0	37.5	13.8	3.2	-21.9	9.1	-	-
	16th-18thC	NPSS 10	Pig	Metapodial	19.7	40.9	15.2	3.1	-21.2	8.7	-15.6	-6.00
	16th-18thC	NPSS 11	Pig	Scapula	12.4	40.3	14.8	3.2	-21.5	9.0	-14.7	-5.06
	16th-18thC	NPSS 12	Pig	Mandible	10.6	39.6	14.1	3.3	-21.3	7.2	-	-
	18th-20thC	NPSS 13	Pig	Ulna	18.4	43.5	16.0	3.2	-21.8	6.7	-	-

Site	*Period*	*Sample*	*Taxon*	*Element sampled*	*Coll Yield (%)*	*%C*	*%N*	*C:N (ratio)*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
	18th-20thC	NPSS 14	Pig	Metapodial	7.3	39.8	14.3	3.2	-22.1	8.1	-15.8	-5.52
	18th-20thC	NPSS 15	Pig	Tibia	12.2	39.9	14.6	3.2	-21.9	6.9	-15.9	-5.60
	18th-20thC	NPSS 16	Pig	Maxilla	9.4	40.9	14.9	3.2	-21.1	9.5	-14.3	-5.36
	18th-20thC	NPSS 17	Pig	Radius	17.7	38.1	13.9	3.2	-21.1	6.6	-14.4	-4.73
	18th-20thC	NPSS 18	Pig	Metapodial	13.9	35.9	13.2	3.2	-20.8	7.1	-	-
	18th-20thC	NPSS 19	Pig	Tibia	9.2	41.5	15.0	3.2	-21.9	5.2	-	-
	18th-20thC	NPSS 20	Pig	Ulna	19.9	37.4	13.6	3.2	-21.2	6.5	-	-
Hungate, York	19th C	HUN 01	Sheep	Mandible	-	39.8	14.3	3.2	-22.3	7.2	-	-
	19th C	HUN 02	Sheep	Mandible	-	43.3	15.9	3.1	-21.9	4.5	-	-
	19th C	HUN 03	Sheep	Mandible	-	41.0	15.3	3.1	-22.1	9.2	-	-
	15th C	HUN 027	Sheep	Metacarpal	-	39.3	14.3	3.2	-22.6	6.2	-	-
	18th-19th C	HUN 064	Sheep	Metacarpal	-	39.3	14.1	3.2	-21.6	4.6	-	-
	18th-19th C	HUN 080	Sheep	Metacarpal	-	39.2	14.2	3.2	-22.1	6.1	-	-
	18th-19th C	HUN 082	Sheep	Metacarpal	-	40.0	14.4	3.2	-22.2	6.5	-	-
	20th C	HUN 101	Sheep	Metacarpal	-	39.2	14.3	3.2	-22.2	4.1	-	-
	20th C	HUN 102	Sheep	Metacarpal	-	39.3	14.2	3.2	-22.3	5.3	-	-
	20th C	HUN 105	Sheep	Metacarpal	-	38.8	14.3	3.2	-22.2	7.0	-	-
	20th C	HUN 108	Sheep	Metacarpal	-	40.0	14.5	3.2	-23.0	7.0	-	-
	19th C	HUN 160	Sheep	Metacarpal	-	39.0	14.0	3.2	-22.1	4.7	-	-
	19th C	HUN 161	Sheep	Metacarpal	-	38.4	13.9	3.2	-22.5	6.7	-	-
	18th-19th C	HUN 162	Sheep	Metacarpal	-	43.0	15.5	3.2	-22.3	6.1	-	-
	18th-19th C	HUN 163	Sheep	Metacarpal	-	36.2	13.2	3.2	-22.0	8.9	-	-
	18th-19th C	HUN 165	Sheep	Metacarpal	-	43.0	15.6	3.2	-22.7	8.2	-	-
	16th-17th C	HUN 241	Sheep	Metatarsal	-	44.5	16.1	3.2	-22.5	6.4	-	-
	16th-17th C	HUN 267	Sheep	Metatarsal	-	43.1	15.6	3.2	-21.5	6.1	-	-
	18th-19th C	HUN 422	Sheep	Metacarpal	-	42.7	15.6	3.2	-21.8	3.6	-	-
	18th-19th C	HUN 426	Sheep	Metatarsal	-	39.6	14.5	3.2	-21.7	6.9	-	-
	18th-19th C	HUN 331	Sheep	Metacarpal	-	38.9	14.3	3.2	-22.1	3.9	-	-

Site	*Period*	*Sample*	*Taxon*	*Element sampled*	*Coll Yield (%)*	*%C*	*%N*	*C:N (ratio)*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
The Bedern, York	15th C	BED 01	Sheep	Metacarpal	-	37.6	13.9	3.2	-22.1	3.9	-	-
	15th C	BED 02	Sheep	Pelvis	-	40.8	15.2	3.1	-22.3	8.8	-	-
	15th C	BED 03	Sheep	Metacarpal	-	35.8	13.5	3.1	-21.8	5.5	-	-
	15th C	BED 04	Sheep	Scapula	-	38.7	14.2	3.2	-22.3	9.0	-	-
	15th C	BED 05	Sheep	Metacarpal	-	35.7	13.6	3.1	-22.1	5.0	-	-
	18th-19th C	BED 07	Sheep	Tibia	-	38.5	14.5	3.1	-21.5	5.6	-	-
	18th-19th C	BED 08	Sheep	Humerus	-	37.2	14.2	3.1	-21.8	5.4	-	-
	18th-19th C	BED 09	Sheep	Tibia	-	38.4	14.5	3.1	-21.5	4.8	-	-
	18th-19th C	BED 15	Sheep	Metacarpal	-	37.9	14.3	3.1	-20.8	8.7	-	-
	18th-19th C	BED 16	Sheep	Metacarpal	-	36.2	13.7	3.1	-22.2	8.0	-	-
	18th-19th C	BED 18	Sheep	Metacarpal	-	34.5	12.9	3.1	-22.3	5.2	-	-
Walmgate, York	18th-19th C	WAL 01	Sheep	Mandible	-	43.0	5.5	3.2	-20.8	6.3	-	-
	18th-19th C	WAL 02	Sheep	Mandible	-	39.1	13.3	3.2	-21.7	4.4	-	-
	18th-19th C	WAL 03	Sheep	Mandible	-	42.8	15.6	3.2	-21.7	5.4	-	-
Oulton, Leeds	18th-19th C	Flee (061)(062)	Cattle	Metacarpal	14.4	36.8	13.5	3.2	-21.7	7.9	-	-
	18th-19th C	Flee 053	Cattle	Metacarpal	10.3	33.3	12.2	3.2	-23.3	9.1	-	-
	18th-19th C	Flee 053-1	Cattle	Tibia	12.5	39.5	14.5	3.2	-22.5	8.3	-	-
	18th-19th C	Flee 053-3	Cattle	Metacarpal	11.8	34.0	12.7	3.1	-22.7	8.5	-	-
Otley, Leeds	18th-19th C	GPMO 037	Cattle	Long bone	19.3	40.0	14.7	3.2	-22.1	4.4	-13.0	-5.68
	18th-19th C	GPMO 159	Pig	Femur	21.5	39.8	14.6	3.2	-11.5	9.3	-9.9	-4.97
	18th-19th C	GPMO 168	Sheep	Metapodial	21.0	37.4	13.7	3.2	-21.7	5.5	-	-
	18th-19th C	GPMO 169	Sheep	Radius	9.5	39.6	14.5	3.2	-22.3	6.7	-	-
	18th-19th C	GPMO UC1	Sheep	Radius	15.1	40.4	14.7	3.2	-22.4	6.5	-	-
	18th-19th C	GPMO UC1-2	Cattle	Rib	22.9	42.1	15.3	3.2	-22.2	7.3	-	-
	18th-19th C	GPMO UC2	Cattle	Femur	20.4	40.4	14.9	3.2	-22.7	7.1	-	-
	18th-19th C	GPMO UC3	Pig	Tibia	17.9	40.1	14.7	3.2	-8.6	6.6	-10.3	-3.89
Sand-le-Mere	16th - 19th C	SALM T8 801	Cattle	Pelvis	15.7	39.9	14.6	3.2	-21.7	6.7	-	-
	16th - 19th C	SALM T8 803	Cattle	Maxilla	4.8	35.7	13.0	3.2	-21.3	8.1	-	-

Site	*Period*	*Sample*	*Taxon*	*Element sampled*	*Coll Yield (%)*	*%C*	*%N*	*C:N (ratio)*	*$\delta^{13}C_{coll}$ (‰)*	*$\delta^{15}N$ (‰)*	*$\delta^{13}C_{carb}$ (‰)*	*$\delta^{18}O_{carb}$ (‰)*
Square Chapel Halifax	16th - 19th C	SALM T8 807	Cattle	Ulna	11.8	21.1	7.8	3.2	-22.2	4.9	-	-
	18th - 19thC	SQC 1006	Sheep	Femur	12.3	37.9	13.7	3.2	-20.5	10.2	-13.2	-4.49
	18th - 19thC	SQC 1012-1	Cattle	Long bone	23.6	42.5	15.5	3.2	-21.7	8.1	-6.8	-6.33
	18th - 19thC	SQC 1012-2	Pig	Tibia	24.7	40.6	14.9	3.2	-22.3	10.1	-12.5	-4.71
	18th - 19thC	SQC 1012-3	Sheep	Tibia	13.3	39.1	13.9	3.3	-22.7	7.8	-13.4	-3.70
	18th - 19thC	SQC 1012-4	Sheep	Calcaneus	9.5	42.2	15.4	3.2	-21.7	8.1	-12.8	-3.89
	18th - 19thC	SQC 1250	Sheep	Tibia	17.1	37.3	13.7	3.2	-22.4	6.2	-15.3	-6.07
	18th - 19thC	SQC 1355	Cattle	Metatarsal	7.6	21.1	7.7	3.2	-22.7	3.7	-	-

Appendix C

C.1 FTIR-ATR results for all post-medieval samples in this study. Each sample was measured in triplicate

Sample	*Human/Animal*	*IRSF (Mean ± σ)*	*C/P (Mean ± σ)*
FEW 53	Human	3.72 ± 0.03	0.19 ± 0.00
FEW 77	Human	3.59 ± 0.02	0.19 ± 0.00
FEW 130	Human	3.77 ± 0.05	0.17 ± 0.00
FEW 156	Human	3.52 ± 0.01	0.23 ± 0.00
FEW 177	Human	3.67 ± 0.09	0.17 ± 0.00
FEW 238	Human	3.37 ± 0.04	0.25 ± 0.00
FEW 241	Human	3.58 ± 0.06	0.20 ± 0.00
HGM 1	Human	3.91 ± 0.03	0.13 ± 0.00
HGM 2	Human	3.47 ± 0.03	0.20 ± 0.00
HGM 3	Human	3.77 ± 0.03	0.14 ± 0.00
HGM 4	Human	4.05 ± 0.03	0.11 ± 0.00
HGM 5	Human	3.49 ± 0.03	0.21 ± 0.00
HGM 6	Human	3.82 ± 0.03	0.16 ± 0.00
HGM 8	Human	3.65 ± 0.03	0.15 ± 0.00
HGM 9	Human	3.77 ± 0.02	0.16 ± 0.00
HGM 10	Human	3.71 ± 0.02	0.16 ± 0.00
HGM 11	Human	4.01 ± 0.02	0.12 ± 0.00
HGM 12	Human	3.75 ± 0.02	0.15 ± 0.00
HGM 13	Human	4.00 ± 0.02	0.12 ± 0.00
HGM 15	Human	3.93 ± 0.01	0.13 ± 0.00
HGM 16	Human	3.63 ± 0.02	0.14 ± 0.00
HGM 17	Human	3.97 ± 0.03	0.12 ± 0.00
HGM 19	Human	3.88 ± 0.03	0.13 ± 0.00
HGM 20	Human	3.64 ± 0.03	0.15 ± 0.00
HGM 21	Human	3.54 ± 0.02	0.19 ± 0.00
HGM 22	Human	3.60 ± 0.02	0.16 ± 0.00
HGM 23	Human	3.61 ± 0.02	0.16 ± 0.00
HGM 24	Human	3.69 ± 0.02	0.17 ± 0.00
HGM 26	Human	3.90 ± 0.02	0.13 ± 0.00
HGM 27	Human	3.75 ± 0.02	0.16 ± 0.00
HGM 28	Human	3.84 ± 0.02	0.13 ± 0.00
HGM 29	Human	3.72 ± 0.04	0.16 ± 0.00
HGM 30	Human	3.84 ± 0.02	0.13 ± 0.00
HGM 31	Human	3.77 ± 0.02	0.15 ± 0.00
HGM 32	Human	4.06 ± 0.03	0.11 ± 0.00
HGM 33	Human	3.76 ± 0.03	0.14 ± 0.00
HGM 34	Human	3.90 ± 0.02	0.13 ± 0.00
HGM 35	Human	3.77 ± 0.01	0.13 ± 0.00
HGM 36	Human	3.65 ± 0.01	0.15 ± 0.00
VGL 2	Human	3.72 ± 0.04	0.18 ± 0.00

Sample	*Human/Animal*	*IRSF (Mean ± σ)*	*C/P (Mean ± σ)*
VGL 6	Human	3.55 ± 0.02	0.20 ± 0.00
VGL 12	Human	3.49 ± 0.03	0.22 ± 0.00
STGC 1003	Human	3.87 ± 0.03	0.13 ± 0.00
STGC 1006	Human	3.53 ± 0.02	0.15 ± 0.00
STGC 1010	Human	3.39 ± 0.03	0.17 ± 0.00
STGC 1014	Human	3.49 ± 0.03	0.17 ± 0.00
STGC 1017	Human	3.45 ± 0.03	0.17 ± 0.00
STGC 1020	Human	3.43 ± 0.03	0.17 ± 0.00
STGC 1024	Human	3.40 ± 0.02	0.17 ± 0.00
STGC 1029	Human	3.45 ± 0.02	0.16 ± 0.00
STGC 5003	Human	3.80 ± 0.03	0.13 ± 0.00
SBK 7	Human	3.75 ± 0.02	0.17 ± 0.00
SBK 8	Human	3.99 ± 0.03	0.13 ± 0.00
SBK 9	Human	3.65 ± 0.03	0.17 ± 0.00
SBK 10	Human	3.68 ± 0.02	0.19 ± 0.00
SBK 11	Human	3.80 ± 0.02	0.16 ± 0.00
SBK 12	Human	3.65 ± 0.02	0.19 ± 0.00
SBK 15	Human	3.60 ± 0.01	0.19 ± 0.00
SBK 17	Human	3.71 ± 0.01	0.17 ± 0.00
SBK 18	Human	3.53 ± 0.03	0.23 ± 0.00
SBK 21	Human	3.69 ± 0.03	0.20 ± 0.00
SBK 26	Human	3.76 ± 0.01	0.17 ± 0.00
SBK 30	Human	3.43 ± 0.01	0.24 ± 0.00
SBK 34	Human	3.64 ± 0.02	0.20 ± 0.00
SBK 36	Human	3.75 ± 0.02	0.15 ± 0.00
SBK 43	Human	3.66 ± 0.02	0.17 ± 0.00
SBK 44	Human	3.65 ± 0.02	0.17 ± 0.00
SBK 45	Human	3.60 ± 0.02	0.20 ± 0.00
SBK 46	Human	3.50 ± 0.02	0.19 ± 0.00
SBK 48	Human	3.47 ± 0.02	0.20 ± 0.00
SBK 53	Human	3.47 ± 0.02	0.23 ± 0.00
SBK 54	Human	3.48 ± 0.02	0.24 ± 0.00
SBK 57	Human	3.56 ± 0.02	0.21 ± 0.00
SBK 58	Human	3.50 ± 0.01	0.21 ± 0.00
SQC 35	Human	3.56 ± 0.01	0.22 ± 0.00
SQC 39	Human	3.51 ± 0.02	0.21 ± 0.00
SQC 59	Human	3.60 ± 0.03	0.20 ± 0.00
SQC 80	Human	3.70 ± 0.02	0.18 ± 0.00
SQC 88	Human	3.72 ± 0.02	0.18 ± 0.00
SQC 92	Human	3.74 ± 0.03	0.16 ± 0.00
SQC 98	Human	3.93 ± 0.06	0.13 ± 0.00
SQC 119	Human	3.64 ± 0.03	0.16 ± 0.00
SQC 140	Human	3.57 ± 0.02	0.18 ± 0.00
SQC 191	Human	3.80 ± 0.01	0.12 ± 0.00
SQC 1144	Human	4.07 ± 0.02	0.13 ± 0.00
SQC 1146	Human	3.96 ± 0.02	0.15 ± 0.00
SQC 1159	Human	4.04 ± 0.02	0.15 ± 0.00

Sample	*Human/Animal*	*IRSF (Mean ± σ)*	*C/P (Mean ± σ)*
SQC 1171	Human	3.57 ± 0.03	0.18 ± 0.00
SQC 1202	Human	3.68 ± 0.02	0.18 ± 0.00
SQC 1232	Human	3.65 ± 0.02	0.18 ± 0.00
SQC 1247	Human	4.03 ± 0.04	0.14 ± 0.00
SQC 1268	Human	4.07 ± 0.02	0.13 ± 0.00
SQC 1329	Human	3.66 ± 0.02	0.18 ± 0.00
SQC 1337	Human	3.85 ± 0.02	0.16 ± 0.00
SQC 1347	Human	3.56 ± 0.03	0.18 ± 0.00
SQC 1357	Human	3.73 ± 0.02	0.16 ± 0.00
SQC 1377	Human	4.03 ± 0.01	0.14 ± 0.00
SQC 1381	Human	3.92 ± 0.02	0.14 ± 0.00
SQC 1384	Human	3.88 ± 0.01	0.16 ± 0.00
SQC 1415	Human	4.15 ± 0.02	0.13 ± 0.00
SQC 1452	Human	3.52 ± 0.03	0.19 ± 0.00
SQC 1470	Human	3.65 ± 0.03	0.17 ± 0.00
SQC 1482	Human	3.73 ± 0.02	0.17 ± 0.00
SQC 1513	Human	3.76 ± 0.02	0.17 ± 0.00
SQC 1525	Human	3.76 ± 0.02	0.16 ± 0.00
SQC 1546	Human	3.59 ± 0.03	0.19 ± 0.00
ROM 03	Human	3.75 ± 0.03	0.18 ± 0.00
ROM 07	Human	3.91 ± 0.04	0.15 ± 0.00
ROM 09	Human	3.82 ± 0.03	0.17 ± 0.00
ROM 11	Human	3.84 ± 0.03	0.15 ± 0.00
ROM 13	Human	3.88 ± 0.01	0.15 ± 0.00
ROM 17	Human	4.06 ± 0.02	0.13 ± 0.00
ROM 23	Human	3.48 ± 0.02	0.20 ± 0.00
ROM 24	Human	3.61 ± 0.05	0.15 ± 0.00
ROM 25	Human	3.50 ± 0.03	0.24 ± 0.00
ROM 28	Human	3.72 ± 0.02	0.16 ± 0.00
ROM 31	Human	3.66 ± 0.03	0.20 ± 0.00
ROM 35	Human	3.66 ± 0.03	0.20 ± 0.00
ROM 37	Human	3.86 ± 0.02	0.15 ± 0.00
ROM 44	Human	3.70 ± 0.01	0.18 ± 0.00
ROM 55	Human	3.54 ± 0.01	0.21 ± 0.00
ROM 56	Human	3.43 ± 0.03	0.21 ± 0.00
ROM 57	Human	3.84 ± 0.01	0.16 ± 0.00
ROM 58	Human	3.72 ± 0.05	0.16 ± 0.00
ROM 60	Human	3.90 ± 0.02	0.15 ± 0.00
QCS 122	Human	3.58 ± 0.05	0.16 ± 0.00
QCS 124	Human	3.27 ± 0.07	0.25 ± 0.00
QCS 163	Human	4.09 ± 0.07	0.14 ± 0.00
QCS 534	Human	3.99 ± 0.07	0.16 ± 0.00
QCS 589	Human	3.55 ± 0.07	0.19 ± 0.00
QCS 1123	Human	4.23 ± 0.01	0.12 ± 0.00
QCS 1804	Human	3.88 ± 0.01	0.15 ± 0.00
QCS 1810	Human	3.89 ± 0.01	0.17 ± 0.00
QCS 1817	Human	3.64 ± 0.07	0.17 ± 0.00

Sample	*Human/Animal*	*IRSF (Mean ± σ)*	*C/P (Mean ± σ)*
QCS 1998	Human	3.07 ± 0.02	0.25 ± 0.00
CSM 2.03	Human	3.46 ± 0.04	0.21 ± 0.00
CSM 2.05	Human	3.76 ± 0.02	0.14 ± 0.00
CSM 2.07	Human	3.68 ± 0.08	0.18 ± 0.00
CSM 2.12	Human	3.73 ± 0.02	0.14 ± 0.00
CSM 2.15	Human	3.68 ± 0.06	0.18 ± 0.00
CSM 2.16	Human	3.80 ± 0.03	0.18 ± 0.00
CSM 2.18	Human	3.80 ± 0.02	0.17 ± 0.00
CSM 2.2	Human	3.76 ± 0.02	0.15 ± 0.00
CSM 2.21	Human	3.75 ± 0.04	0.18 ± 0.00
CSM 2.25	Human	3.92 ± 0.06	0.16 ± 0.00
CSM 2.29	Human	3.92 ± 0.06	0.17 ± 0.00
CSM 2.3	Human	3.74 ± 0.01	0.18 ± 0.00
CSM 2.31	Human	3.58 ± 0.07	0.20 ± 0.00
CSM 2.32	Human	3.70 ± 0.11	0.18 ± 1.00
CSM 2.34	Human	4.05 ± 0.09	0.15 ± 0.00
CSM 2.35	Human	3.74 ± 0.00	0.18 ± 0.00
CSM 2.36	Human	4.01 ± 0.08	0.14 ± 0.00
CSM 2.37	Human	4.01 ± 0.04	0.14 ± 0.00
CSM 2.4	Human	4.02 ± 0.15	0.15 ± 1.00
CSM 2.41	Human	3.74 ± 0.00	0.18 ± 0.00
CSM 2.43	Human	3.94 ± 0.07	0.14 ± 0.00
CSM 2.49	Human	3.87 ± 0.03	0.16 ± 0.00
CSM 2.51	Human	3.77 ± 0.03	0.15 ± 0.00
CSM 2.52	Human	3.44 ± 0.01	0.23 ± 0.00
CSM 2.54	Human	3.61 ± 0.02	0.17 ± 0.00
CSM 3	Human	3.87 ± 0.01	0.16 ± 0.00
CSM 3.25	Human	3.84 ± 0.09	0.16 ± 0.00
CSM 3.33	Human	4.00 ± 0.07	0.14 ± 0.00
CSM 3.34	Human	4.01 ± 0.02	0.14 ± 0.00
CSM 3.36	Human	4.07 ± 0.01	0.14 ± 0.00
CSM 3.43	Human	3.66 ± 0.01	0.15 ± 0.00
CSM 3.45	Human	3.51 ± 0.07	0.19 ± 0.00
CSM 3.48	Human	3.79 ± 0.07	0.15 ± 0.00
CSM 3.53	Human	3.81 ± 0.02	0.16 ± 0.00
CSM 4.05	Human	4.08 ± 0.00	0.14 ± 0.00
CSM 4.11	Human	3.60 ± 0.03	0.18 ± 0.00
CSM 4.12	Human	3.81 ± 0.02	0.16 ± 0.00
CSM 4.24	Human	4.02 ± 0.02	0.15 ± 0.00
CSM 4.28	Human	3.56 ± 0.14	0.19 ± 0.00
CSM 4.38	Human	3.85 ± 0.06	0.17 ± 0.00
CSM 4.53	Human	3.88 ± 0.09	0.16 ± 0.00
CSM 5.05	Human	3.53 ± 0.05	0.19 ± 0.00
CSM 5.07	Human	3.91 ± 0.05	0.14 ± 0.00
CSM 5.09	Human	3.60 ± 0.01	0.18 ± 0.00
CSM 5.16	Human	3.80 ± 0.02	0.18 ± 0.00
CSM 5.23	Human	3.62 ± 0.04	0.17 ± 0.00

Sample	*Human/Animal*	*IRSF (Mean ± σ)*	*C/P (Mean ± σ)*
CSM 5.36	Human	3.72 ± 0.02	0.15 ± 0.00
CSM 61.03	Human	3.30 ± 0.02	0.25 ± 0.00
CSM 61.04	Human	3.81 ± 0.04	0.18 ± 0.00
CSM 62.02	Human	3.86 ± 0.04	0.16 ± 0.00
CSM 7	Human	3.42 ± 0.04	0.21 ± 0.00
CSM 12	Human	3.87 ± 0.00	0.16 ± 0.00
CSM 27	Human	3.91 ± 0.03	0.16 ± 0.00
CSM 37	Human	3.50 ± 0.03	0.21 ± 0.00
CSM 41	Human	3.80 ± 0.01	0.18 ± 0.00
RLH 103	Human	3.77 ± 0.01	0.17 ± 0.00
RLH 135	Human	3.44 ± 0.01	0.24 ± 0.00
RLH 208	Human	3.66 ± 0.02	0.20 ± 0.00
RLH 340	Human	3.76 ± 0.02	0.14 ± 0.00
RLH 349	Human	3.65 ± 0.02	0.17 ± 0.00
RLH 356	Human	3.64 ± 0.02	0.17 ± 0.00
RLH 367	Human	3.59 ± 0.02	0.19 ± 0.00
RLH 386	Human	3.51 ± 0.02	0.19 ± 0.00
RLH 397	Human	3.47 ± 0.02	0.20 ± 0.00
RLH 421	Human	3.52 ± 0.02	0.24 ± 0.00
RLH 572	Human	3.48 ± 0.02	0.24 ± 0.00
SBL 1203	Human	3.57 ± 0.03	0.21 ± 0.00
SBL 1207	Human	3.50 ± 0.03	0.21 ± 0.00
SBL 1215	Human	3.76 ± 0.03	0.17 ± 0.00
SBL 1244.1	Human	4.02 ± 0.03	0.13 ± 0.00
SBL 1526	Human	3.65 ± 0.02	0.19 ± 0.00
SBL 1558	Human	3.67 ± 0.03	0.18 ± 0.00
SBL 1641	Human	3.67 ± 0.04	0.16 ± 0.00
SBL 1653	Human	3.64 ± 0.04	0.19 ± 0.00
SBL 1785	Human	3.59 ± 0.04	0.19 ± 0.00
SBL 1799	Human	3.73 ± 0.04	0.17 ± 0.00
SBL 1872	Human	3.52 ± 0.04	0.24 ± 0.00
SBL 1932	Human	3.68 ± 0.03	0.19 ± 0.00
SBL 2049	Human	3.60 ± 0.04	0.19 ± 0.00
SBL 2134	Human	3.71 ± 0.04	0.17 ± 0.00
SBL 2296	Human	3.61 ± 0.04	0.19 ± 0.00
CSM 2056	Animal	3.77 ± 0.03	0.14 ± 0.00
CSM 2061	Animal	3.68 ± 0.03	0.17 ± 0.00
CSM 3006	Animal	3.66 ± 0.02	0.18 ± 0.00
CSM 3008	Animal	3.76 ± 0.02	0.17 ± 0.00
CSM 4050	Animal	3.87 ± 0.04	0.13 ± 0.00
CSM 4054	Animal	3.68 ± 0.02	0.16 ± 0.00
CSM 4055	Animal	3.70 ± 0.02	0.16 ± 0.00
CSM 4062	Animal	3.52 ± 0.02	0.18 ± 0.00
CSM 4238	Animal	3.69 ± 0.01	0.17 ± 0.00
CSM 5030	Animal	3.97 ± 0.03	0.12 ± 0.00
CSM 6134	Animal	3.70 ± 0.03	0.16 ± 0.00
CSM 6323	Animal	3.73 ± 0.03	0.15 ± 0.00

Sample	*Human/Animal*	*IRSF (Mean ± σ)*	*C/P (Mean ± σ)*
GPMO 037-	Animal	3.54 ± 0.01	0.19 ± 0.00
GPMO 159-	Animal	3.50 ± 0.02	0.19 ± 0.00
GPMO U/SC 3	Animal	3.81 ± 0.05	0.14 ± 0.00
NPSS 10	Animal	3.69 ± 0.02	0.17 ± 0.00
NPSS 11	Animal	3.88 ± 0.01	0.13 ± 0.00
NPSS 14	Animal	3.69 ± 0.02	0.16 ± 0.00
NPSS 15	Animal	3.53 ± 0.03	0.18 ± 0.00
NPSS 16	Animal	3.70 ± 0.03	0.17 ± 0.00
NPSS 17	Animal	3.71 ± 0.02	0.15 ± 0.00
SQC 1012-1	Animal	3.70 ± 0.02	0.15 ± 0.00
SQC 1012-2	Animal	3.87 ± 0.02	0.15 ± 0.00
SQC 1012-3	Animal	3.66 ± 0.01	0.18 ± 0.00
SQC 1012-4	Animal	3.75 ± 0.01	0.17 ± 0.00
SQC 1012	Animal	3.69 ± 0.03	0.17 ± 0.00
SQC 1250	Animal	3.69 ± 0.03	0.16 ± 0.00

Appendix D

D.1 Tests of normality for all isotope data

H_0: The sample comes from a normal distribution
H_1: The sample does not come from a normal distribution

- Where P-value is smaller than 0.05, the null hypothesis is rejected
- Some sites do not come from a normal population so nonparametric tests will be used for comparisons (see results below)

Isotopes	Sites	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
$\delta^{13}C_{coll}$ (‰)	CSM	.086	54	.200*	.979	54	.446
	HGM	.127	31	.200*	.934	31	.057
	FEW	.203	7	.200*	.940	7	.638
	SCH	.104	32	.200*	.976	32	.691
	SGC	.238	9	.149	.838	9	.055
	VGL	.253	3	.	.964	3	.637
	ROM	.115	21	.200*	.977	21	.872
	QCS	.466	10	.000	.449	10	.000

Isotopes	Sites	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
$\delta^{15}N$ (‰)	SBK	.162	23	.122	.937	23	.157
	RLH	.197	11	.200*	.946	11	.596
	SBL	.241	15	.019	.799	15	.004
	CSM	.100	54	.200*	.950	54	.026
	HGM	.137	31	.148	.892	31	.004
	FEW	.274	7	.121	.824	7	.070
	SQC	.178	32	.011	.896	32	.005
	STGC	.138	9	.200*	.924	9	.431
	VGL	.385	3	.	.750	3	.000
	ROM	.139	21	.200*	.953	21	.387
	QCS	.188	10	.200*	.972	10	.908
	SBK	.122	23	.200*	.955	23	.370
	RLH	.118	11	.200*	.982	11	.977
	SBL	.125	15	.200*	.970	15	.863

Isotopes	Sites	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
$\delta^{13}C_{carb}$ (‰)	CSM	.100	54	.200*	.969	54	.182
	HGM	.102	31	.200*	.975	31	.659
	FEW	.184	7	.200*	.944	7	.679
	SQC	.114	32	.200*	.953	32	.173
	STGC	.130	9	.200*	.969	9	.889
	VGL	.321	3	.	.881	3	.328
	ROM	.091	21	.200*	.972	21	.787
	QCS	.240	10	.109	.745	10	.003
	SBK	.185	23	.040	.839	23	.002
	RLH	.136	11	.200*	.947	11	.604
	SBL	.159	15	.200*	.936	15	.340

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

D.2 Tests of normality for all FTIR data

FTIR Parameter	Sites	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
IRSF	CSM	.083	55	.200*	.975	55	.296
	HGM	.110	32	.200*	.978	32	.752
	FEW	.147	7	.200*	.964	7	.851
	SQC	.146	32	.082	.930	32	.038
	SGC	.288	9	.030	.762	9	.008
	VGL	.287	3	.	.929	3	.485
	ROM	.124	19	.200*	.971	19	.802
	QCS	.170	10	.200*	.964	10	.826
	SBK	.101	23	.200*	.947	23	.256
	RLH	.183	11	.200*	.924	11	.355
	SBL	.173	15	.200*	.859	15	.023
	Animals	.181	27	.024	.924	27	.049

FTIR Parameter	Sites	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
C/P ratio	CSM	.153	55	.002	.899	55	.000
	HGM	.158	32	.042	.922	32	.023
	FEW	.214	7	.200*	.893	7	.291
	SQC	.135	32	.146	.963	32	.323
	SGC	.317	9	.009	.729	9	.003
	VGL	.175	3	.	1.000	3	1.000
	ROM	.208	19	.029	.907	19	.066
	QCS	.255	10	.064	.869	10	.097
	SBK	.154	23	.164	.958	23	.422
	RLH	.182	11	.200*	.904	11	.204
	SBL	0.226	15	0.038	0.934	15	0.308
	Animals	0.168	27	0.048	0.949	27	0.207

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

D.3 Post hoc results for the northern and London sites.

The significance level is 0 .05 and the significance values have been adjusted by the Holm's Sequential Bonferroni correction for multiple tests. *Posthoc analysis showing significant differences between sites

Sample 1-Sample 2	$\delta^{13}C_{coll}$	$\delta^{15}N_{coll}$	$\delta^{13}C_{carb}$
HGM-VGL	1.000	0.705	1.000
HGM-FEW	1.000	0.663	1.000
HGM-SQC*	0.021	0.000	0.093
HGM-ROM*	0.011	0.069	1.000
HGM-CSM*	0.000	0.000	1.000
HGM-SGC*	0.035	0.004	1.000
VGL-FEW	1.000	1.000	1.000
VGL-SQC	1.000	1.000	1.000
VGL-ROM	1.000	1.000	1.000
VGL-CSM	1.000	1.000	1.000
VGL-SGC	1.000	1.000	1.000
FEW-SQC	1.000	1.000	1.000
FEW-ROM	1.000	1.000	1.000
FEW-CSM	1.000	1.000	1.000
FEW-SGC	1.000	1.000	1.000
SQC-ROM*	1.000	1.000	0.005
SQC-CSM	1.000	1.000	1.000
SQC-SGC	1.000	1.000	1.000
ROM-CSM	1.000	1.000	0.250
ROM-SGC	1.000	1.000	0.804
CSM-SGC	1.000	1.000	1.000

Sample 1-Sample 2	$\delta^{13}C_{coll}$	$\delta^{15}N_{coll}$	$\delta^{13}C_{carb}$
SBL-RLH*	1.000	1.000	0.019
SBL-SBK	0.419	1.000	0.572
SBL-QCS*	0.010	1.000	1.000
RLH-SBK*	1.000	0.008	0.550
RLH-QCS	0.344	1.000	0.728
SBK-QCS*	0.436	0.014	1.000

Appendix E

E.1 Ethical Approval Documents

THE UNIVERSITY OF TENNESSEE KNOXVILLE

January 02, 2019

Amy Mundorff
UTK - College of Arts & Sciences - Anthropology

Re: UTK IRB-18-04888-XM
Study Title: Isotope analysis of human dental calculus carbonate: investigating a potential new proxy forsugar consumption.

Dear Amy Mundorff:
The Human Research Protections Program (HRPP) reviewed your application for the above referenced project and determined that your application is eligible for **exempt** review under 45 CFR 46.101(b) Category 4: Research involving the collection or study of existing data, documents, records, pathologicalspecimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded in a de-identified manner.

Your application has been determined to comply with proper consideration for the rights and welfare ofhuman subjects and the regulatory requirements for the protection of human subjects. This letter constitutes approval of your application v1.0 for the above referenced study.

In the event that volunteers are to be recruited using solicitation materials, such as brochures, posters,web-based advertisements, etc., these materials must receive prior approval of the IRB.

Any alterations (revisions) in the protocol or other approved materials must be promptly submitted to andapproved by the UTK Institutional Review Board prior to implementation of these revisions. You have individual responsibility for reporting to the Board in the event of unanticipated or serious adverse eventsand subject deaths.

Sincerely,

Colleen P. Gilrane

Colleen P. Gilrane, Ph.D.
Chair

Biology Ethics Committee
Department of Biology
University of York
Wentworth Way
York, YO10 5DD
Tel: 01904 328520
Email: biol-ethics@york.ac.uk
Website: https://www.york.ac.uk/biology/intranet/ethics/bec/

UNIVERSITY of York

Department of Biology Ethics Committee

Memorandum of Ethical Approval for Research

To: **Michelle Alexander, Blessing Chidimuro**
From: **Victoria Beale; Ethics Committee Senior Administrator**
Date: **11 January 2019**
Re: **Ethical Approval**
Title of study: **Isotope analysis of human dental calculus carbonate: investigating a potential new proxy for sugar consumption.**
Ethics Reference: **BC201812**

Dear Blessing and Michelle

I am pleased to inform you that the above research application has been reviewed by the Department of Biology Ethics Committee and I can confirm a favourable ethical opinion as of the date of this letter. The following documentation was considered:

Document	Version	Date
Chidimuro BC201812 BEC Human Tissue v2 (VB BC 7.12.18).docx	2	7.12.18
Body_Donation_Packet_2017 (PIS&consent).pdf	6:9_2017	
Alexander_York_Project Description.pdf		
Collections_Research_Request_Form_Speller.pdf		5.7.18
Request BOX 8.pdf		
Request_for_Destructive_Sampling_Form_Speller.pdf		15.7.18
Email explaining FAC Licencing.pdf (from Amy Mundorff to BC)		7.12.18
Email re FAC approval of AM's request.pdf (from Dawnie Steadman to VB)		3.1.19
IRB_Outcome_Letter re AM's application.pdf		2.1.19
NERC Outcome Letter June 2018.pdf		6.6.18

Please be aware that, before the study progresses, any other ethical permission(s) required must be in place (e.g. local and national REC). Should you require further confirmation of which, if any, other permissions are necessary please contact biol-ethics@york.ac.uk. You are expected to keep a record of all your approved documentation and other documents relating to the study, including any risk assessments.

Please notify the committee of any changes, including extensions to the study.

We welcome your suggestions for improvement of the ethical review process. Please email any comments to biol-ethics@york.ac.uk.

Yours sincerely,

Professor Dawn Coverley (Chair)
Department of Biology Ethics Committee, University of York

This is to certify that

Blessing Chidimuro

completed the following e-learning with an assessment (England, Wales and Northern Ireland) score of

80%

Research and human tissue legislation

Overview of Human Tissue Act 2004 and Human Tissue (Scotland) Act 2006

When the Acts apply

What constitutes best practice

Top tips to support compliance

Where to find help

November 26, 2018 MRC Regulatory Support Centre