Post-medieval Poverty: An Integrated Investigation

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Abstract

This study stemmed from our contested state of knowledge regarding under- and malnutrition in long-18th century England. The project aims to connect environment, nutrition and health, through the combined approach of osteological, biomolecular and historical research methods, and was motivated by three main research questions: (1) Is the potential scurvy biomarker identified during earlier work a true marker for scurvy in Human Skeletal Remains (HSR)? (2) Can we track potato consumption (a good source of Vitamin C) during this period through evidence of potato starch granules in human dental calculus? (3) Can we use a combination of HSR and historical documentary evidence to trace dietary and social change? A variety of different methods for extracting collagen from HSR were systematically tested, and a new technique has subsequently been established. This was applied to HSR from five post-medieval sites. These extractions - along with those of control samples - were run using MALDI-TOF-MS, and the resulting data analysed to a level of detail that has not previously been carried out, in the search for a scurvy biomarker. These analyses ruled out the potential biomarker, but revealed information that may help with the biomolecular identification of scurvy in the future. Dental calculus samples from individuals buried at one of the sites included here were analysed using light microscopy, but this element of the project was terminated as the data that could be produced was of limited use to the central research questions. Historical documentary evidence related to the sites included here has revealed the complexity of the factors influencing burial ground demographics. All five sites were identified by archaeologists as being linked to ‘poverty’, but this is an oversimplification when historical debate is taken into account. Throughout, this project evidences the benefits of a wider adoption of interdisciplinary approaches to historical research questions.

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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.

Chapter One: Introduction

The project detailed in this thesis aimed to investigate poverty in England during the long eighteenth century (c. 1660 - 1830), primarily through the consideration of diet, dietary deficiency, and under- and malnutrition during this period. An integrated approach combining archaeological and historical research techniques was employed, with a particular focus throughout on the dietary deficiency disease 'scurvy', and consumption of the potato.

Background to the Project

The work presented here stems from our contested state of knowledge regarding under- and malnutrition in England during the long eighteenth century – both analytically and in terms of chronic conditions and the nature of diet for the poor or impoverished.

We as archaeologists often say that we can identify poverty and that archaeology is made for poor people; while history deals with the rich, archaeology deals with the poor. There may be some truth to that, but there are inherent pros and cons to both archaeological and historical research, purely due to their nature.

The work presented in this project combines different disciplinary areas of research: namely archaeology - particularly bioarchaeology - and post-medieval history. The bioarchaeological elements of the project take us beyond demography, and beyond historical records. However, it could be argued that archaeology as a discipline has a traditional tendency to oversimplify things, which the historical elements of this project can help to readdress. By focusing on poverty from the angle of diet, dietary deficiency, and under- and malnutrition, we can bring together these

different lines of evidence and use this approach to answer questions that are of interest to both disciplines.

For example, scurvy – the name given to the disease that results from a prolonged period of Vitamin C deficiency in the diet - is a chronic condition that has been a focus of both archaeological and historical research, but which we actually know relatively little about. This condition can be particularly difficult to identify in human skeletal remains, as it causes no pathognomonic lesions. However, if an accurate, reliable method for identifying this disease in archaeological populations could be established, then this would enable us to connect individuals to their environment, and to explore the nutritional health of past populations.

For the purposes of this project, scurvy is presented as evidence of malnutrition. Malnutrition can be defined as a lack of the proper nutrition that our bodies require in order to function as they should, and as we will see in later chapters, a prolonged lack of Vitamin C in the diet prevents the human body from functioning properly. Furthermore, it is worth nothing that, as Vitamin C was not discovered until the 1930s, any absence of this from the diet of individuals living in England during the long-eighteenth century cannot have been an intentional choice.

This project was formulated in part on the basis that there is truth to the widely accepted theory that those living in poverty, or close to the poverty line, during our period of interest would have had a narrower field of choice with their diets than the wealthier members of society, and that this would subsequently have resulted in an unbalanced diet that was missing key nutritional elements, such as Vitamin C. In that sense, we can say that we are using scurvy as a proxy for poverty - however, this assumption will be considered in more detail in later chapters of this thesis.

The potato - well known as a staple of the British diet, particularly for the poor - is a surprisingly good source of Vitamin C. As such, their consumption could be useful in staving off scurvy. However, the tuber has not always been popular in England; when potatoes first arrived here, they were viewed with a sense of caution (to say the least), and it took a long time for public opinion to change. There is currently a gap in our knowledge of the potato's history in England - we know that they arrived here prior to the start of the long eighteenth century, and we know that they were a popular, commonly consumed food by the end of that period, but it is still unknown as to when they became widely accepted and people started willingly eating them regularly.

While issues of under- and malnutrition have been considered by both historians and archaeologists, this is arguably one of the first projects that has aimed from the outset to use an integrated approach in order to address questions that are of interest and importance to both disciplines.

Outline of the Project

Aims and Objectives

This project aimed to address the following research questions:

- Is the potential scurvy biomarker identified during a previous research project a true marker for scurvy in human skeletal remains?

- Can we track potato consumption (a good source of Vitamin C) during the 17th - 19th centuries through evidence of potato starch granules in human dental calculus?

- Can we use a combination of human skeletal remains and historical documentary evidence to trace dietary and social change?

An integrated approach combining science and the humanities was taken in order to answer these questions; a combination of osteological and biomolecular research data was combined with documentary historical research in order to analyse, understand and contextualise evidence pertaining to under- and malnutrition in human skeletal remains from five post-medieval archaeological sites in England.

Key goals of the project, related to the research questions just outlined, were:

- To connect environment, nutrition and health, through the combined approach of osteological, biomolecular and historical research methods.

- To attempt to identify under- and malnutrition in 17th-19th century skeletal collections using this combined analytical approach.

- To systematically test existing methods for isolating collagen peptides from archaeological bone, and to develop and test potential new methods for this purpose, ultimately with the aim of identifying the best minimally destructive method

- To test a low-cost, minimally-destructive method for the biomolecular identification of scurvy in human skeletal remains, which will further our understanding of the biological processes behind how scurvy affects collagen

- To use the potential scurvy biomarker to take our understanding of the nutritional status of people living in England during the 17th-19th centuries beyond what documentary sources tell us

- To research the history of the areas in which the different skeletal populations to be analysed lived, in order to attempt to explain the osteological and biomolecular findings, and to provide a social, political and cultural context for the research.

The Relevance and Importance of the Project

As has been mentioned, scurvy can be particularly difficult to identify from human skeletal remains, which will inevitably mean that this condition is currently underrepresented in the archaeological record. What we mean by the word 'scurvy' today is also challenging to trace in historical records with any certainty, due to the fact that the meaning and use of language can change over time. However, being able to accurately identify cases of scurvy in the past is important in order for the true scale of its influence to be established. There are two notable quotes of particular relevance to this, the first by Stone and the second by Carpenter:

"*In the long period of human prehistory and history, scurvy has caused more deaths, created more human misery and has altered the course of history more than any other single cause*"
(Stone 1966, p.345)

"*If we exclude straightforward famine, scurvy is probably the nutritional deficiency disease that has caused most suffering in recorded history*"
(Carpenter 1988, p.vii)

Each of these serves to highlight the importance of being able to accurately identify scurvy in the archaeological and historical records. If these quotes are based in truth, then we are definitely not seeing this accurately reflected in human skeletal remains. However, if it is possible to establish an accurate, reliable, low-cost technique for identifying scurvy from archaeological human bone then this would completely

change our understanding of this disease and would enable us to start building a true picture of the scale of malnutrition in the past.

While scurvy is generally thought of as an ancient disease, it has been making news headlines in recent years, with a number of medical experts linking this to a rise in food poverty (Taylor-Robinson et al. 2013, p.e.g.; Anon 2017a; Anon 2017b). If we can gain a greater understanding of under- and malnutrition in the past, and the potential links that this had with poverty, then we can apply this knowledge to modern day issues regarding food supply and health.

Thesis Structure

The main body of this thesis will begin with Chapter Two, in which we will explore the history and current status of bioarchaeological collection extraction techniques. A large part of this project focuses on extracting collagen from human skeletal remains in order to establish and analyse potential evidence of a biological marker for the presence or absence of scurvy. Subsequently, it is important to have a clear understanding of the work that has been done to-date, and the successes and limitations of the methods currently being employed. We will then build on this with Chapter Three, in which the most commonly used collagen extraction techniques were tested against a number of new methods, with the aim of establishing the 'best' option for collagen extraction, which was then applied to the individuals sampled from the five sites analysed as part of this project. The current collagen extraction techniques that are used routinely in archaeological research have never been systematically tested against each other, to establish their different effects on collagen yield, and so this chapter will detail work not only of key importance to this project, but also to the wider field of archaeological proteomics.

Having discussed the existing status of collagen extraction within archaeological research, and having identified the most appropriate methods to be used here, Chapters Four and Five turn our attention to the research contexts that frame the project aims and objectives. In Chapter Four the histories and scurvy and diet in long eighteenth century England will be discussed. Chapter Five then considers each of the five sites included in this project; St Thomas's Hospital, Farringdon St Bride's Lower, Cross Bones, the Bow Baptists, and Priory Yard. The first four of these sites are today located in London (although the village of Bow was considered to be located in Middlesex during our period of interest, being incorporated into London much later), with Priory Yard being located in Norwich.

With a greater understanding of the social, political and economic landscape in which the individuals sampled for this project were living, Chapter Six goes on to discuss the various steps taken in the search for a scurvy biomarker. This is the central chapter of the thesis, detailing the systematic analysis work that was undertaken both to meet the aim of testing the potential scurvy biomarker previously identified, and also to establish whether any other accurate, reliable markers could be found.

Chapter Seven provides a research context for the use of light microscopy in studies of archaeological dental calculus samples. The methodological information gathered and presented in this chapter is then applied in Chapter Eight, whereby dental calculus samples from individuals excavated from the Farringdon St Bride's Lower site were analysed. The main aim of studying these samples using light microscopy was to search for evidence of potato starch, in line with the research question regarding the tracking of potato consumption during the long eighteenth century.

We will end with a consideration of the findings presented in each chapter, and how that relates to the aims, objectives and research questions for the project. Limitations of the project and potential directions for future work will also be discussed.

Chapter Two: Research Context - Methods of Extraction

Bone consists of two components - the organic, collagen, and the inorganic, mineral. In a living human, the organic component (with water) accounts for roughly 30%, with the inorganic component making up the rest (Boskey 2013, 2). Here we are most interested in collagen, as it can provide us with a wealth of information about an individual and the life they once led.

With that in mind, a key part of this project was extracting collagen from human skeletal remains in order to analyse that collagen and to attempt to establish the presence or absence of scurvy in the individuals that it was taken from. Before discussing the work that was done as part of this project to test and develop collagen extraction techniques, or to search for a scurvy biomarker, this chapter will detail and discuss the structure of collagen, the role of Vitamin C in the production of healthy collagen, and bone turnover rates of collagen in humans. The existing methods used for collagen extraction in archaeological research, and likely directions for future work, will also be explored.

The Structure of Collagen

As a first step, we need to understand the structure of collagen - specifically that of Type I collagen, as that is the most abundant type found in humans, being present in bones, organs, skin and tendons.

One of 28 known types, Type I collagen fibrils are made up of three polypeptide chains - two α_1(I) chains and one α_2(I) chain (see Figure 1). Each individual chain assumes a left-handed helical conformation, with three residues per turn, and the three then wind around each other with

a right-handed twist, forming the triple helix, termed ‘tropocollagen’ (Currey 2002, 5; Voet et al. 2012, 138; Whitford 2013, 94).

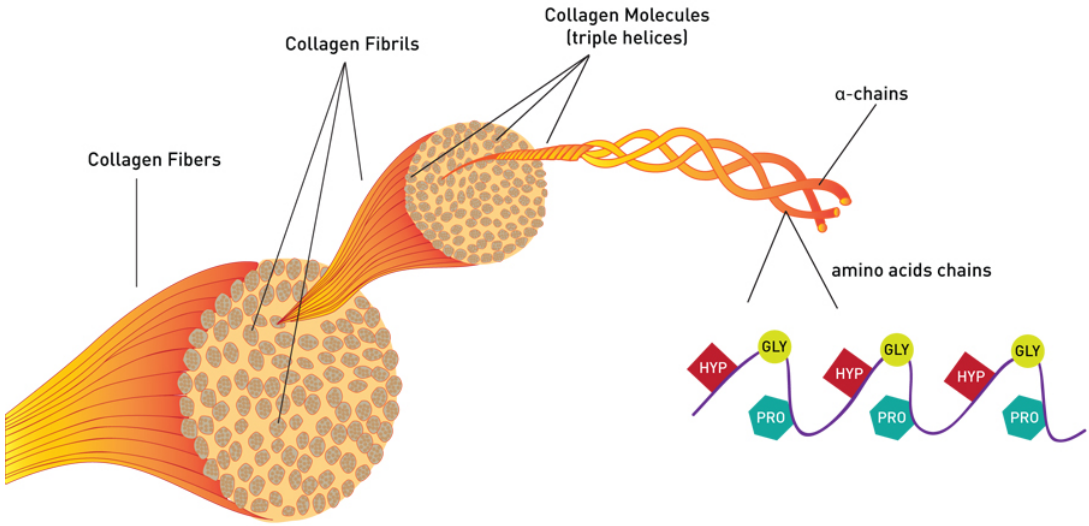


Figure 1: The structure of Type I collagen (proto-col 2014)

Proteins can undergo a process termed ‘post-translational modification’, in which they are modified after being synthesised. One of these changes - and the one most relevant to us here - is the hydroxylation of proline and lysine to hydroxyproline and hydroxylysine, respectively. Hydroxylation involves the replacement of a hydrogen atom with a hydroxyl group on the side chain of proline. These hydroxyl groups are also involved in forming hydrogen bonds between polypeptide chains, connecting the NH groups of Glycine residues with the CO groups of other residues in other chains, which further stablises the collagen structure (Kawahara et al. 2005, 15812; Berg et al. 2012, 44; Whitford 2013, 95 & 289). If an individual is suffering from scurvy then they logically do not have these hydroxyl groups (please see subsection ‘The Role of Vitamin C in Collagen Synthesis and Proline Hydroxylation’, later in this chapter) and subsequently will produce poor quality collagen, due to those bonds not being created (Berg et al. 2012, 44). However, it should be noted that there remains controversy over the precise mechanism of stabilisation (Nishi et al. 2005, 6041).

The configuration of amino acids - the building blocks of peptides and subsequently, proteins - along the polypeptide chains is very regimented, with Glycine appearing at every third position, followed by an X-position amino acid (regularly proline) and then a Y-position amino acid (often hydroxyproline). This distribution of prolines in the X- or Y-position, but hydroxyprolines only ever in the Y-position has been widely accepted as fact (e.g. Park et al. 2005, 1612; Krane 2008, 704; Wu et al. 2011, 1054; Chow et al. 2015, 1). However, Weis and colleagues (2010, 2583), Eyre and colleagues (2011, 7733), and Yang and colleagues (2012, 40598), have all reported findings that showed hydroxyprolines occurring in the X-position as well as in the Y-position - this is something that we will revisit in Chapter Four of this thesis. Thinking again about the G-X-Y structure of collagen, it is necessary to have a Glycine in every third position as it is the only amino acid small enough to fit into the centre of the triple helical structure just described (Voet et al. 2012, p138–9; Whitford 2013, 95). Both proline and hydroxyproline are much larger than Glycine, and far more inflexible. They therefore need to sit on the outside of the triple helix. Their large, inflexible nature, however, helps to contribute to the rigidity of the tropocollagen structure (Berg et al. 2012, 44; Voet et al. 2012, 139; Whitford 2013, 95).

Different researchers have published different estimates regarding the percentage of tropocollagen that proline and hydroxyproline make up. For example, Voet and colleagues (2012, 138) state that somewhere between 15 and 30% of the polypeptide chains in Type I collagen are accounted for by these two imino acids, while Brodsky and Ramshaw (1997, 546) suggest that they account for around 20%. To date, there has been no consensus reached on the actual percentage of proline and hydroxyproline present in the human Type I collagen triple helix. This has a knock-on effect for any biomolecular study of scurvy. This is because the presence or absence of hydroxyproline is believed to be indicative of

the absence or presence of scurvy, respectively. Therefore it is necessary to know how much hydroxyproline should be present in healthy human collagen, before a lack of it can be accurately identified; if we don't know what the 'right' amount is, then we can't know what too little is, and subsequently can't identify scurvy.

The Role of Vitamin C in Collagen Synthesis and Proline Hydroxylation

Having considered the basics of the structure of collagen, we will now look at this in more depth, specifically focusing on the role that Vitamin C plays in collagen synthesis and proline hydroxylation.

Vitamin C – also termed 'ascorbic acid' – is of key interest to this project at least in part because of its vital involvement in the formation of healthy collagen; without it, the human body produces collagen that is unstable, and it is that which eventually leads to the presentation of visible symptoms of scurvy in living humans (these are described in Chapter Four).

In order for a collagen triple-helix to have the appropriate tensile strength, hydroxyproline and hydroxylysine are required – these create the intermolecular cross-links that stabilise the structure (Murad et al. 1981, 2879; Montgomery et al. 2012, 5900). As was mentioned briefly earlier in this chapter, hydroxyproline and hydroxylysine are produced from the hydroxylation of the imino acids proline and lysine. This post-translational modification reaction requires the presence of a particular enzyme – namely, hydroxylase - and a particular co-factor (a co-factor being a non-protein chemical that enables a biochemical reaction) – in this case, ascorbic acid. Without the presence of ascorbic acid to fulfil the co-factor role, the hydroxylase enzyme cannot perform the reaction that adds the hydroxyl group to the particular prolines and lysines, and subsequently

the triple-helix will be missing the intermolecular cross-links that give it its rigid structure.

We can see, therefore, that the presence of Vitamin C in the diet is crucial for humans to be able to synthesise healthy collagen.

Unfortunately there would appear to be nothing in the literature published to date that defines at what point the laying down of new bone stops as the result of scurvy. Individuals living in the past could have experienced varying levels of Vitamin C deficiency (both within their own experience and in comparison to others). However defining those levels, or putting them into categories, would be somewhat subjective - particularly as we still do not know at what point this deficiency becomes visible in bone collagen, and so we would have no concrete basis for defining different levels of deficiency.

We need to understand the way in which scurvy affects human collagen before we can confidently establish when this might be observable, and the work that will be discussed in Chapter Six hopes to take us closer to that point.

Bone Turnover Rates of Collagen

Having established the basic structure of stable collagen, along with the role of Vitamin C in its production, we will now consider the rate at which collagen in human bones is replaced throughout life. This is termed the ‘bone turnover rate’. Traditionally within archaeological science work, this information has been of primary importance for radiocarbon dating, but it can also be potentially useful when considering a biomolecular investigation of pathology, as is the case here. By knowing how long it takes bone collagen to completely turnover, we may be able to establish

the period of an individual's life that we are looking at when we study collagen extracted from their skeleton. However, it is important to note that turnover rates are affected by a variety of factors, such as age, sex and skeletal element.

In their investigations of the effects of age on turnover rates, Hedges and colleagues (2007, 814) and Geyh (2001, 727) concluded that this rate declines with age. Hedges and colleagues detail that they used only femoral mid-shaft samples for their study, with the results revealing that adolescents displayed a 5-15% turnover rate between the ages of 10 and 15, whereas the average adult turnover rate in the same bone was only 1.5-4% (Hedges et al. 2007, 814). This result is perhaps unsurprising, as adolescent skeletons are still growing and developing, unlike fully formed adult skeletons. Geyh (2001, 727) also observed this finding, however the specific skeletal element(s) tested was not noted at publication and so this limits any useful application of this result.

In terms of differences that are influenced by biological sex, Hedges et al.'s 2007 study that looked at femoral collagen turnover compared adult males to adult females, and found that males displayed a fairly consistent decline from 3% down to 1.5% between the ages of 25 and 80, whereas females displayed a steady decline from 4% down to 3% over a similar period (Hedges et al. 2007, 814). This suggests that the average adult female turnover rate is always higher than the average observed in adult males. However, before any significance in relation to sex can be reliably determined, data from a wider range of skeletal elements would need to be gathered.

Overall, though, these findings on the influences of age and sex are important contextual information to be aware of when comparing collagen data extracted from subadults to adults, and adult males to adult females, as it would appear that the effects on turnover rates caused by both age

and sex differences could mean that different life history representations are being observed. This could potentially affect the validity of comparing data for different individuals. However, it has also been noted that considerable variation is observed between individuals that is not attributable to age or sex (e.g. Hedges et al. 2007, 815). With this in mind, we should therefore show caution when attempting to draw conclusions on bone turnover rates and the subsequent life period that is being observed.

In regards to bone collagen turnover rates differing between skeletal elements, the generally accepted theory is that larger, denser bones – such as the femur – will take longer to completely turnover than smaller, less dense bones – such as the ribs. Much of the work that has been published on collagen turnover rates to date appears to compare bone with soft tissue, but for most archaeological purposes – and certainly for the purposes of this project – knowledge of collagen turnover rates for different skeletal elements would be a more valuable comparison. Ubelaker and colleagues did attempt to compare two different skeletal elements – the femur and lumbar vertebrae – from the same individuals in their study, the results of which conformed to the aforementioned theory that larger, denser bones take longer to turnover than smaller, less dense ones (Ubelaker et al. 2006, 485 – 486). However, there were only two individuals included in this study, and so ideally this would need to be repeated with a greater number of individuals in order to test the validity of the results.

When taking into account the potential variability in turnover rates caused by age, sex and skeletal element, along with the fact that there is variance between individuals anyway, this picture becomes particularly complex and it becomes apparent that there is unfortunately no uniform set rate for bone collagen turnover in humans (Matsubayashi & Tayasu 2019, 37). An awareness of the fact that collagen extracted from one individual

will not be directly comparable to another individual in terms of life history representation enables us to more fully appreciate the complete picture of our investigations. However, it is important to note that in relation to this project, the aim was primarily to establish whether a potential biomarker for scurvy could be successfully identified. It would unfortunately be beyond the scope of this project to also accurately establish the different life history representations of the individuals analysed and account for the differences between them.

The archaeological human bone samples collected as part of this project were all rib samples. In terms of collagen turnover rate, ribs most likely provide a narrower life history representation than a denser bone, such as the femur: in this sense, it may appear to make the most sense to choose to look for biomolecular evidence of pathology in a bone that provides the widest life history representation. However, it was decided to focus on rib bones for two reasons: first, the theory that ribs having a higher turnover rate may mean that any changes in the collagen due to scurvy may be visible sooner, and second, the practical reason that rib bones are more accessible for sampling than femora.

A Brief History of Collagen Extraction from Archaeological Bone

Collagen is extracted from archaeological bone - both human and animal - in order to enable us to learn about the lives of past individuals and populations. One of the most frequently employed biomolecular techniques involving collagen extraction is stable isotope analysis, which is most regularly used to gain insight into the diets of past populations. However, stable isotope data would not have been suitable for this project as the dietary information that it provides is particularly vague; it gives only an overview of the food groups consumed, and so could not have contributed meaningfully to answering the questions that are being

asked here. However, collagen extracted for stable isotope analysis can also be used successfully for proteomic work. This is the area of analysis that we will primarily focus on here, as it is most relevant to this particular project. Proteomics is the term given to the study of proteins, with research usually focusing on rates of protein degradation or the ways in which proteins are modified (e.g. post-translational modifications, as discussed above).

A widely employed form of proteomic analysis in archaeological research is known as Zooarchaeology by Mass Spectrometry (ZooMS). This technique was first introduced in 2009 (Buckley et al. 2009), and enables the biomolecular determination of species from archaeological bone samples. ZooMS is based on the premise that different species will have different amino acid sequences making up their Type I collagen (Collins et al. 2010, 8). Amino acid sequences are generally established by running collagen from a known species sample, enzyme-digested into peptides, through liquid-chromatography mass spectrometry (LC-MS/MS) (Ewles & Goodwin 2011), which will then break down the peptides to reveal the individual amino acids that make up those peptides. Different amino acids have different masses, so the combination of different amino acids forming different peptides will give those peptides particular masses. If there are peptide mass differences between species when looking at the same point on the collagen sequence, then this can be identified using a cheaper, faster mass spectrometry technique than LC-MS/MS, usually matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). This technique is much less detailed than LC-MS/MS in that it only involves the enzyme-digested collagen peptides, recording the masses of those but not then going on to break the peptides down into the individual amino acids.

Knowing what the sequences and peptide masses are for different species, and knowing where to look for the differences, enables us to determine the species when it cannot be established using more traditional methods, such as zooarchaeological analyses, or when there are too many samples for traditional zooarchaeology to be time- and/or cost-effective. Perhaps the most well-known application of the ZooMS method is its use in distinguishing between archaeological sheep and goats (e.g. Buckley et al. 2010; Martins et al. 2015), which are almost identical in their osteology.

Both a destructive and a non-destructive method for extracting collagen from bone samples as part of the ZooMS process have been published (Buckley et al. 2010; van Doorn et al. 2011). The destructive method involves the use of acid to demineralise the bone sample, removing the mineral component and leaving behind only the collagen. The non-destructive method involves the exploitation of natural degradation processes that have already taken place, enabling the gentle removal of collagen that is no longer being held rigidly in the triple-helix, but which is often of good enough quality to enable a species identification to be made. It should be noted that the destructive method is much more commonly used, as it is believed to give clearer results. This idea will be discussed in more detail in a later chapter and is important in relation to the method development aspect of this project.

This technique has recently been further modified and applied to parchment (as parchment is made from animal skin, itself essentially pure collagen, Fiddyment et al. 2015). The modifications led to the development of a non-invasive method for the extraction and analysis of archaeological collagen. However, when tested on archaeological bone and ivory samples in localised lab experiments it was found that the technique was not as successful as it is on parchment (Coutu et al. 2016).

Despite the fact that these methods for collagen extraction from archaeological samples have all been published – some more than a decade ago - and are now widely used within the discipline, there has to date been no published systematic testing of these different variations. As part of this project, it was decided that systematic testing of existing methods, along with a number of variations on these, should be undertaken, in order to understand the capabilities of the different protocols and ultimately to search for the 'best' method.

In later chapters, this systematic testing and its findings will be detailed, and we will then consider how a proposed variation on the existing extraction methods can be used in order to attempt to trace a biomarker for scurvy in humans, based on hydroxylation of the imino acid proline.

The Future of Collagen Extraction from Archaeological Bone

In recent years, museums, curators and collections managers have been calling for the development and implementation of non-destructive techniques in bioarchaeological research, particularly in work that involves human skeletal remains. This can be linked in part to the change in the way that burial law is applied to archaeological human remains. Between 2008 and 2011, all human skeletal remains excavated from archaeological sites had to be reburied within two years (with permission generally given for sub-samples to be taken for biomolecular analysis). From February 2011, the legislation was offered more flexibly, with extensions on the standard two-year period being granted. In 2012 licences were introduced that enable archaeological human skeletal remains to be kept out of the ground indefinitely.

A move to non-destructive techniques would have many benefits. For example, biomolecular research would no longer have any negative effect on traditional osteological analyses, as the bones would remain whole. Currently, museum and collection curators have to be careful to oversee any sampling of human skeletal remains, to ensure that key areas of bones, such as the medial ends of ribs, which can be used for osteological ageing, are not damaged or removed. This is something that they very often do not have time for, which can in turn result in the refusal of requests for sampling.

Moreover, as biomolecular techniques are refined and developed, they tend to require less and less sample material in order to obtain data. For example, radiocarbon dating of archaeological bone required up to 5g of bone in the 1980s, but can now be undertaken with no more than 1g, often much less (Gillespie et al. 1984, 167; Brock et al. 2010, 106). In bypassing the need for destructive sampling, we avoid the possibility of a finite resource being wasted by 'too much' being taken for biomolecular work. There are much-debated ethical issues surrounding the sampling and destructive analysis of human skeletal remains (as discussed in Mays et al. 2013, 4–5; Zuckerman, Kamnikar, et al. 2014, 514); the development of successful non-destructive techniques would also mean that these issues could largely be avoided, with the bones remaining intact but new information still being learned.

There are still a number of biomolecular techniques that are not - and likely will not be, for the foreseeable future, if ever - able to be carried out non-destructively. For example, stable isotope analysis requires such a large quantity of protein in comparison to proteomics that, even if mass spectrometry technology was refined, it may always remain impossible to extract enough using non-destructive techniques. In addition, ancient DNA analysis - especially involving humans - is so susceptible to contamination that the best way to avoid this is to remove the top layers

of sample to reach the protected, uncontaminated layers. Rohland and colleagues have proposed a non-destructive technique for the extraction of mitochondrial DNA, but admit that this was not successful with nuclear DNA (Rohland et al. 2004, 818–9). Ultimately, if *some* non-destructive techniques can be developed and implemented, such as that which is an aim of this project, then at the very least this will decrease the number of samples being taken from archaeological human skeletal remains, and will help to protect and preserve them for the future.

Collagen Extraction in Relation to the Study of Scurvy

When attempting to study pathological differences in the collagen sequence, it is crucial to establish whether or not the extraction method being used will cause any changes. If there is the possibility that it might, then this will affect the ability to reliably use the resulting data to say anything about the effects of the disease in question. While we will not discuss *how* scurvy affects human collagen here - that will be discussed in some detail in a later chapter - it is important to be aware that severe Vitamin C deficiency causes the body to produce defective collagen. Therefore, in aiming to use a collagen extraction technique in order to look for the presence or absence of scurvy, we have to be sure that the data being analysed has not been affected by that technique in such a way that could be misleading.

A further consideration of potential importance here is the effect of taphonomic processes on bone collagen survival in the archaeological record, and the subsequent influence that they may have had on the amount of collagen still available for extraction, including which areas of the collagen chain may or may not be present. Taphonomy is a complex area of study, and unfortunately the many different variables that can affect the skeleton between the point of burial and the point of analysis

do not affect all bones, or all individuals, in the same way. In that sense it is unknown exactly what specific effects taphonomic processes have on collagen survival rates. The extraction protocol developed as part of this project could have been designed to include a step that establishes how much collagen has been extracted, but this would have slowed down the overall process and would have increased the cost, and ultimately wouldn't have told us which collagen fraction had been extracted – we needed the MS analysis to reveal that – and so it was deemed to be unnecessary, particularly in relation to the aim of developing a fast, cheap technique for extraction.

To date, most publications regarding scurvy in the archaeological record have made little mention of the biomolecular aspects of the disease, instead focusing almost entirely on the proposed osteological evidence (e.g. Ortner & Putschar 1985; Stark 2014; Zuckerman, Garofalo, et al. 2014; Brickley et al. 2016; Moore & Koon 2017). There are some notable exceptions, however. For instance, Travis (2008, 368–9) details a talk given by Hannah Koon at the Third International Symposium on Biomolecular Archaeology in 2008, where she outlined the potential for studying the hydroxylation of proline and lysine to determine whether an individual had been suffering from scurvy. More recently, this idea was discussed again by Pendery and Koon (2013, 63), by Armelagos and colleagues (2014, 10), and also by Crandall and Klaus (2014, 5). In all of these cases it was acknowledged that a biomolecular method for the identification of scurvy would be incredibly useful in enabling reliable study of the disease, but it was also evident that being able to routinely apply such a technique was still some way off.

Discussion & Conclusions

The unique structure of Type I collagen and its high level of tensile strength have direct links with the Vitamin C deficiency disease scurvy; as will be discussed in later chapters, when Vitamin C is removed from the human diet for a long enough period of time, it makes it impossible for this high level of tensile strength to be maintained, subsequently leading to a gradual breakdown of the body. However, we still at present do not know why it is that scurvy leads to such a remarkable destabilisation of collagen-based tissues.

Knowing that there is a link between the lack of Vitamin C in a human diet and the breakdown of Type I collagen makes it possible to study archaeological Type I collagen to investigate the incidence of scurvy in past individuals and populations, particularly given collagen's ability to survive well in the ground under most circumstances.

We have seen that there are a number of successful techniques already in existence for the extraction of collagen from archaeological bone. However, methods such as ZooMS only look at nine peptides when in reality there are over 700 that could be observed. Depending on which of those is/are the peptide(s) that are relevant to and necessary for a diagnosis of scurvy, it is possible that the currently employed techniques might not be capable of extracting this data. It is even possible - as we will go on to discuss in later chapters - that the existing extraction methods may be destroying some parts of the collagen sequence.

To date, there is one known published work addressing the concept of analysing collagen through proteomic work for palaeopathological purposes – the chapter by Pendery and Koon (2013) – however this does not consider the potential affects that the collagen extraction technique used may have on the resulting collagen yield, and so these existing

methods needed to be tested (to establish whether they would negatively affect any resulting data) and further developed, to ensure that the best possible method, producing the largest amount of reliable, usable data, was employed as part of this project.

The ways in which collagen-based research within archaeology has been carried out has changed and developed since its inception, partly due to scientific advancements, such as improvements in mass spectrometry, and partly as a result of changes influenced by the discipline itself, such as the changes regarding burial laws in the UK. Logically it would seem that future method development work within bioarchaeology should focus on developing non-destructive methods. Proteomics is a good candidate for this: in the next two chapters we will look at systematically testing existing methods, as well as modifying and developing them, with the aim of establishing an effective non-destructive option.

Chapter Three: Systematic Method Development

This chapter addresses one of the central objectives of this project, which has already been discussed in Chapter Two; systematic testing of existing methods for isolating collagen peptides from archaeological bone, and the development and testing of potential new methods for this purpose, ultimately with the aim of identifying the best minimally destructive method. Once established, this method was then used to extract collagen from the thesis samples collected for this project.

At this point, it is important to address what is meant by the term ‘best’: different methods produce different amounts of data and are capable of targeting different areas of the collagen chain. This essentially means that there is no one best method for all research questions involving archaeological collagen; which method is best depends on what you want to achieve. With this in mind, it was decided that the ‘best’ method for this project would be that which produces the largest number of peptides where those peptides are also of a high intensity, to provide the best chance of observing hydroxylation differences between individual samples and/or sample groups.

The need for a functional non-destructive method is also an issue of importance here: the growing call for non-destructive methods of analysis within archaeology generally has already been discussed (in Chapter Two), but in relation to this project specifically, a large proportion of the samples collected for analysis were only permitted to be analysed non-destructively. Subsequently it was hoped that the systematic testing of different methods would reveal a non-destructive method at least as effective as the more commonly employed destructive methods.

This leads us to a consideration of what constitutes a destructive versus a non-destructive method for the extraction of collagen from human skeletal remains. A destructive method is relatively easy to define in that a visible and irreversible change occurs to the bone being processed, almost exclusively via the use of acid to destroy the mineral component, leaving only the collagen. A non-destructive method, on the other hand, is somewhat harder to characterise. The Oxford English Dictionary defines non-destructive as something “That does not destroy or involve destruction”, or in regards to computing, something “that does not result in the erasure of data”, which is also particularly applicable when considering what constitutes a non-destructive biomolecular method. However, there are different levels of destruction and different kinds of data that can be erased. ‘Non-destructive’ can refer to a method that leaves a sample visually the same as it was before the process was carried out, however that does not account for any biomolecular changes that may have occurred. When considering biomolecular changes, there is DNA as well as protein that could be affected. With all of this in mind, ‘non-destructive’ within the scope of this project refers to a method that leaves the sample in visually the same condition as it was prior to the analysis being carried out, and which would allow for further collagen-based analyses to be undertaken on that sample in the future.

The systematic testing of a variety of collagen extraction techniques was carried out in two stages: Phase One, which involved testing a broad range of methods on a small number of samples, and Phase Two, which took a small number of the methods tested in Phase One - those deemed most promising or of most interest for further analysis - and re-tested them on a larger number of samples.

Phase One

Materials

Samples

For the first phase of collagen extraction experiments, a minimum of five human bone samples from the Priory Yard burial ground in Norwich, dated to the 18th-19th centuries AD, were used. Details of these samples, and which methods they were used to test, can be found in Table 1.

BioArCh LOT No.	Skeleton No.	Skeletal Element	Methods tested
13329	665	Distal right tibia	All
13330	10552	Tibia midshaft	All except K and Kd
13331	11346(A)	Cranial fragment	All except O, P, Q, R, S and T
13332	11346(B)	Cranial fragment	All except J
13333	11348	Occipital fragment	All
13334	11348	Distal right tibia	All

Table 1: Details of the Priory Yard samples used in the first phase of collagen extraction experiments.

Chemicals and Equipment

- Hydrochloric acid (HCl) (VWR International)
- Ammonium bicarbonate (AmBic) buffer solution (NH_4HCO_3, pH 8.0)
- Acetonitrile (Sigma)
- Sodium hydroxide solution (0.1M NaOH)
- Hydrochloric acid (HCl, pH3)
- a-cyano-4-hydroxycinnamic acid matrix powder (Fisher Scientific)
- Six calibration standard peptides (Sigma)
- Trypsin (Promega)
- Trifluoroacetic acid (VWR)

Samples were spotted in triplicate onto a Bruker steel target plate, and run on a Bruker Ultraflex III mass spectrometer.

Methods

This first phase of experiments involved testing 24 different methods, the details of which are listed in Table 2.

Method label/identifier	Details	Destructive (D) / Non-destructive (ND)
A*	Samples in AmBic rinse at room temperature for 24 hours	ND
B*	Samples in AmBic rinse at room temperature for 168 hours	ND

C*	Samples demineralised in 0.6M HCl, no sodium hydroxide rinse	D
D	Samples in AmBic rinse at room temperature for 24 hours, heated in AmBic for 3 hours	ND
E	Samples in AmBic rinse at room temperature for 24 hours, heated in AmBic for 9 hours	ND
F	Samples in AmBic rinse at room temperature for 24 hours, heated in AmBic for 9 hours	ND
G	No AmBic rinse. Sample heated in AmBic for 3 hours, initial AmBic discarded and replaced, sample heated for a further 3 hours	ND
H	No AmBic rinse. Sample heated in AmBic for 3 hours, initial AmBic discarded and replaced, sample heated for a further 9 hours	ND
I*	Samples frozen at -20℃ in 0.6M HCl for 3 weeks, sodium hydroxide rinse	D
J*	Samples frozen at -20℃ in 0.6M HCl for 3 weeks, no sodium hydroxide rinse	D

K*	Samples demineralised in 0.6M HCl, sodium hydroxide rinse	D
N*	Samples in AmBic rinse at room temperature for 168 hours, sodium hydroxide rinse	ND
O*	Sodium hydroxide rinse	ND
P*	Samples in AmBic rinse at room temperature for 24 hours, sodium hydroxide rinse	ND
Q*	Sodium hydroxide rinse, then samples frozen at -20℃ in 0.6M HCl for 3 weeks	D
R*	Samples in AmBic rinse at room temperature for 24 hours, sodium hydroxide rinse, then frozen at -20℃ in 0.6M HCl for 3 weeks	D
S	Samples demineralised in 0.6M HCl, sodium hydroxide rinse, then gelatinised using pH3 solution and ultrafiltered (ultrafilters prepared with NaOH)	D
T	Samples frozen at -20℃ in 0.6M HCl for 3 weeks, sodium hydroxide rinse, gelatinised using pH 3 solution and ultrafiltered (ultrafilters prepared with NaOH)	D
Cd	⅛ dilution of C	D

Id	⅛ dilution of I	D
Jd	⅛ dilution of J	D
Kd	⅛ dilution of K	D

Table 2: Details of the different collagen extraction methods tested as part of Phase One. Samples with an asterisk next to their identifier had AmBic added and were heated for one hour at 65℃ following the treatment described in the table.

Methods A, B and C were chosen because they are the standard in-house methods used for collagen extraction as part of the Zooarchaeology by Mass Spectrometry (ZooMS) process (based on methods first detailed in Buckley et al. 2009 and van Doorn et al. 2011, although Methods A and B are particularly different from the method detailed in van Doorn et al. 2011, most notably due to the inclusion of a room temperature buffer soak step). ZooMS being a quick and cheap technique that has proved successful in the extraction of collagen from archaeological bone, these methods were taken as the starting point for the analyses that will form part of this project.

Methods D, E and F relate to Method A, but aim to determine whether or not heating for a longer period of time extracts more collagen.

Methods G and H aim to determine whether replacing the AmBic rinse at room temperature with a three-hour heating rinse will increase the amount of collagen extracted.

Methods I and J aim to test whether freezing the samples in acid allows for a more controlled demineralisation, leaving more of the bones' collagen component intact and available for analysis. The inclusion of the sodium hydroxide rinse in Method I was intended to test whether this

could help reduce the problems introduced by humic substances, which negatively affect the appearance of the resulting MALDI spectra.

Method K is essentially the same as Method B, but with the addition of the sodium hydroxide rinse. Similarly, Methods N and P are the same as Methods B and A, respectively, but with the sodium hydroxide rinse step included.

Method O tests whether a sodium hydroxide rinse, with no AmBic rinse or demineralisation can still result in the extraction of a useful amount of collagen.

Method Q aims to test whether applying a sodium hydroxide rinse before freezing in acid can eliminate the humic substances problems and also provide a controlled demineralisation. Method R takes a similar approach, but asks if the addition of a 24-hour AmBic rinse prior to the sodium hydroxide rinse would improve the overall quality of the final spectra.

Methods S and T compare the difference between standard demineralisation in 0.6M HCl at 4℃ and a slower demineralisation at -20℃, while also investigating whether gelatinisation and subsequent ultrafiltering would result in a high collagen yield and clean spectra.

Methods Cd, Id, Jd and Kd are all dilutions of their original method. This was done in order to test whether a dilution would produce better spectra, which could be the case if there was a particularly high concentration of collagen in the original method solutions.

Each of the methods listed in Table 2, excluding Methods Cd, Id, Jd and Kd, were treated according to the details provided, then the supernatant separated into a new, labelled eppendorf tube. 1µl of 0.4µg/µl

sequencing grade modified porcine trypsin was then added, before samples were placed on a heat block at 37℃ overnight. Following this, samples were spun down in a centrifuge for one minute at 13000rpm, and 1ul TFA was added to each. The samples were then cleaned up and the collagen peptides isolated using 100 µL C18 resin ZipTip® pipette tips (EMD Millipore), after which they were spotted in triplicate onto a Bruker ground steel target plate (1µl sample to 1µl matrix) and run on the Ultraflex in reflector mode. Samples for Methods Cd, Id, Jd and Kd were diluted, spotted in triplicate (1µl sample to 1µl matrix) and run on the Ultraflex in the same way as the other samples.

Results & Discussion

The results from the first phase of extraction experiments can be seen in Figures 2 and 3.

Beginning with a discussion of the peak count results for each method tested, it is important to be aware that the graph shown in Figure 2 contains data that has been 'peak-picked' at a signal:noise ratio of three. This level was chosen as it is the standard accepted as being reliable within ZooMS research at York, and means that noise peaks have been filtered out, leaving only real peptides remaining. Peak-picking at this level therefore accounts for the potential issue of non-destructive methods being falsely perceived as being better than destructive techniques simply because they typically produce more noise.

Figure 2 shows that Method C (the standard destructive method that is commonly used for collagen extraction from archaeological bone) is definitely not one of the best performing methods in regards to peak count. Surprisingly, the two most commonly used non-destructive methods, Methods A and B, produced a notably higher number of peaks

and Method B actually produced the highest number of peaks of all methods tested.

Non-destructive Methods D, E and F also outperformed the standard destructive method in terms of the number of peaks that they produce, but they were not as effective as Method B, suggesting that the increased heating time does not result in more collagen being extracted from the bone samples.

Method G produced one of the worst peak counts of all of the methods tested during Phase One: this would suggest either that the initial AmBic rinse at room temperature is important and should not be skipped, or that discarding the AmBic from the first three-hour heating meant discarding any collagen that was extracted, and little more was extracted during the second heating stage. Method H also skipped the initial room temperature AmBic rinse, but gave better results than Method G, suggesting that a longer heating time could be beneficial in regards to increasing the number of collagen peaks produced. However, both Method G and Method H produced lower overall numbers of peaks than the other non-destructive methods already discussed. This could be due to the three-hour heating stage in Methods G and H causing some collagen to be extracted but discarded.

Methods I and J, two of the methods proposing a controlled demineralisation at -20℃, produced some of the lowest numbers of peaks. The reason for this is currently unknown; it could be because more damage is being done to the collagen component of the samples than was originally thought, or possibly because collagen is being discarded when the acid is discarded and they are rinsed with ultrapure water. Interestingly, Method J, which does not include the sodium hydroxide rinse, performs better than Method I, which does. This is also seen in the difference between Method C (no rinse) and Method K (rinse) - with

Method K being one of the best methods in terms of overall number of peaks produced - and between Method A (no rinse) and Method P (rinse). However, Methods B (no rinse) and N (rinse), show the reverse.

Methods Q, R, S and T, the remaining -20℃ methods, were also some of the worst methods in terms of the low number of peaks that they produced; Method Q was in fact the worst performing of all 22 methods tested in that sense.

Method O, another of the worst performing methods in regards to the number of peaks produced but perhaps the simplest of all the methods, did appear to be better than any of the freezer methods.

The dilution methods (Cd, Id, Jd and Kd) did not produce more peaks than their undiluted counterparts, suggesting that there was not an issue of having too high a collagen concentration in the samples employing those original methods.

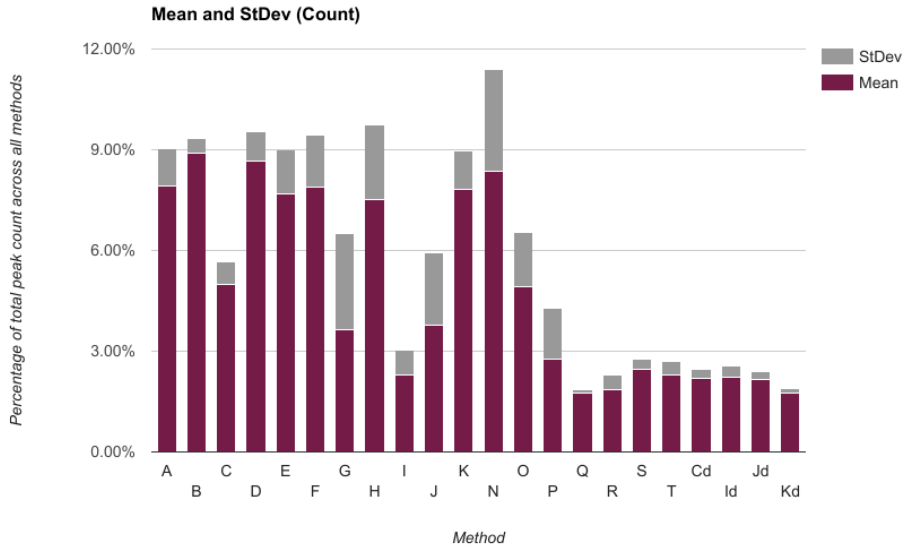


Figure 2: Graph showing the total peak count for each method tested during Phase One of the collagen extraction experiements

With all of that in mind, the peak count for each method is not the only factor to be considered when working to determine the 'best' method; we must also look at the intensity of the peaks produced. Figure 3 shows that Method C, a commonly used destructive method, is not only one of the worst methods in terms of the number of peaks produced, but is also the worst in regards to the intensity of those peaks. This is potentially concerning as it shows a significant weakness in a method that has been used by a large number of researchers over the last eight years, but it does evidence the validity of carrying out collagen extraction experiments prior to proceeding with data collection from the samples collected for this project.

The most variable results in terms of peak intensity come from the two main 'freezer' methods, Methods I and J. However, it should be noted that the standard deviation for Method I is particularly large due to one extremely intense peak in one of the samples tested.

The most successful method in regards to peak intensity is arguably Method N, although there is still a potential issue with the large standard deviation. Methods A and B were also favourable in their intensities when taking into account the standard deviations.

From the data presented in Figure 3, there is no clear result that confirms whether or not the inclusion of the sodium hydroxide rinse affects final peak intensity: Methods A and J (both no rinse) perform better than Methods P and I (both with a rinse), respectively. However, Methods K and N (both with a rinse) perform better than Methods C and B (both no rinse), respectively.

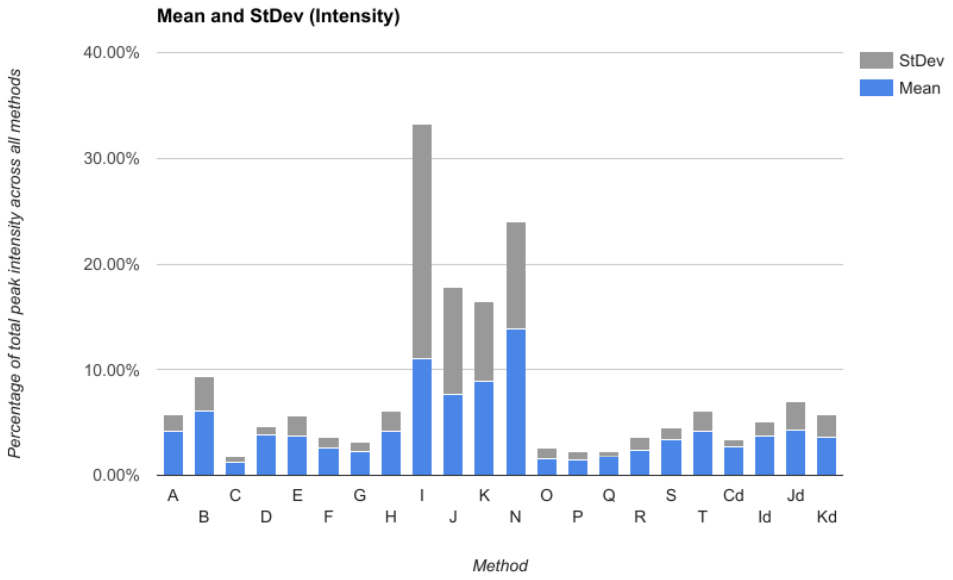


Figure 3: Graph showing the total intensity of the peaks for each method tested during Phase One of the collagen extraction experiments

Taking into account the data on both peak count and peak intensities, it was decided that five methods - B, C, I, K and N - would be investigated further.

Phase Two

Materials

Samples

For the second phase of extraction experiments, the same five samples used throughout phase one were used, with the addition of six human samples from Italy and nine human samples from Portugal (see Table 3).

Site	BioArCh LOT No.	Skeleton No.	Skeletal Element
Priory Yard, UK	13329	665	Distal right tibia
Priory Yard, UK	13330	10552	Tibia midshaft
Priory Yard, UK	13332	11346(B)	Cranial fragment
Priory Yard, UK	13333	11348	Occipital fragment
Priory Yard, UK	13334	11348	Distal right tibia
Basilica Ardeatina, Italy	15735	TB233 US10484	Right rib
Basilica Ardeatina, Italy	15726	TB196 US9214	Right rib
Basilica Ardeatina, Italy	15731	TB204 US9221/9261/9299	Right rib
Basilica Ardeatina, Italy	15734	TB233 US10484	Right rib
Basilica Ardeatina, Italy	15733	TB233 US10569	Right rib
Basilica Ardeatina, Italy	15729	TB291 US10296	Right rib

Beja, Portugal	13365	BEJ1008	Right rib
Beja, Portugal	13402	BEJ7217	Right rib
Beja, Portugal	13410	BEJ1029	Right rib
Beja, Portugal	13494	BEJ1145	Right rib
Beja, Portugal	13459	BEJ3073	Right rib
Beja, Portugal	13477	BEJ1036	Right rib
Beja, Portugal	13499	BEJ1152	Left rib
Beja, Portugal	13507	BEJ1117	Right rib
Beja, Portugal	13532	BEJ1108	Left rib

Table 3: Details of the samples used in the second phase of the collagen extraction experiments.

Chemicals and Equipment

- Hydrochloric acid (HCl) (VWR International)
- Ammonium bicarbonate (AmBic) buffer solution (NH_4HCO_3, pH 8.0)
- Acetonitrile (Sigma)
- Sodium hydroxide solution (0.1M NaOH)
- a-cyano-4-hydroxycinnamic acid matrix powder (Fisher Scientific)
- Six calibration standard peptides (Sigma)
- Trypsin (Promega)
- Trifluoroacetic acid (VWR)

As with Phase One, samples were spotted in triplicate onto a Bruker steel target plate, and run on a Bruker Ultraflex III mass spectrometer.

Methods

The five methods selected for further testing in Phase Two of the collagen extraction experiments are presented in Table 4. The aim of the second phase was to produce a greater amount of data pertaining to these methods, in order to both test the results from the first phase and to establish which would be the best method to take forward for processing and analysing the project collections.

Method label/identifier	Details	Destructive (D) / Non-destructive (ND)
B*	Samples in AmBic rinse at room temperature for 168 hours	ND
C*	Samples demineralised in 0.6M HCl, no sodium hydroxide rinse	D
I*	Samples frozen at -20℃ in 0.6M HCl for 3 weeks, sodium hydroxide rinse	D
K*	Samples demineralised in 0.6M HCl, sodium hydroxide rinse	D
N*	Samples in AmBic rinse at room temperature for 168 hours, sodium hydroxide rinse	ND

Table 4: Details of the different collagen extraction methods tested as part of Phase Two. Samples with an asterisk next to their identifier had

AmBic added and were heated for one hour at 65℃ following the treatment described in the table.

Method B was chosen because it produced the highest number of peaks out of all 22 methods that were included in Phase One and it also showed a promising peak intensity level. In order to further investigate the potential effects of including a sodium hydroxide rinse, Method N - essentially the same as Method B but with that one difference - was also included. In its own right Method N also produced one of the higher count rates and a good intensity level.

Method C was included in the second phase as it is a commonly employed method for collagen extraction from archaeological bone for proteomic research, having been used extensively by a number of researchers and traditionally thought to be more effective than non-destructive techniques. The results of the Phase One experiments dispute this, and so it was decided that further analysis of this method, in comparison with non-destructive methods, would be useful in trying to resolve this issue. Similarly to Methods B and N, Method K was included as it differs from Method C only in its inclusion of a sodium hydroxide rinse ste

Method I was the final Phase One method chosen for inclusion in Phase Two. While neither Method I nor Method J (the no rinse counterpart to Method I) looked particularly promising overall from the initial results, the potential for the method to produce fantastic peak intensities was demonstrated in one of the samples analysed, and so it was decided to retest the method on a larger number of samples.

The decision to further test these five methods facilitated three lines of enquiry. Firstly, it enabled the difference between including or excluding a sodium hydroxide rinse to be investigated. Secondly, it enabled a long-

standing and commonly used destructive technique to be tested against non-destructive alternatives that would previously have been thought of as inferior options. Finally, it allowed the investigation of a new destructive technique that could, if successful, provide an alternative to the established method.

Each of these methods was processed according first to the details outlined in Table 4, and then following the same technique outlined above in the Methods section for Phase One.

Results & Discussion

The results from the second phase of extraction experiments can be seen in Figures 4 and 5.

Figure 4 shows clearly that the non-destructive methods, Methods B and N, produce the most peaks. Note that, as was the case with the Phase One data, the Phase Two data has been peak-picked at a signal:noise ratio of three.

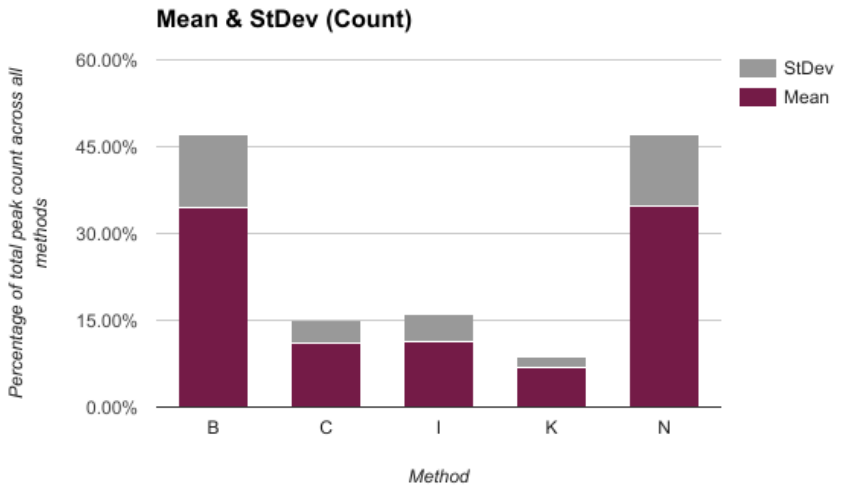


Figure 4: Graph showing the total peak count for each method tested during Phase Two of the collagen extraction experiments

In contrast to the first phrase of extraction experiments, Method N displays a slightly better peak count than Method B. When comparing the data from the two phases in terms of peak count, this brings us no closer to resolving how useful or otherwise the sodium hydroxide rinse step is.

When we look at the destructive methods tested, Method C is better than it's sodium hydroxide rinse counterpart, Method K, again in contrast with the Phase One data. Method I performs similarly to Method C and marginally better than Method K in regards to peak count, but is once again one of the worst performing methods overall

The Phase Two intensity results (Figure 5) show a similar pattern to the Phase Two count results just discussed in that Methods B and N, the non-destructive methods, are by far the best when compared to the destructive methods.

Method I, similar to its performance Phase One, shows relatively low peak intensities with a large standard deviation. It is important to highlight that, unlike in Phase One, there was no anomalously high peak produced in the second phase of experiments.

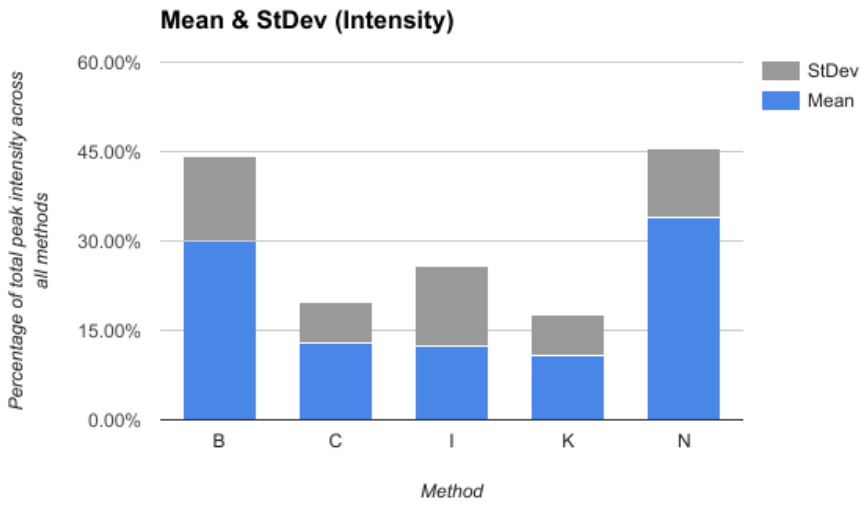


Figure 5: Graph showing the total intensity of the peaks for each method tested during Phase Two of the collagen extraction experiments

From this second phase of collagen extraction experiments, especially when the data is compared to that from the first phase, it appears that we still cannot say with any certainty what influence the inclusion or exclusion of a sodium hydroxide rinse has on either the peak count or the peak intensity that is observed. What we can say, though, is that non-destructive methods are capable of outperforming the destructive method, which was previously generally considered an impossibility. While the new non-destructive method, Method N, has been surprisingly successful, the newly proposed destructive method, Method I, did not perform well in either phase of testing. Based on the results presented here, it would not be recommended that Method I be considered an alternative to either the currently used destructive method or either of the non-destructive methods tested.

Chapter Conclusions

One of the key findings from the systematic experiments detailed in this chapter was that even relatively minor changes to a collagen extraction protocol can have a significant effect on the overall quality of the resulting mass spectrometry data, and can even influence which peptides can be observed. This finding is of fundamental importance for any researcher whose work involves the extraction of collagen from archaeological bone for subsequent mass spectrometric analysis, as it highlights the need for an appropriate protocol to be applied consistently in order for the resulting data to be both reliable and comparable to similar datasets.

Despite the extraction experiments detailed here providing inconclusive results regarding the use of a sodium hydroxide rinse, Method N gave a good result for peak count in Phase One, the best result for peak count in Phase Two, and the best result for overall peak intensity in both. This is significant both generally and in relation to this project. More generally,

this goes against long-standing beliefs that non-destructive methods cannot be as good as - certainly not better than - destructive techniques for the extraction of collagen from archaeological bone (e.g. van Doorn et al. 2011, 288). This viewpoint is perfectly understandable and arguably logical; the theory of removing the mineral component and subsequently having only collagen to work with would, one would think, produce better results than a method that does not get rid of the mineral component, which ultimately means that much of the collagen is still being held in place and is therefore less accessible for extraction (Collins et al. 2010, 6). One possibility is that the standard destructive method (labelled Method C above), is too aggressive, and is destroying some of the collagen in the time that it takes to fully demineralise the samples. This could make it appear as though the method is extracting less than the non-destructive Method N, as Method N is not destroying anything, whether that be mineral or collagen.

Regardless of the reason why, the fact that museums and collections managers are increasingly requesting that researchers use non-destructive methods makes these findings particularly important, as they suggest that proteomic researchers may be able to implement a non-destructive approach while also producing good quality data.

In terms of this project specifically, Method N was chosen as the best method to be used for the processing of all samples. As has been mentioned previously, many of the samples obtained for inclusion in the project required a non-destructive approach in order for access to be granted, so it is both important and fortunate that a non-destructive method displayed such positive results in these tests.

This chapter has addressed the issue of how to best extract collagen from archaeological human bone. Now that the method of extraction has been established, we can move on to consider the archaeological

questions to which the data produced can be applied. With this in mind, the next chapter will discuss the history of scurvy and diet in post-medieval England.

Chapter Four: Research Context - Diet and Scurvy in England

The relationship between nutrition, ill health and mortality is an area of long-standing debate. This is especially true of England during the eighteenth and nineteenth centuries, a period that sees major shifts in disease patterns and population.

This chapter will investigate the histories of diet and the nutritional deficiency disease, scurvy, in England during the long eighteenth century. It is important to state that malnutrition is a wide topic, and there are certainly other indicators of malnutrition that could be considered in this chapter. However, as this thesis focuses on the different ways to approach the study of one specific nutritional deficiency, to discuss others would distract from that central theme, and so they will unfortunately not be considered here.

In regards to the history of diet, we will first briefly explore how the relationship between nutrition and ill health has been and is currently perceived and understood, before moving on to consider the ongoing 'McKeown debate' and its relationship to the themes of this project, the percentage of household budget spent on food by the labouring poor during our period of interest, ways of obtaining food outside of the cash economy, and how debates between optimists and pessimists in the history of diet in England are relevant to this project. Special consideration will be given to the potato in this chapter, both because it is a cheap source of Vitamin C that was available in England during the long eighteenth century, and so could have helped to guard against scurvy, and because attitudes towards this particular food changed dramatically over this time period. We will then move on to consider the potential pitfalls of writing the history of a disease, ask what is meant by the term 'scurvy' today, and look at the Bills of Mortality and early

nineteenth century newspaper evidence for the presence of ‘scurvy’ in the past. We will discuss the general focus of existing research on scurvy to date - which has largely focused on scurvy at sea, or as part of the Irish potato famine. The current status of osteological capabilities in determining the presence or absence of scurvy in human skeletal remains will be detailed, before finally moving on to consider the potential benefits of a biomolecular marker for identifying this disease in archaeological individuals and populations.

Diet

Nutrition and its Effects on Health

Fundamentally, modern day research has proved that the foods we eat, and their nutritional compositions, can have a profound affect on growth and development in subadults, and on the functional capabilities and overall health of both adults and subadults (e.g. Krehl 1983, 9; Ohlhorst et al. 2013, 1349). While diet alone cannot prevent or account for all instances of ill health or disease, it is now understood that there is a link between the two. Whether this link was considered by those living through our period of interest is realistically unknown, however it is noteworthy that there is considerable evidence of ‘remedies’ for a variety of ailments being offered for sale at this time (as will be discussed later in this chapter with a particular focus on scurvy), while documentary evidence relating to the possible effects of particular foods on these same conditions appears to be scarce. This would suggest that diet was not widely viewed as a way to prevent or cure instances of ill health.

However, it is important to remember that our own current understanding of the link between nutrition and health is not absolute, and is only possible as the result of on-going scientific progress and discovery that

has occurred since our period of interest. With this in mind, we should remember that for people living in England in the long-eighteenth century, it would not necessarily have been possible for them to know that there was a link between nutrition and health.

The McKeown Debate

When discussing the history of diet in England, the debate surrounding the work of Thomas McKeown must be considered. The 'McKeown Thesis', as it has been termed, centres around population growth in England from the eighteenth century onwards. McKeown identifies the root cause of this as being falling mortality rates, caused by improved nutrition resulting from increasing economic prosperity (McKeown & Record 1962, 121; McKeown 1979, 59; McKeown 1983, 227).

As just mentioned, at a basic level McKeown believed that the rise in population numbers was due to falling mortality (caused by an increase in the standard of living), rather than to increasing fertility. He argued that it would be difficult to explain how a higher standard of living from the eighteenth century onwards could selectively increase fertility levels but not decrease mortality levels (McKeown 1988, 85). However, this is assuming that it was the standard of living, and not an entirely different cause, that fuelled the change. The falling mortality versus rising fertility issue has been much debated by scholars (e.g. Habakkuk 1953, 269 & 281; Krause 1958; Benson 1976; Hollingsworth 1976, 350–1; McKeown 1978b; Wrigley & Schofield 1989, 228), and some convincing arguments have been made in this area. For example, Wrigley and Schofield (1989, 255), and later Wrigley and colleagues (1997, 194), have presented evidence supporting their theory that a combination of earlier age at first marriage, shorter intervals between pregnancies, and a reduction in the number of individuals never marrying caused a rise in population

numbers. We will not discuss further the potential arguments for rising fertility levels during the eighteenth century, as it is not directly relevant to this project. However it is important to note that this theory has a place in the debate, and that it is important to our overall understanding of population levels in England at that time.

Thinking again about McKeown's focus on declining mortality levels being the reason for population growth, his argument stemmed from his reluctance to believe that this was solely the result of medical advancements preventing many otherwise inevitable deaths from disease (McKeown et al. 1972, 349; McKeown 1978a). He plotted the numbers of deaths per year from tuberculosis, for example, and found that the number was falling prior to the introduction of the medical advancements that were targeted at treating this disease (Grundy 2005, 529). This lead him to identify and consider what he believed to be all other possible explanations for the change; decline of disease, better hygiene standards, and improved nutrition.

McKeown and colleagues acknowledged that the incidence of some diseases - for example, scarlet fever - may have declined due to a change in the interaction between pathogen and human host, but that this would not have accounted for nearly enough individuals to explain the growth in population that was observed (McKeown et al. 1972, 349).

Regarding improvements in hygiene standards, McKeown ranked this as the second most important factor (after nutrition) in influencing a decline in mortality levels (McKeown 1979, 76). However he also points out that the Public Health Act was not passed until 1875, and asserts that, with more and more people moving into cities during this period, the hygiene standards in areas such as London - and presumably Norwich - would not have been improving (McKeown 1979, 76).

McKeown arrived at the conclusion that nutrition was the single most important factor behind the decline in mortality levels largely through a process of elimination, which appears to be one of the main points that his critics take issue with. He believed that improved nutritional standards enabled the population to be more resistant to infectious diseases, and that medical vaccinations may not have been effective on a malnourished population; seemingly admitting the positive impact of immunisation, but saying that nutrition is the primary factor that enables medicine to work (McKeown 1979, 64, 119 & 162; McKeown 1983, 227).

It should be noted that McKeown did not believe nutrition to be the only factor which caused a decline in mortality levels - he acknowledged the role that medicine and improvements in hygiene played, but concluded that improvements in nutrition would have had the biggest impact (McKeown et al. 1972, 351). In this sense, McKeown had acknowledged the potential effects of nutrition on health, and had linked that on a wider scale to population mortality levels.

A number of scholars support McKeown's theory that improved nutrition was the determining factor in a decline in mortality at this time. For example, Harris and colleagues (2010, 20), Floud and Harris (Floud & Harris 1997, 96 & 101–2), and Fogel and colleagues (1982, 417) have all found that average height was increasing in England at the same time as mortality levels were dropping, which they have attributed to improved nutritional standards.

Link and Phelan (2002, 730 & 731) have noted their support for McKeown's conclusions, albeit in a slightly broader fashion, stating their view that it is "social conditions" such as nutrition that are ultimately responsible for population growth or decline, whether that be in the past or today. Kim (2000, 1382) also notes that while there undoubtedly were medical advancements made during this period in England, these

advancements did not necessarily filter down the social classes quickly enough to explain the observed overall decline in mortality levels.

However there has also been much criticism of McKeown's work, and various scholars have raised issues of other environmental factors, as well as social intervention. This criticism ranges from the mild to the absolute. For example Colgrove (2002, 4–5) presents the argument that McKeown was wrong to attribute the growth in population numbers during the eighteenth century solely to improvements in nutrition (and it should be noted that McKeown himself did eventually acknowledge the role that factors such as medical science played in some circumstances (McKeown & Record 1962, 227–8)), but also points out the applicable nature of his work to problems currently being experienced in the developing world. Simon Szreter, on the other hand, declared McKeown's work to be conceptually lacking in definition, asserted that he had made major errors in interpreting the data that he had used - even going as far as re-interpreting the data himself, and consequently drawing some notably different conclusions - and implied that he had taken a biased approach to his research due to his assumptions about the usefulness or otherwise of medical science (Szreter 1988, 11, 12–13, 33–34).

Harris (2004, 380) and Schwarz (1996, 298) have both argued that it was improvements in hygiene and sanitation levels that caused the drop in mortality levels during this period. Several other scholars have agreed that sanitation improvements played a role, but have also asserted the importance of medical advancements (Preston 1975; Preston 1980; Preston 1996; Cutler et al. 2006, 106, 116–7; Deaton 2006, 113; Deaton 2013, 93).

In terms of this project, McKeown's work is important because he is talking about our specific period of interest and focusing on nutrition, the

effects of nutrition on overall health, and nutritional change within that period. This is the classic industrial revolution period and the classic mortality reduction period: as a result, there's a lot of scholarly interest. However, to date, no consensus has been reached on what really did cause such a large population growth from the eighteenth century, and some researchers are now calling for the McKeown debate to be put to bed (e.g. Grundy 2005, 532). The real issue concerning this debate, though, is the equation of 'real wages' and nutrition, which has inspired a huge amount of work on levels of nutrition in this period, produced by the optimists and pessimists in the history of diet.

This project will not solve the McKeown debate - arguably there are data deficiencies which mean it will never be solved - but it aims to contribute to our understanding of diet and experience of nutritional deficiency in this period.

Thinking again about the equation of 'real wages' and nutrition, we shall now move on to consider the two main ways in which people obtain food: first, that which is purchased using individual or household income, and second, that which is procured outside of the cash economy.

Percentage of Household Budget Spent on Food

The first thing to note when searching for information from the household budgets of poor families living in England during the long 18th century is that the existence of these budgets is rare. With levels of literacy at this time generally low across the population as a whole - and the ability to read much more common than the ability to write (Sutherland 1990,124–5) - it is not surprising that the poor were not keeping records of their annual expenditure. However, Boulton (2005, 121) proclaims that food would have been the biggest single expense incurred during this period. This conclusion has been echoed more recently by Muldrew (2011, 29),

who suggests that both labouring families and those living in poverty could have spent up to 75 per cent of their annual expenditure on food. Sharpe (2016, 118) goes further than this, suggesting that the poorest members of society frequently bought food on credit, often resulting in debts that could not easily be repaid.

The amount spent on food by families living in England during this period will have partially been determined - as is still the case today - by how many members of the family there were. This could be affected by a number of factors: the number of children born into a family, the number of those children that survived childhood, the age at which a child left home, whether the mother herself survived childbirth, and whether the father remained with the family (Sharpe 1995, 362; Boulton 2005, 92).

Access to Food Outside of the Cash Economy

Purchasing food was of course not the only option for obtaining food - we will now discuss the possibilities of growing food, theft in relation to food, and poor relief.

Labourers working in more rural areas of England during this period may have had access to common land on which they could grow food, either for their own consumption or to sell on to others (Johnson 1996, 33; Griffin 2018, 90). However there are two issues with this in relation to this project; first, it is unlikely that the individuals living at the sites being considered here would have had access to such land. Second, the food produced in such a way would likely have gone unrecorded, and so there is no accurate way of knowing how much extra this would have contributed to the diet (Griffin 2018, 88).

Theft relates to food consumption in two different ways - either food itself can be stolen, or more commonly, something that can be sold to then pay

for food is stolen. A number of scholars have noted that in times of poor harvest - such as 1782-3 - when food prices rose as a consequential result of demand outstripping supply, the number of convictions for theft also rose (Beattie 1975, 103; Hay 1982, 130; MacKay 1999, 623; Horrell et al. 2013, 258). Convictions for theft have also been noted to rise - in London in particular - immediately following the end of periods of war (Horrell et al. 2013, 258). This has been ascribed to an increase in the amount of unemployment, due to high numbers of servicemen being discharged at once but no provision being made for their subsequent employment (Beattie 1975, 103). Prosecution numbers for thefts of food itself tended to be low, likely due to their generally low value (Hindle 2004, 82–4). However there are records detailing food and liquor thefts in London between 1780 and 1789, and these show that a higher number of these crimes were committed by men than by women. Closer inspection of these records reveals that many of these thefts related to sugar and tea, and may have been come by through working at - or pretending to work at - docks where these commodities were received (MacKay 1999, 625). As both of these were incredibly popular across all classes at this point, it is possible that these items were not taken for personal consumption, but in order to sell them on - Horrell and colleagues (2013, 256) note that some eighteenth century shopkeepers would knowingly buy and sell stolen tea, presumably to maximise their own profits.

For the truly poor in England at this time, there was the option to request poor relief. Throughout this period this was linked to the Poor Laws - initially to the Old Poor Law (passed in 1601) and later to what is known as the New Poor Law (following the Poor Law Amendment Act of 1834). Under the Old Poor Law, people were given ‘in kind’ support, either instead of or as well as financial relief, which enabled them to stay in their own homes. For example, items such as clothes, fuel or food could be provided when it was deemed necessary (Hindle 2004, 265; Sharpe

2016, 134). It should be noted that while this ‘outdoor relief’ was an option under the Old Poor Law, any able-bodied person refusing work when it was available to them was denied this and was sent to a House of Correction. The Old Poor Law was organised and financed at a parish level, with overseers responsible for ensuring that relief was administered appropriately (Solar 1995, 3). While this may sound like an effective, even generous system, we must question how effective it actually was if there were still cases of theft being recorded.

The New Poor Law - brought about partly as a result of continued complaints about the old system, and partly in response to the changing economic and social nature of England thanks to the Industrial Revolution - completely replaced the 1601 Old Poor Law, and fundamentally changed the country’s poverty relief system (Solar 1995, 18; Wood 1991, 52). The New Poor Law was intended to cut the cost of poor relief, and to address the perceived abuses of the old system. Without question the biggest change was the abolishment of ‘outdoor relief’; all poor relief now involved entrance into the workhouse (Walker 2004, 93; Burnett 2013, 28).

Having considered the ways in which people may have been able to obtain food in England during the long eighteenth century, it would now be desirable to discuss potatoes, tea and sugar - three of the most important influences on diet in this period - in relation to the diets of the labouring poor at this time. However, as it is currently impossible to directly study the consumption of tea or sugar through archaeological means, these two items will be left out of consideration. Suffice to say that it has been proposed that many of the women who could be classed as ‘labouring poor’ supplemented their daily diet with weak, sweet tea, to distract from the insufficient amount of food that they could afford for themselves (Otter 2012, 813).

Potato Consumption

Potatoes are thought of as a key component of the British diet and they have been described many times as a staple of the poor - we know that by the end of the long eighteenth century, this was certainly the case (Rule 1981, 124). However, it has never been accurately established when the potato arrived in England, or when people began to eat them (Reader 2008, 78–9). It is most commonly believed that either Sir Walter Raleigh or Sir Francis Drake brought them here in the 1580s. However, Walter Raleigh never actually visited the part of North America where it is claimed he brought the potato back from, and it has been established that the tubers would not have survived the journey that Francis Drake took to get back to England, so it seems the potato's introduction to England is unlikely to be attributable to either of them (Macbeth 1997, 104; Zuckerman 1998, 10; McNeill 1999, 73; Messer 2000, 190; Reader 2008, 87). All that we know for certain regarding the potato's arrival in England is that it was here - but not being eaten - by the start of the seventeenth century.

In contrast to the end of our period of interest, when potatoes first arrived in England they were regarded with suspicion (Salaman 1952, 51; Carpenter 1988, 100). No one wanted to eat them; they were grown primarily for their flowers (Eskin 1989, 4). This suspicion was undoubtedly fuelled by books such as the 'Herbals' and the 'Doctrine of Signatures', which purported the rumour that eating potatoes would cause leprosy in the consumer, due to tubers resembling the appearance of leprous hands and feet (Salaman 1952, 51; Zuckerman 1998, 15; Reader 2008, 111). Added to this, religious preachers are recorded as having forbidden their parishioners from growing potatoes; they were not named in the Bible, and therefore must have links with the devil (Salaman 1952, 51; Reader 2008, 111).

Some slightly more practical reasons for the slow adoption of the potato in England are seen through the consideration of farming practices. Land rotation patterns had already been established for growing crops and grazing livestock, and it initially seemed that the potato could not be accommodated in these cycles (McNeill 1999, 74; Reader 2008, 114). It was also more labour-intensive to plant a whole field of potatoes than it was to sow a whole field of wheat (Reader 2008, 114). While we now know that a field of potatoes provides four times the calories that the same field of cereal would provide, this was an unknown fact at that time (McNeill 1999, 79; Reader 2008, 117).

As has been mentioned, exactly when potatoes were accepted as a main component of English diet, particularly for the poor, is currently unknown. Historical accounts suggest that northern England adopted them first, and that by the mid-1700s any excess being produced was shipped to other countries, such as Gibraltar (Zuckerman 1998, 57; Reader 2008, 122; Burnett 2013, 10).

We know that at some point in the potato's history in England, public opinion was changed, and it went from being something that was feared to a common staple. However we still don't know when, how, or why. To date, no historical documentation has been able to answer these questions, and investigations are made all the more complex by the term 'potato' also being used to refer to sweet potatoes, and jerusalem artichokes. Archaeological methods might not be able to reveal how or why people began eating potatoes in England, but by studying the physical remains of the past, it is possible to contribute to the question of when.

We have so far in this chapter focused on the change in consumption patterns of just one foodstuff, but we will now move on to consider the the over-arching changes that occurred to the diet of the labouring poor

over the course of the long eighteenth century, and how that may have linked to health at that time.

Optimists vs. Pessimists in the History of Diet

A huge amount of work has been undertaken by historians on changes in levels of nutrition during the long eighteenth century - far more than it could be hoped to cover here - but it would seem that no overall consensus has been reached on whether these levels increased or declined. Scholars generally fall into one of two camps on this issue; the 'optimists', who propose that the Industrial Revolution increased wages, which in turn brought about an overall higher standard of living and therefore a better diet (e.g. Allen 2009, 29; Floud et al. 2011, 138; Meredith & Oxley 2014, 171), and the 'pessimists', who argue that the Industrial Revolution actually damaged overall quality of life for the labouring poor, including decreasing the nutritional quality of their diets (e.g. Muldrew 2011, 322; Humphries 2013, 708).

This ongoing debate as to the overall improvement or decline in standards of nutrition is made particularly complicated when allocation of resources is taken into account. Division of food between men and women during this period is related in part to gender division of labour. Prior to this period, women in the working classes were engaged in many similar labour roles to men (Houston & Snell 1984, 487; Humphries 1987, 931). However by the eighteenth century, the idea that a man should earn enough money each year to ensure that his wife did not need to work was arguably becoming commonplace in England (Burnette 2011, 174) - this was a clear marker of socioeconomic status. Labouring poor families would most certainly not have been able to survive on one wage alone, and so women from these families must have continued to work in order to add to the overall household income (Bohstedt 1988, 95 & 97; Humphries 2013, 708). Around the same time, cottage industries began

to emerge, largely employing women in tasks such as weaving, embroidery or lace-work, which enabled them to work from the home and in theory care for their children at the same time (Burnette 2011, 181). This then left men to take on the more labour intensive roles, which in turn would have required a greater calorific intake than was needed by women (Burnette 2011, 173; Humphries 2013, 703).

Estimates on average calorie intake throughout this period are many and varied (Clark et al. 1995, 223; Muldrew 2011, 156; Kelly & Ó Gráda 2012, 6; Schneider 2013; Harris et al. 2015), and link heavily with the theme of optimistic and pessimistic attitudes towards nutrition and health during the Industrial Revolution in England. Much more work needs to be done in this area before any kind of meaningful conclusion can be reached.

As has already been alluded to, when considering female labour in the past, we must also consider the constraints that childcare requirements could have put on this, particularly breastfeeding and subsequent weaning practices. While the wealthy had optional access to wet nurses, this was not a luxury that the labouring poor could afford (Stone 1977, 269 & 271). However if working in one of the cottage industries, it would have been possible for a woman to continue breastfeeding whilst in employment (Humphries 1987, 935).

Historical records pertaining to the age at which weaning occurred tend to vary in their accounts: Stone (1977, 269) suggests that breastfeeding in the eighteenth century could have continued until the child was as old as 18 months. Forsyth (1911, 119 & 128) records recommendations from the end of the seventeenth century, which state that infants should be breastfed for up to two years, and also the start of the nineteenth century, by which time it is advised that the weaning process can start at between six and eight months old. The issue of weaning ages is one that both historical records and bioarchaeological methods can contribute to.

Stable isotope analysis of 72 individuals from the Spitalfields site in London, dated to the eighteenth-nineteenth centuries, revealed that all individuals had had solid food introduced to their diets by the age of one, and had completely stopped breastfeeding before the age of two (Nitsch et al. 2011, 622). There were a minority of individuals who appeared to have never been breastfed (Nitsch et al. 2011, 622.) This would seem to broadly fit with the eighteenth century guidelines cited by Stone and Forsyth.

As part of the weaning process, many children in England throughout the long eighteenth century were fed on pap or panada (Wood 1955, 475; Fildes 1986, 213). Pap was a type of paste made from bread that had been soaked or boiled in water or milk (Radbill 1981, 617; Weinberg 1993, 2020). Panada was a similar concoction, made by boiling cereals (and sometimes breadcrumbs) in water or, more commonly, broth (Wickes 1953, 238; Radbill 1981, 617). There was little if any nutritional value to either of these, they were simply used as a way of ensuring that the child was receiving enough food (Wood 1955, 480). A mother's breastmilk should contain enough Vitamin C to ensure that her child does not develop scurvy (Thompson & Howard 1998, 4); therefore if subadult individuals in the archaeological record are found to have had scurvy, this would suggest either that they had already been weaned onto a Vitamin C-deficient diet including foods such as pap or panada, or that the mother was severely deficient herself.

A 'pap spoon' or 'pap boat' was commonly used to feed the child, which was essentially a spoon with a hollow handle that enabled the feeder to blow the food into the child's mouth and down their throat, apparently speeding up the whole process (Wickes 1953, 238; Weinberg 1993, 2016 & 2020; Stevens et al. 2009, 35). These spoons were impossible to sterilise and subsequently most likely contributed to infant mortality in this period (Stevens et al. 2009, 35).

In this ongoing debate surrounding the change in mortality levels over the course of the eighteenth century, and the extent to which that relates to nutritional levels, many different sources have been and continue to be used, such as biological data on height, contemporary records on mortality figures, and individual household accounts. However, as we have discussed here, some of these sources are particularly difficult to access (if they exist at all), none are simple to interpret, and caution needs to be taken in attempting to use them to make statements about the wider population or across a time period of such enormous variation and change.

We know very little about morbidity during this period, especially in regards to chronic conditions, which were probably common but didn't lead to hospital admissions. Added to this, we are still very uncertain about nutritional status, especially when people may have been weak, or may have had dietary deficiencies but aren't dying from them. This reinforces the point made earlier that it is particularly difficult to establish whether or not a link between nutrition and ill health had been identified at this time. Of interest though, is that some scholars have suggested that in the early 19th century, as much as 20% of the population of England were consuming enough food to survive, but just barely (Hay 1982, 132; Fogel 1994, 373–4; Floud et al. 2011, 72–77). The question that this brings us to, but which it may be impossible for us to answer, is to what extent the population were chronically but not fatally undernourished.

Of particular relevance to this project is the fact that we know surprisingly little about scurvy as a chronic deficiency disease, and it is this that we will now turn our focus to.

Scurvy

Writing the History of a Disease

Before we can consider the history of scurvy in England, we need to think about inherent challenges and potential pitfalls that writing the history of a disease involves.

Possibly the most important challenge to be aware of is that cultural framing changes the way that language is used and applied over time (Anderson 1998, 31; Patterson 1998, 9–10; Wilson 2000, 271; Cooter & Stein 2013, 160); words do not necessarily mean the same thing today that they meant in the early nineteenth century (Patterson 1998, 10; Mitchell 2012a, 314) - indeed they may not have meant the same thing in the early nineteenth century as they meant at the end of the seventeenth. We cannot, therefore, simply search archival documents and records for 'scurvy' - or any other medical condition - and expect that what we find concerns the same ailment that we are referring to today. We are very often guilty in archaeological research of doing just this, however in recent years scholars such as Piers Mitchell - an NHS Consultant also trained in medical history - have started to draw attention to the importance of being rigorous in our investigation and application of historical accounts to modern day research (Mitchell 2011; Mitchell 2012b; Mitchell 2017).

Almost the flip-side of this problem is also true, and is another potential pitfall to be accounted for. When investigating the history of a disease, it is especially important to remember the difference between a disease and a symptom: symptoms are described, diseases are diagnosed. If a historical record contains a list of symptoms, we may be able to ascertain what that would today be diagnosed as, but we have to also be aware

that this is not necessarily what that condition would have been diagnosed as in the past (Cooter & Stein 2013, 161 & 164). This is not to say that we should not try to learn about the history of diseases, just that we need to be aware of the constraints that changing language use places on this kind of research.

Another potential problem in investigating the history of any disease is the time gap between the discovery of a cure and the point at which the general population have access to that cure and it is making a notable difference to mortality levels (Leavitt 1990, 1471). History tends to note the date on which cures are discovered but it is less easy to trace when they were made widely available to the general population. For example, James Lind is credited as discovering the cure for scurvy in 1747 and he published his nominal work, 'Treatise on the Scurvy', in 1753. However the navy did not implement his ideas until 1795, a year after his death, and it could have been longer still until this information reached the general population (Wilson 1975, 46; Dunn 1997, F64; Lamb & Rigby 2013, 4). We must therefore be careful to remember that just because a cure for a disease had been discovered by a certain date, mortality levels will not necessarily have noted the effects until some time later.

Finally, we must remember that medical knowledge as it stands today is not the pinnacle of achievement, and is not a point from which to look back on those researching and writing about disease in the past with any veil of superiority (Leavitt 1990, 1473; Cooter & Stein 2013, 166). Eighteenth century knowledge of particular diseases may have been limited in comparison to the knowledge that we have today, but it was on past knowledge that ours has been built, and we still do not have all of the answers (Leavitt 1990, 1484; Patterson 1998, 13).

What do we mean by ‘scurvy’?

On the face of it, this may seem like a straightforward question. When we use the term scurvy today, we are referring to a dietary deficiency disease whereby there is an inadequate amount of Vitamin C being consumed (Pimentel 2003, 328; Halligan et al. 2005, 688). This disease can affect humans, other primates, guinea pigs, bats, the bulbul bird, and some fish (Armelagos et al. 2014, 10; Hirschmann & Raugi 1999, 898; Jacob & Sotoudeh 2002, 66; Mays 2014, 55; Nishikimi & Udenfriend 1977, 111; Stone 1966, 345–6).

In humans, the first noticeable symptoms are usually fatigue, weakness and irritability (Olmedo et al. 2006, 911; May 2013, 97). Continued absence of Vitamin C from the diet can lead to joint and limb pain, the sufferer experiencing bleeding gums, and bruising easily. A consistently scorbutic diet can ultimately lead to the loss of teeth, old wounds opening up again, and a hemorrhage or heart attack that could cause death (Stone 1966, 345; Jaffe 1972; Geber & Murphy 2012, 514; Crandall & Klaus 2014, 3; Waldron 2009, 132).

However, while this is what we mean by ‘scurvy’ today, we know from our considerations regarding the history of diseases that language use changes over time, and so ‘scurvy’ in the long-eighteenth century may not always - or ever - have meant the same thing as we take it to today. Certainly the way that we define scurvy would not have been applicable then, as Vitamin C was not discovered until the 1930s.

Scurvy in the Bills of Mortality

The Bills of Mortality for London are a collection of weekly records that detail how many individuals died each week in the city, and their causes

of death. They first started in the 1650s and ran until the 1830s. Figure 6 provides details of how many individuals are recorded as having died in London from scurvy between 1657 and 1758, according to the Bills of Mortality. The volume detailing 1759 to the 1830s is currently undergoing digitisation, and so it has not been possible for the author to view the figures for those years. However, as will be detailed below, this was not critical to the point that reference to the Bills was making.

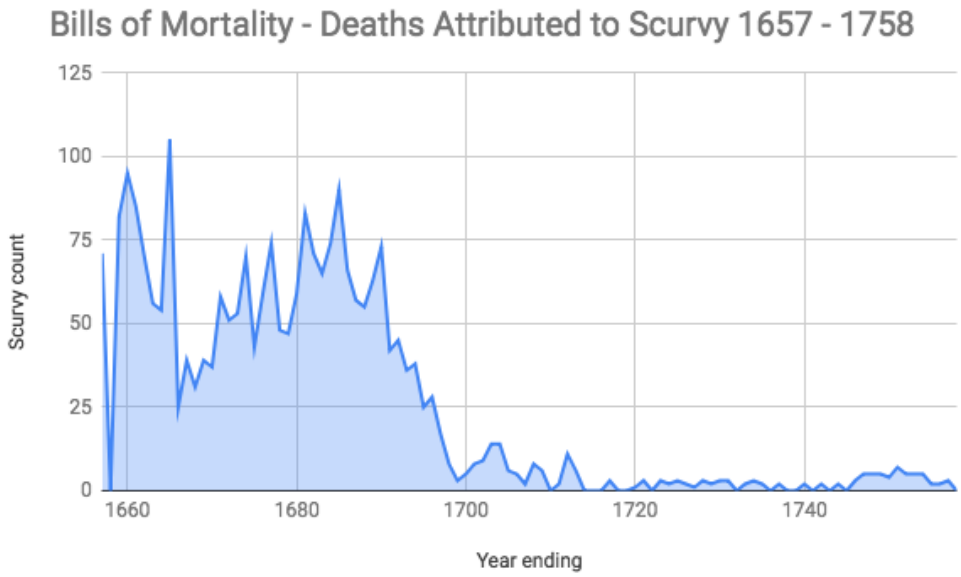


Figure 6: Graph showing the number of deaths attributed to scurvy each year in the Bills of Mortality for London, from 1657 - 1758 (data from Postlethwayt et al. 1759)

While we cannot say that 'scurvy' as noted in the Bills of Mortality was referring to the same disease that we would mean by that term today, the purpose of Figure 6 is to evidence that people in the long eighteenth century were noted as dying of scurvy.

We can see that this number falls quite rapidly in the 1690s, which raises questions as to whether this was because of a change in the use of the term 'scurvy' that made it less applicable, or because the number of deaths from that ailment actually did decrease? If the number of deaths

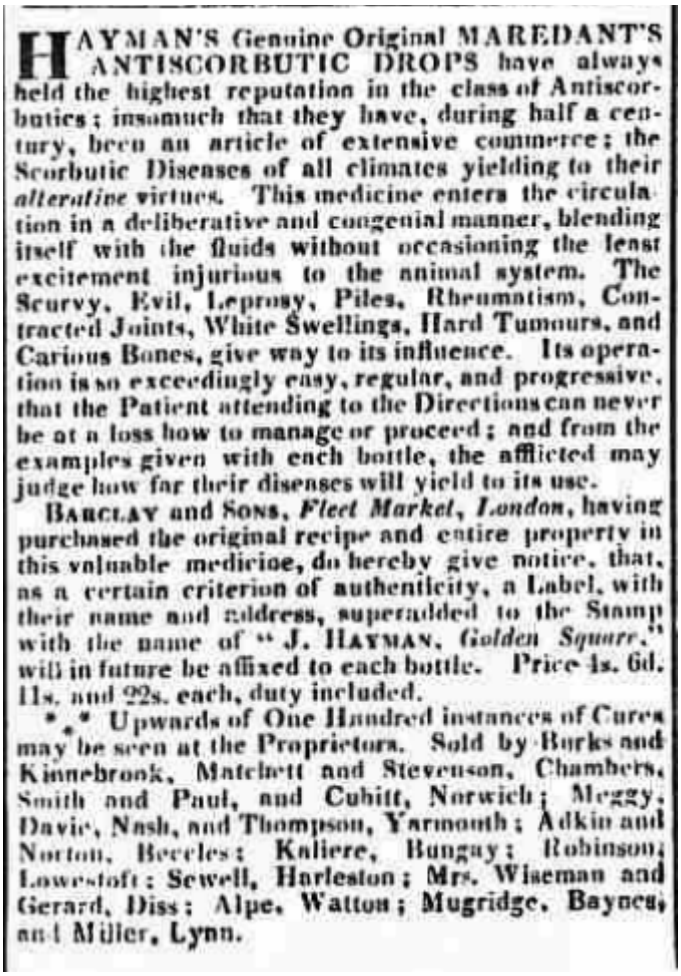
attributable to scurvy really did drop, why was that? It should also be noted that a decrease in the number of people dying from scurvy does not necessarily equate to a decrease in the number of people suffering from scurvy; it could have been the case that the same number of, or even more, people experienced the disease, just not to the extent that it caused death. This becomes much more difficult to trace through historical records, as no one was systematically recording numbers of individuals suffering from diseases which did not kill them. Here, then, is where it would be hoped that archaeologists could step in to provide osteological or biomolecular evidence to help solve these mysteries. However, as has been mentioned earlier, and as will be discussed in more detail below, there is no pathognomonic skeletal evidence of scurvy, and no existing accurate, reliable biomarker.

The author is aware that a number of scholars have questioned the accuracy and usefulness of the Bills of Mortality (e.g. Heberden 1801; Ogle 1892; Woods 2006; Boulton & Schwarz 2010). However this debate is not directly relevant to this project, and so this will not be discussed here. Regardless, the fact remains that the Bills of Mortality provide direct evidence that some individuals in London during the long eighteenth century were recorded as having died from something being termed ‘scurvy’.

Antiscorbutic Remedies in Long Eighteenth Century England

Throughout the long eighteenth century, antiscorbutic remedies were being sold all over the country - we see evidence of this in contemporary newspapers, which reveal adverts for a variety of these products (Figure 7).

These adverts detail lengthy lists of ailments that the antiscorbutic remedy will supposedly cure. As already discussed, due to the potential change in the use of language over time, we cannot be sure that any of these mean the same thing today as they did when the advert was written. However the existence of adverts such as that seen in Figure 7 is evidence that 'scurvy' was of concern in England at this time. This in turn raises the important question of how far the population were scorbutic, even if they didn't die from it, and what effect that would have had on their day-to-day lives.



HAYMAN'S Genuine Original MAREDANT'S ANTISCORBUTIC DROPS have always held the highest reputation in the class of Antiscorbutics; insomuch that they have, during half a century, been an article of extensive commerce; the Scorbutic Diseases of all climates yielding to their *alterative* virtues. This medicine enters the circulation in a deliberative and congenial manner, blending itself with the fluids without occasioning the least excitement injurious to the animal system. The Scurvy, Evil, Leprosy, Piles, Rheumatism, Contracted Joints, White Swellings, Hard Tumours, and Carious Bones, give way to its influence. Its operation is so exceedingly easy, regular, and progressive, that the Patient attending to the Directions can never be at a loss how to manage or proceed; and from the examples given with each bottle, the afflicted may judge how far their diseases will yield to its use.

BARCLAY and SONS, *Fleet Market, London*, having purchased the original recipe and entire property in this valuable medicine, do hereby give notice, that, as a certain criterion of authenticity, a Label, with their name and address, superadded to the Stamp with the name of "J. HAYMAN, *Golden Square*," will in future be affixed to each bottle. Price 4s. 6d. 11s. and 22s. each, duty included.

*** Upwards of One Hundred instances of Cures may be seen at the Proprietors. Sold by Burks and Kinnebrook, Matchett and Stevenson, Chambers, Smith and Paul, and Cubitt, Norwich; Meggy, Davie, Nash, and Thompson, Yarmouth; Adkin and Norton, Beccles; Kaliere, Bungay; Robinson, Lowestoft; Sewell, Harleston; Mrs. Wiseman and Gerard, Diss; Alpe, Watton; Mugridge, Baynes, and Müller, Lynn.

Figure 7: Advertisement in the Norfolk Chronicle from Saturday 5th March 1825, for 'Antiscorbutic Drops' (Anon 1825)

Interestingly, these adverts largely date to many years after it had been established that the consumption of citrus fruits will cure scurvy: general

thinking seems to have been that what would cure ‘sea scurvy’ was not the same as what was required to cure ‘land scurvy’ (Woodall et al. 1984, 61; Hughes 1990, 53).

General Historical Focus of Scurvy Studies in England

This brings us to an important point in the study of the history of scurvy in England: the general historical focus to date has been on scurvy at sea, rather than land scurvy.

James Lind - already mentioned in this chapter - is possibly the most famous individual to have carried out research into scurvy. In 1747 he carried out what has been credited as being the first clinical trial, with the aim of discovering a cure for scurvy in sailors (Baron 2009, 318). He found that citrus fruits cured the disease, but it was not known why, and he believed that these could only be used to cure the disease, and not to prevent it (Lamb 2016, 30). The existence of Vitamin C being unknown until the 1930s, Lind cited poor diet, poor hygiene, a cold, damp climate, and a lack of discipline as the causes of scurvy, rather than a lack of vital nutrients (Harrison 2013, 9–10; Hirschmann & Raugi 1999, 897; Waldron 2009, 131).

Prior to the eighteenth century, British sailors would not have developed scurvy as a result of being away at sea, as the voyages were not long enough - this will be a large contributing factor to why no potential evidence of scurvy was noted in the human skeletal remains from the fifteenth century Mary Rose shipwreck, for example (Simon Mays 2008, 130). To date, the author knows of only one British naval burial population that lived and died during the long eighteenth century, and which has subsequently been excavated and studied by osteologists. Excavations at the British Royal Naval Hospital at Greenwich involved the exhumation of 97 male skeletons of retired naval sailors, who had been in service during the eighteenth century (Mays 2014, 57). Lesions on the greater

wing of the sphenoid and/or the long bone shafts in the majority of these individuals has lead to the osteological conclusion that they may have previously suffered from scurvy, but recovered prior to death (Mays 2014, 57). However, as has been mentioned, the issues surrounding the accuracy of osteological identification of scurvy is a central theme of this project, and will be discussed in more detail later in this chapter. Furthermore, it would be useful to know the likelihood that any period(s) of scurvy experienced by these individuals were related to their time in the navy versus the likelihood that they occurred as land scurvy during their retirement.

While there is limited available archaeological evidence for British naval scurvy, there has been a strong focus on this within historical research. For example, in 2013 the Journal for Maritime Research held a conference on scurvy at sea, in recognition of the 260th anniversary of James Lind's 'Treatise on the Scurvy' being published (Blyth 2013, 1). This was then followed by the production of an entire edition dedicated to the historical study of scurvy (Lamb & Rigby 2013, 3).

Archaeological studies that have focused on scurvy at sea, but which have not focused on the British navy, have largely concerned Dutch whalers, but these cannot directly contribute to studies regarding life in England. The Arctic preservation conditions at one location were so good - the whalers having been buried in Spitsbergen - that blood staining on the bones of 39 out of the 50 whalers interred there could still be seen, proving that they had been experiencing active scurvy around the time of death (Maat 2004, 79; Mays 2014, 57). Unfortunately preservation conditions such as these are particularly unusual and would not be experienced in England. As such, it is unlikely that the data from these studies could realistically be compared to data from any English populations in a straightforward, meaningful way.

Land Scurvy in England During the Long-Eighteenth Century

When land scurvy is considered in academic research, the primary focus to date has been the Irish potato famine, which lasted from 1845 - 1852, and is believed to have killed over a million people. Even in recent years, many osteology-based investigations that have concerned scurvy have shared this focus. For example, from 2012 to date, three papers have been published by Jonny Geber on scurvy in individuals from the Kilkenny workhouse (Geber & Murphy 2012; Geber 2016; Geber 2017), and two more on the same population by Janet Montgomery and Julia Beaumont (Beaumont et al. 2013; Beaumont & Montgomery 2016). This could suggest that when land scurvy is a focus of research, there is a predilection for the study of human skeletal remains collections relating to this known catastrophic event, rather than populations where many individuals were potentially scorbutic to some extent as part of day-to-day life. However alternative explanations could be that these researchers were simply working with the skeletal remains that were available to be studied - archaeologists frequently face problems of access to appropriate sample material; an issue that we will discuss at various points throughout this thesis.

It is worth noting at this point that Beaumont and Montgomery included potato consumption in relation to malnutrition – as we will do here - in their 2016 study on the Kilkenny workhouse population (Beaumont & Montgomery 2016). However this involved stable isotope analysis, rather than the direct dental calculus evidence that will be the main focus here – the usefulness of this will be discussed briefly later, in Chapter Seven.

Mark Harrison's recent paper, 'Scurvy on sea and land: political economy and natural history, c. 1780–c. 1850' discusses land scurvy, but only in relation to prisons, with no mention of it affecting the general population (Harrison 2013, 15–7).

Contemporary early-modern research into land scurvy in England did not begin until the 1820s, and this also focused on prisons (French 1993, 1002–3). One of the first ever recorded outbreaks of land scurvy occurred at Millbank prison in the early 19th century, the report on which notes a surety that the disease observed was the same as that which had previously been known as 'Sea Scurvy' (Latham 1825, 5; Carpenter 1988, 99; Magiorkinis et al. 2011, 148; Harrison 2013, 15–7). It has since been called into question whether this was indeed an outbreak of scurvy (Higgins 2006, 530). The symptoms recorded would largely seem to conform to what we would today refer to as scurvy, but there are admittedly some symptoms described that are more akin to what we would label dysentery - it is possible that the prisoners were experiencing both, but that these got labelled as the same condition. Ultimately, the modern day questioning of the historical diagnosis serves as a further reminder of the ways in which language use can change, and how we need to be cautious in retrospectively diagnosing medical conditions from historical symptoms, as this is done out of context.

Further work on scurvy in prisons was carried out in the 1840s by William Baly, who studied the different diets provided to different classes of prisoners and found that scurvy outbreaks only afflicted those whose diet did not include potatoes or onions (Baly 1843, 4; Carpenter 1988, 99–100; Harrison 2013, 17).

Palaeopathology of Scurvy

Osteological Evidence of Scurvy

Scurvy cannot be identified osteologically with any certainty, as there are no pathognomonic changes that affect the skeleton (Brickley et al. 2016, 93). Scurvy causes the production of defective collagen, which negatively

impacts the tensile strength of bones and weakens the capillaries (Brickley 2000; Jaffe 1972; Ortner 2003; Roberts & Manchester 2007; Scully & Hegarty 2003; Stuart-Macadam 1989). If scorbutic for a long enough period of time, even the most minor movements of or pressure on the body can result in haemorrhaging as a result of capillaries bursting (Ortner 2003; Ortner & Ericksen 1997; Roberts & Manchester 2007; Stuart-Macadam 1989). If this haemorrhaging occurs adjacent to bone then the periosteum can raise, which stimulates new bone growth. This new bone growth is known as a 'sub-periosteal haematoma' (Brickley 2000; Jaffe 1972; Lewis 2007; Mays 2008; Ortner & Ericksen 1997; Ortner 2003; Roberts & Manchester 2007; Steinbock 1976).

Some scholars have proposed that a combination of skeletal lesions may be used to indicate the presence of scurvy, but these are not always present (Ortner & Theobald 2000, 37; Waldron 2009, 132; Brown & Ortner 2011; Brickley et al. 2016, 93). It should also be noted that scholars have not yet been able to agree on which combinations of lesions are required for a diagnosis of scurvy to be made. For example, a number of researchers propose that lesions on the greater wing of the sphenoid are the most common lesion present with scurvy (Brickley et al. 2016, 93; Geber & Murphy 2012, 515 & 516), but Brown and Ortner (2011, 205) note that this particular lesion does not have to the present in order for a diagnosis of scurvy to be made. Recently, Moore and Koon (Moore & Koon 2017, 92) proposed that porosity on the basilar portion of the occipital bone could be indicative of scurvy in sub-adults, but again it is stressed that this lesion is not pathognomonic.

Even when some of these proposed osteological signs of the disease can be seen, in sub-adults these can be easily confused with other pathologies, such as rickets - a completely different condition that appears to produce some similar skeletal lesions to those linked with scurvy (Armelagos et al. 2014, 12; Bourbou 2014, 91; Geber & Murphy

2012, 515; Ortner & Theobald 2000, 37). Scholars have acknowledged that establishing what is evidence of rickets and what is evidence of scurvy can be particularly challenging, and sometimes even impossible with currently available techniques (Ortner & Mays 1998, 45; Brickley & Ives 2006, 170; Brickley & Ives 2008, xiv).

An added complication in regards to the confusion of different pathologies is that of co-morbidity, as the conditions that display some of the same, or similar, palaeopathological characteristics as scurvy can also occur alongside it (Armelagos et al. 2014, 12–14; Bourbou 2014, 91; Ortner 2003, 385; Ortner & Mays 1998, 45; Ortner et al. 2001, 349; Roberts & Cox 2003, 400). For example, both scurvy and rickets can cause porous and spiculated bone to form in the eye orbits and on the ectocranial surface of the skull, but it has been known for these two conditions to co-occur in the same individual (Bourbou 2014, 91; Brickley & Ives 2006, 170). It may therefore be impossible in some instances to tell whether there is evidence of one pathology or two present.

The careful analysis and recording of skeletal lesions is unquestionably important in attempting to identify and distinguish between palaeopathological conditions (Ortner 2003, 393). However the fact remains that there is no definitive osteological evidence observable in human skeletal remains that can lead to the determination of scurvy.

The Osteological Paradox

When discussing the osteological evidence of diseases, the osteological paradox (Wood et al. 1992, 343–70) must be remembered: a combination of skeletal lesions thought to be characteristic of scurvy are currently the only method we have for diagnosing the condition in archaeological human populations. However, only those individuals who lived long enough after developing scurvy to begin consuming Vitamin C

again, and subsequently to at least begin to recover from the disease, developed skeletal lesions. Those individuals who died - whether from scurvy or from something else - before being able to recover from the disease, would not have developed any skeletal lesions and would therefore appear from their skeletal remains to have been the healthier individuals (Wood et al. 1992, 352–354).

From this, we know that individuals who show no skeletal signs of pathology may never have suffered from scurvy, may have suffered from scurvy but fully recovered prior to death, may have suffered from scurvy but died from another cause before developing skeletal lesions for scurvy, or may have died from scurvy; the cause of death for those who display pathological lesions consistent with scurvy must have been something else as, in order to form the lesions, their diet must contain some Vitamin C. Ultimately, this means that from the archaeological record we only see evidence for a fraction of the true number of individuals who suffered from scurvy.

A Lack of (Land) Scurvy in the Archaeological and Historical Records

In their book, 'Health and Disease in Britain: From Prehistory to the Present Day', Roberts and Cox (2003, 306) state that no conclusive osteoarchaeological evidence for scurvy occurring in England between c.1550 and c.1850 had been found at the time they were writing. They suggest that this could possibly be due to subtle osteological evidence going unnoticed, or that this could potentially be a reflection of the "generally high socio-economic status" of the skeletal collections known to them for that period (2003, 306). However, in relation to this latter suggestion, some of the collections they included in their study (e.g. Farringdon St Brides Lower and Cross Bones, both of which are included in this project) have been labelled as being of generally low

socioeconomic status. This would therefore suggest that the lack of cases of scurvy identified among these collections was not influenced by socio-economic status, leaving the possibilities that either osteological evidence was not noticed, or there was none to be noticed.

In line with Roberts and Cox's findings, Lomax (1986, 72) and Rajakumar (2001, 2) both separately highlight a complete absence of references to infantile scurvy in the literature from eighteenth century England. Three potential reasons for this are considered by both: the rise of market gardening, a change in post-medieval breastfeeding practices, and the inclusion of potatoes as a weaning food (Lomax 1986, 72; Rajakumar 2001, 2). A fourth possibility could also be possible and should be considered along with these; namely the idea that what we would today call scurvy is being discussed in the contemporary literature, but under a different name.

The concept of market gardening has already been discussed above, but the possibility of this being responsible for the absence of infantile scurvy in the historical record partly hinges on how much the market gardening produce cost, and what percentage of available household income it would have required.

In regards to breastfeeding, both Rajakumar and Lomax claim that the period during which a child was breastfed was extended during the eighteenth century (Lomax 1986, 72; Rajakumar 2001, 2). Breast-milk contains Vitamin C and therefore, prior to weaning, if the mother's Vitamin C levels are adequate, infants should receive enough to avoid developing scurvy (RDA 25-30mg (Thompson & Howard 1998, 4–5, 13, 232)). However, neither Rajakumar or Lomax give details concerning the average age at which children were usually weaned during this period, which is vital in considering the likelihood of their claims that there probably really were no cases of infantile scurvy during the eighteenth

century. They also fail to discuss whether or not there was a difference in weaning ages between the different social classes, and, if there was, what that difference was.

The use of potatoes as a weaning food is perhaps of most interest in regards to this project. However we must remember that there are records of several other weaning foods being used during this period, especially by the poor. For example, 'panada' (mentioned above), is known to have been given to children by mothers who could not afford other options (Fildes 1980, 315; Geber & Murphy 2012, 514; Stevens et al. 2009, 35; Wing & Brown 1979; Wickes 1953, 233). When considering the use of potatoes as a weaning food, it is important to question, as is also the case with the consumption of fresh fruit and vegetables from market gardening, the likelihood of solely infants consuming potatoes, versus the likelihood that other members of the household were also consuming them. As such a small amount of potatoes are needed to obtain the amount of Vitamin C necessary to stave off scurvy, then if adults were also consuming potatoes it would be almost impossible for cases of scurvy to still be appearing in adults. There is no documentary evidence known to the author which suggests that potatoes were used as a weaning food and then not eaten, post-weaning, by older children or adults.

In regards to 'scurvy' in adults, we see mention of this in naval records, but accounts of land scurvy in the contemporary literature seem to be particularly scarce. This could be, as has been mentioned several times now in this chapter, because Vitamin C deficiency was being referred to under a different name, making it somewhat more difficult for us to investigate. Alternatively, it could be the result of the kinds of records that were kept during the eighteenth century; if a person was scorbutic, but they didn't die from it, there is no obvious place where this would be recorded. The Bills of Mortality, as the name suggests, dealt with death,

and there was no equivalent for recording ailments that afflicted the living but which they either continued to live with or recovered from.

Scurvy from a Biomolecular Perspective

The small number of species susceptible to scurvy lack the necessary enzyme, L-gulonolactone oxidase, for converting glucose to ascorbic acid, and so must obtain ascorbic acid from their diet (S. Mays 2008, 223; Nishikimi & Udenfriend 1977, 111; Stone 1966, 346; Weinstein et al. 2001, 3; Wells 1975, 756). Without it, the hydroxylation of the amino acids proline and lysine to hydroxyproline and hydroxylysine, respectively, cannot take place (Akikusa et al. 2003, 76; Brickley & Ives 2006, 163; Hirschmann & Raugi 1999, 898; Steinbock 1976, 253). Hydroxyproline and hydroxylysine are both vital in producing stable, good quality collagen, which is in turn essential for the formation of healthy skin, cartilage, blood vessels and bone (Armelagos et al. 2014, 10; Hirschmann & Raugi 1999, 898; Jacob & Sotoudeh 2002, 67; Ortner & Theobald 2000, 37; Steinbock 1976, 253; Stuart-Macadam 1989, 202).

In recent years, researchers have begun to call for an accurate, reliable biomarker for scurvy, due to the lack of pathognomonic osteological evidence (Armelagos et al. 2014, 15). If this could be established, then it could enable us to understand the true scale of scurvy in the past. Work to understand the ways in which scurvy affects collagen, and the related search for a scurvy biomarker, began some years ago and is still ongoing (Koon 2012; Pendery & Koon 2013, 63). Chapter Six of this thesis will detail attempts as part of this project to systematically test a potential biomarker.

Discussion and Conclusions

This chapter has considered the relationship between nutrition, ill health and mortality in England during the nineteenth century, acknowledging that a lack of available, relevant documentary evidence from that period makes it challenging to know whether these links were acknowledged at the time, or whether we are only able to apply these now, using our current basis of scientific knowledge and understanding. Relatedly, it is known that the population grew massively during this period, but the reasoning for this - as we have seen through the McKeown debate - is still inconclusive.

At a time when 'grocery' options were expanding hugely in England, studying the amount and types of food consumed by the labouring poor comes with a number of challenges - while we know that there were several options in regards to procuring food, evidence for these is scarce. Arguably the most influential and important foodstuff to be considered is the potato; a readily available, good source of Vitamin C, but with the outstanding question of when it became a staple part of the English diet. This question will be revisited in Chapter Seven.

Along with the debate over the reasoning behind population growth, we have considered the ongoing debate between optimists and pessimists in the history of diet. Whether nutritional levels rose or fell overall in relation to the Industrial Revolution calls for more conclusive data regarding real wages, expenditure on food, and calorie requirements during this period, which in turn link to questions about the wider issues of gender division of labour, and child feeding practices.

Research into these different areas has highlighted that we know very little about morbidity. In particular, little is known for sure about scurvy. When investigating occurrences of this disease in the past, we must remember that the use of language can change over time, and so while

we use the term to refer to a Vitamin C deficiency disease, this may not have been the case throughout the long eighteenth century. However, we know from the Bills of Mortality and newspaper advertisements from the period that ‘scurvy’ was a noted condition that could - and did - cause death, and ‘antiscorbutic remedies’ were of interest throughout the country.

Historically, research into scurvy has tended to focus on its occurrence at sea; if land scurvy is considered it is almost always in relation to the Irish potato famine. This project aims to redress this, and to focus on scurvy in populations that may well have been living with this disease in a sub-clinical form as a part of their everyday lives. In regards to what we term to be scurvy, we know how this manifests in the living, but we still have a lot to learn about how it affects the skeleton (both on a macro- and microscopic level). This project can potentially provide evidence of this chronic condition that it has so far not been possible to obtain from historical records or archaeological analyses.

Chapter Five: Research Context - the Site Backgrounds

Introduction

This project grew out of a previous, much smaller study, which looked at the Priory Yard site in Norwich (for map location, see Figure 22 on page 126) (Rowsell 2011). For the current work, this focus has been extended to the metropolitan area, and this chapter will consider the relevant history of the five different sites that have been included; St Thomas' Hospital, Farringdon St Brides Lower, Cross Bones, the Bow Baptists, and Priory Yard. Today, four of these locations are situated in London (see Figure 8). However, during the long-eighteenth century, the village of Bow was located in Middlesex, and so we are essentially looking at four urban sites (three in London, one in Norwich), and one rural location.

Figure 8: Map showing the locations of the four London-based sites included in this study (from left to right: St Thomas' Hospital (green), Farringdon St Brides Lower (purple), Cross Bones (blue) and Bow (red))

Each of these five sites has been recorded in the archaeological literature as being linked to poverty, which we will discuss towards the end of this chapter. The reasoning for selecting five sites archaeologically classed as having links to poverty was twofold; firstly it was hypothesised that poor populations would be more likely to be scorbutic, as a result of having an overall less nutritious diet than the wealthier members of long eighteenth century society, and secondly it was possible to gain access to and sample the human skeletal remains from each of these sites. As is discussed at various points throughout this thesis, much research within the discipline of archaeology is reactive, and we must work with the sample sets that are available.

These sites also covered a long period of metropolitan history, during which diet changed significantly, and so they fit well with the project aims of tracing social and dietary change over the course of the long eighteenth century, and investigating potato consumption during this period through evidence that can become trapped in human dental calculus.

Table 5 provides details of how many individuals were excavated from each site, whether the individuals excavated represent a sub-sample of or the complete burial ground population, the period of use of the site, and the period of use of the site relating to the human skeletal remains excavated.

We will now consider this information in more detail by looking at the age and sex distributions within each burial ground population.

Site	No. of individuals excavated	Sub-sample or complete burial ground population?	Period of use of the site	Period of use of the site relating to HSR excavated, if different
St Thomas' Hospital	227*	Sub-sample	? - 1700	1550 - 1700
Farringdon St Bride's Lower	606**	Complete burial ground population	1624 - 1849	1770 - 1849
Cross Bones	148	Sub-sample	c.1600's - 1853	1800 - 1853
Bow Baptists	416	Complete burial ground population	1814 - 1853	-
Priory Yard	64	Sub-sample***	1697 - 1854	1726 - 1854

Table 5: Details relating to the number of individuals excavated from each site, and the periods of use at each site. *227 individuals excavated but 193 analysed by osteologists **606 individuals excavated but 544 analysed by osteologists * The 64 individuals excavated represent the total post-medieval population of the burial ground, but there were also medieval individuals buried at the site.**

Comparing Demographics at the Different Sites

Age

Figure 9 shows the demographic age distribution at each of the London-based sites included in this study; the Priory Yard data could not be included in this, as different age categories had been assigned during the recording process to those used by the Museum of London Centre for Human Bioarchaeology. This highlights the importance of standardised reporting methods being employed as part of osteoarchaeological work, in order to make comparisons between different human skeletal remains collections possible in a meaningful way.

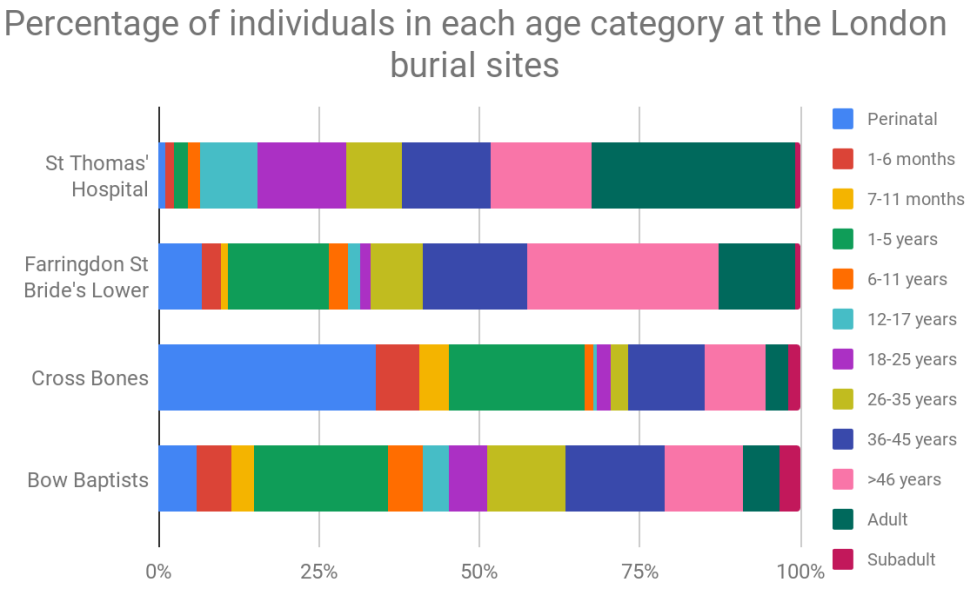


Figure 9: Graph showing the age distributions of all individuals at each of the four London-based sites included in this study

Roberts and Cox (2003, 203) record that roughly 50% of the population in the eighteenth and nineteenth centuries in England would have died before they reached the age of 20. This appears to have been the case at the Bow Baptist site, where just over 50% of the burial ground

population were aged 18 or under. An even higher figure is recorded at the Cross Bones site, where roughly 70% of the burial ground population were judged to be 18 or under at the time of death. Caffell and Holst (2007, 46) state that it is not uncommon to observe a higher rate of infant mortality in the burial grounds of 'lower-class' sections of the population, and this could potentially explain the exceptionally high perinatal numbers excavated from Cross Bones. However, St Thomas' Hospital and Farringdon St Bride's Lower both record much lower levels of sub-adult mortality (16% and 32%, respectively), and yet they have also been archaeologically categorised as being poor or having links with poverty. One possible explanation for this could be that sub-adult bones - being smaller and more fragile than adult bones - do not typically survive as well in the archaeological record (Caffell & Holst 2007, 46), and therefore may be being underrepresented at these two sites.

If we consider a simpler distribution of adults versus non-adults, rather than breaking the populations down into specific age categories, then we are able to compare the four London sites with Priory Yard (Figure 10).

We can see from this that the Priory Yard distribution of adults versus sub-adults sits somewhere between the Bow Baptists and Farringdon St Bride's Lower, at 39%. Again this is below the average of 50% stated by Roberts and Cox, but that could be due to poor preservation (although only nine individuals at Priory Yard (14% of the 63 individuals recovered from the site) were recorded as having a 'poor' or 'very poor' level of preservation, with all others being graded between 'moderately well', 'well' and 'very well' preserved) (Caffell & Holst 2007, 5).

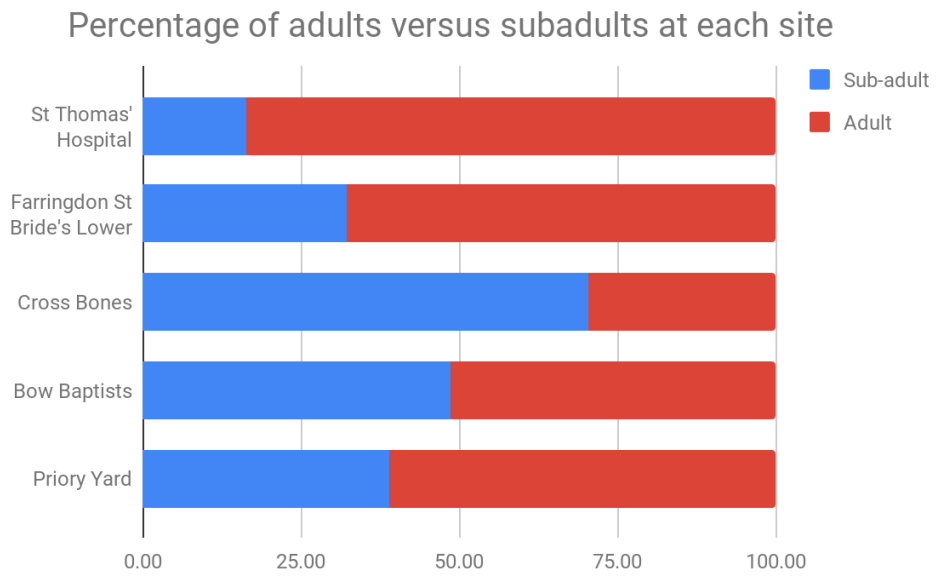


Figure 10: Graph showing the distribution of adults versus sub-adults at each site included in this study, to enable a comparison between Priory Yard and the London-based sites

Both of these graphs - but particularly Figure 9 - show a wide variation in age distributions between the different sites, which arguably makes them difficult to compare, as the populations may have been formed due to different circumstances. St Thomas', for example, has a particularly low number of sub-adults in the burial ground population, which could be due to the nature of it being linked to a hospital. If this is the case, then it would be difficult to use data from this population to say anything meaningful about life in eighteenth century England, or to compare the individuals from this site with non-hospital burial ground groups. There is limited published data on hospital burial grounds in England during this period, but if we compare the St Thomas' data to that from the nineteenth century London Hospital burial ground (Figure 11), then we see that the two are quite different.

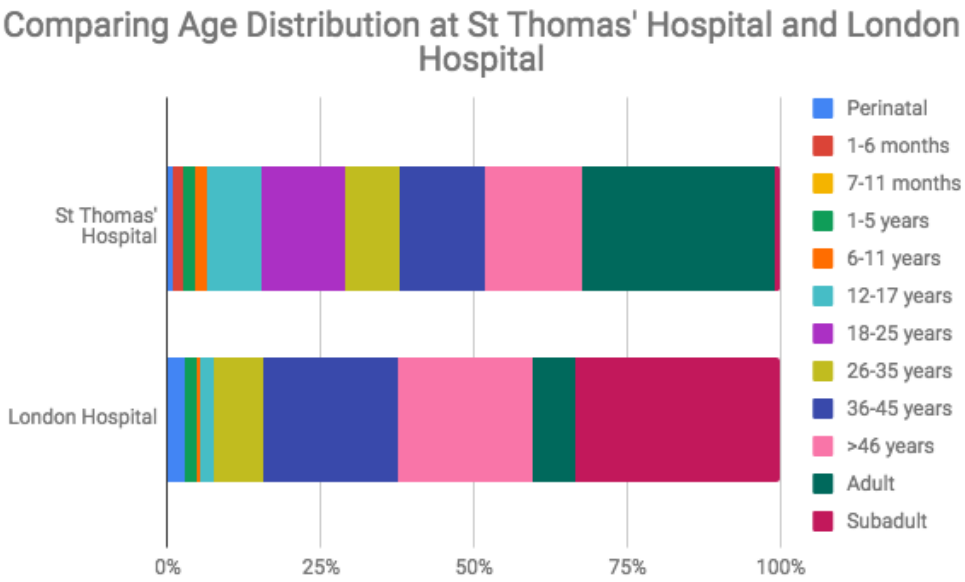


Figure 11: Graph comparing the age distribution at St. Thomas' with that at the London Hospital

Roughly 41% of burials at the London Hospital were aged under 18, in comparison with the much lower figure of 16% at St Thomas'. It should be noted that a large number of disarticulated remains were excavated from the London Hospital, which have not been included in the calculations for Figure 11 (Fowler & Powers 2012, 33). This decision was taken as these would confuse the comparison due to there being no disarticulated remains identified within the three body trenches from St Thomas'. However the presence of these at the London Hospital (resulting from limb removal operations and post-mortem dissections (Fowler & Powers 2012, 34)) and the absence at St Thomas', could be worthy of further consideration in the future.

Sex

Figure 12 shows the differences in sex distribution at the different sites. The most immediate point to note is that almost 50% of the adult individuals excavated from the St Thomas' Hospital burial ground could not be sexed, which makes it difficult to meaningfully compare the sex

distribution for this site with any of the other four. To date, there is no published archaeological information on St Thomas' that explains this.

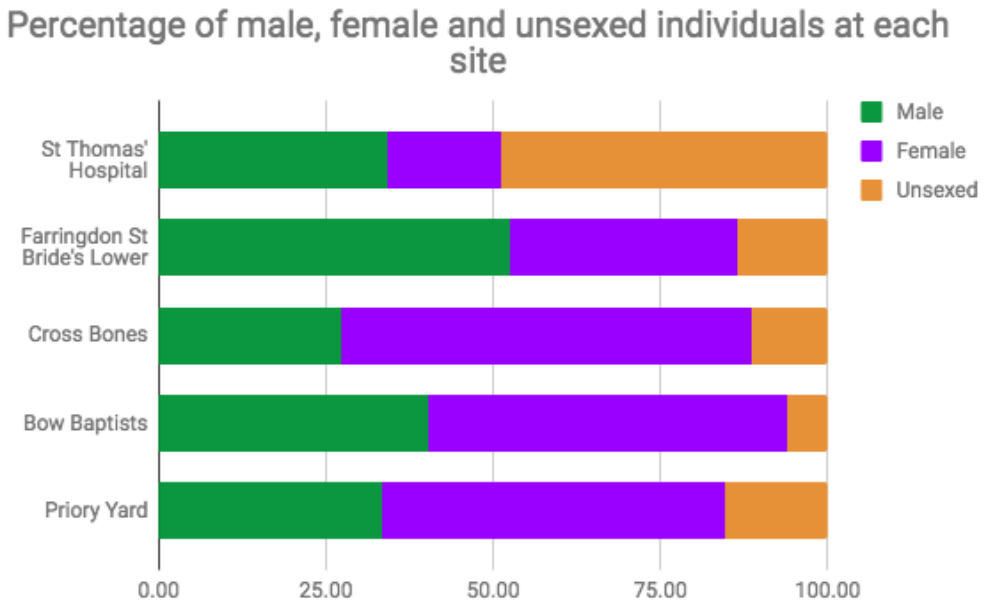


Figure 12: Graph showing the distribution of male, female and unsexed adults at each site included in this study

The Cross Bones, Bow Baptist, and Priory Yard burial ground populations all contained a higher proportion of female individuals than male, whereas Farringdon St Bride's Lower contained a higher number of males.

Historically speaking, the beginning of the seventeenth century saw more males than females living in London, however this had changed by the end of the seventeenth century as a result of expanding employment opportunities for women (Sharpe 2016, 117). Farringdon St Bride's Lower goes against this, as we can see that there were more males than females buried there, but the burial ground was in use during the latter half of the eighteenth century. Cross Bones could potentially fit the pattern just described, as there were more females than males buried there. However if we were to assume that a greater number of females

was linked to women moving into the city due to an increase in employment opportunities, we wouldn't expect them to then be buried in a pauper's burial ground. It is not possible to apply this theory of a shift away from a male-dominated population to either Bow or Priory Yard, as they were both located outside of London.

In regards to the Bow and Priory Yard sites, a potential reason for the higher proportion of females to males could be the fact that they were Baptist burial grounds: McKinley (2008, 102–4) notes that there were political, social, and economic consequences for males joining a Dissenting congregation, even after the Toleration Act of 1689. While this could reasonably have affected some of the individuals buried at Priory Yard, the individuals buried at the Bow Baptist site were living at least 100 years later. It would be hoped that this alleged discrimination would not still have been continuing by that time. Subsequently, this religious discrimination may not be an applicable consideration. Furthermore, in regards to economic consequences it should be noted that Watts (1985, 380) declared General Baptists to be "...probably the poorest of all the Dissenting denominations…", but this cannot have affected all of the individuals attending the two Baptist chapels discussed here, or otherwise the major building work undertaken at each would not have been financially viable.

Sampling Strategy in Relation to Demography

Ideally, all individuals exhumed from all sites would have been sampled for biomolecular analysis (detailed in Chapter Six). However this was not possible, due to particular restrictions placed on sampling by Museum of London Archaeology and the Museum of London Centre for Human Bioarchaeology; namely that a maximum of 25% of each age category be sampled, that no rib with visible pathology be sampled, that no individual with only one rib be sampled, that no complete ribs be sampled,

that no fragment with either a distal or proximal end present be sampled (as these are used for osteological age determinations), and that no named individuals be included. The Museum of London Centre for Human Bioarchaeology and Museum of London Archaeology receive a far larger number of research access requests every year than is received by any other organisation or institution housing human skeletal remains (HSR) in the UK. HSR are a finite resource, and these restrictions help to ensure that there can be analytical opportunities for future researchers (both osteological and biomolecular). A further restriction that was completely out of anyone's control was whether or not an individual that could potentially be sampled had existing rib bones. While obviously this unavoidable restriction of ribs being initially present was also placed on the Priory Yard samples, the Norfolk Museums Service's only other specific sampling restrictions were that no rib with visible pathology be sampled, and that no individual with only one rib be sampled.

With this in mind, the maximum numbers of samples were collected for all age categories from all sites. Much of the time, after taking all of the restrictions into account, there was less than 25% available for sampling and therefore no choices on which specific individuals should be sampled had to be made. However in the instances where more than 25% was available, two different approaches were used for subadults versus adults. With adults, the number of individuals available for sampling was filtered firstly by whether or not it had been possible to determine sex: the individuals recorded as either male or female were prioritised over those where sex could not be established. This was in order to allow potential comparison of males and females during the data analysis phase of the project. The next step of the sampling strategy designed for use here was that if more than 25% still remained, that there should be an even split between the number of males and the number of females sampled, for the same reason as just described. The specific individuals would be

chosen using a random number generator, to avoid any form of unconscious bias. The strategy for subadults was somewhat simpler, as the sex issue did not need to be accounted for: a random number generator was used to determine the specific individuals to be sampled, again avoiding any potential unconscious bias.

Table 6 compares the number of individuals excavated at each site to the number of individuals sampled as part of this project.

Figures 13, 14, 15 and 16 compare the age distribution for each site population with the age distribution of the actual samples collected.

All Priory Yard samples where an appropriate rib was available for analysis were sampled. Unfortunately, as has already been mentioned, the way in which the age of each individual was recorded does not allow for a similar graph to those in Figures 13, 14, 15 and 16 to be produced.

Site	No. of individuals excavated	No. of individuals sampled	Percentage of individuals excavated that were sampled
St Thomas' Hospital	227*	38	16.74%
Farringdon St Bride's Lower	606**	84	13.86%
Cross Bones	148	40	27.03%
Bow Baptists	416	61	14.66%
Priory Yard	64	22	34.38%

Table 6: Details of the number of individuals excavated versus the number sampled for each site

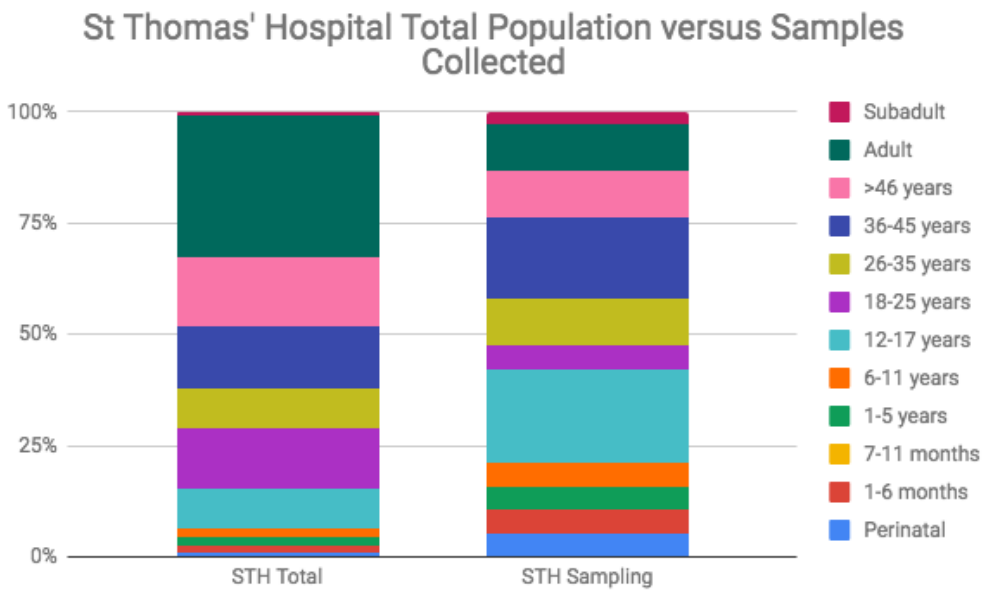


Figure 13: Graph showing the age distribution for the total human skeletal population excavated from the St Thomas' Hospital burial ground, versus the age distribution for the samples that were actually collected

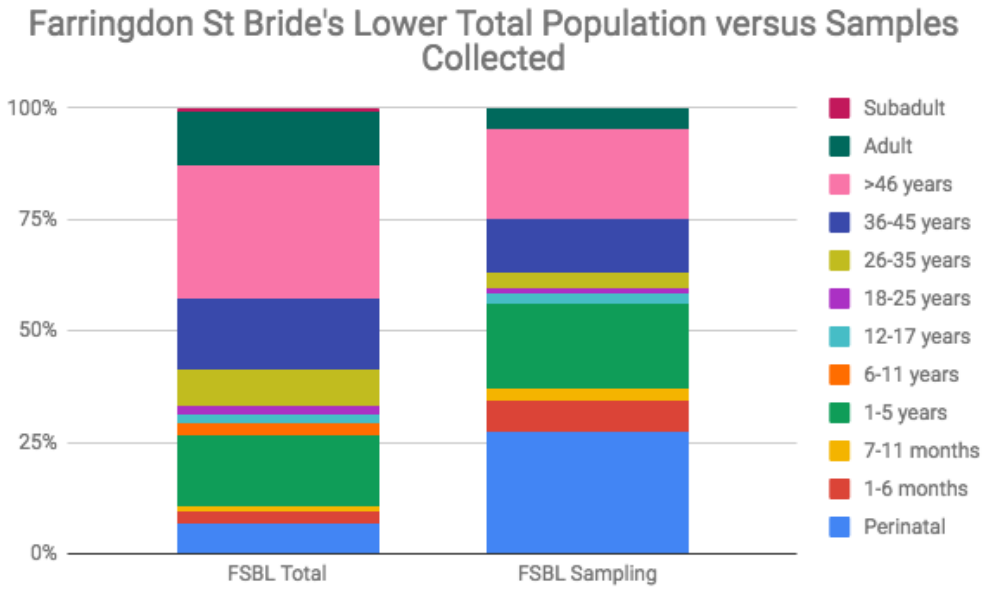


Figure 14: Graph showing the age distribution for the total human skeletal population excavated from the Farringdon St Bride's Lower burial ground, versus the age distribution for the samples that were actually collected

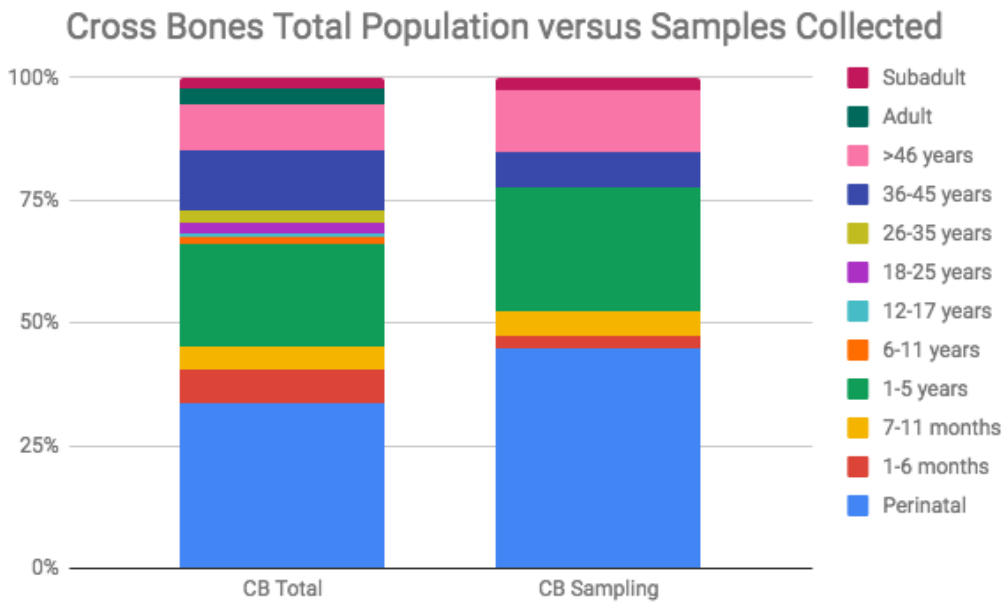


Figure 15: Graph showing the age distribution for the total human skeletal population excavated from the Cross Bones burial ground, versus the age distribution for the samples that were actually collected

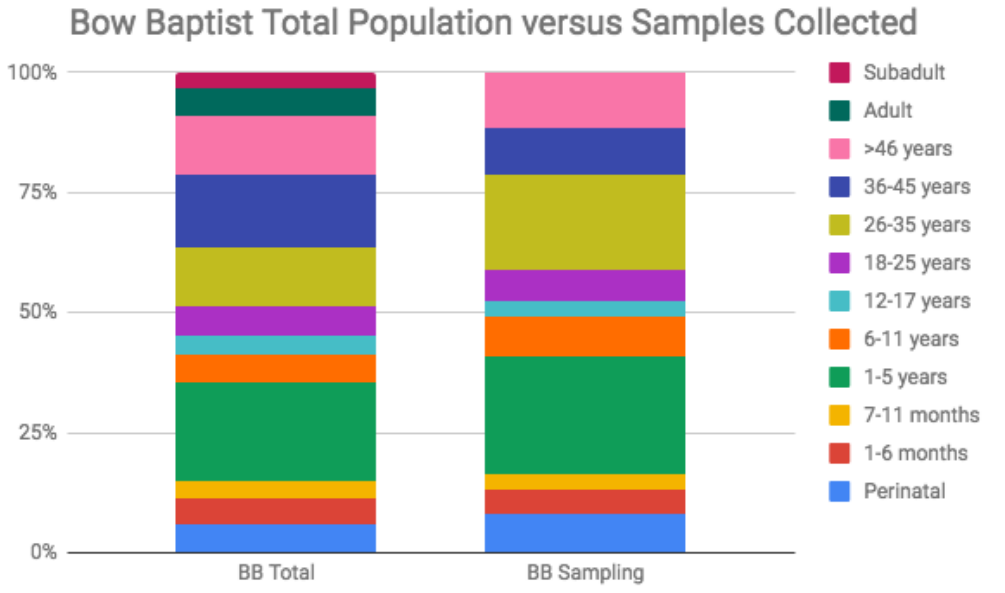


Figure 16: Graph showing the age distribution for the total human skeletal population excavated from the Bow Baptist burial ground, versus the age distribution for the samples that were actually collected

These figures show that the distribution of age across the sample sets collected do not match the distributions of the total populations. The aim was to obtain the maximum number of rib samples possible within the sampling restrictions, in order to have the best chance of saying something meaningful with the biomolecular data produced as part of Chapter Six. The sampling restrictions would not have allowed for the sample set distribution to match the population distribution while also obtaining the maximum number of samples (e.g. in the case of Cross Bones, nearly 50% of the samples at that site were perinates, but the sampling limit for each age category was 25%, so this would have drastically limited the potential sampling numbers for the other age categories at that site). An alternative approach, to sample an even number of individuals from each age category, would also not have been possible as some age categories had no individuals with a suitable rib that could be analysed.

Having explored the biological breakdowns of the different burial ground populations, we will now consider the osteologically identified prevalence of scurvy at each of these sites.

Palaeopathology – Scurvy

While the osteologists who analysed the human skeletal remains at each of the five sites recorded much information on the different pathologies that they observed, it would unfortunately be beyond the scope of this project to detail and compare them all, and could detract from the central focus. As we are primarily concerned with evidence of scurvy, we will subsequently only consider the noted instances of this particular pathology here.

Figure 17 compares the percentage of individuals from each population who were osteologically determined to have scurvy, versus those who seemingly showed no potential signs of this disease in their skeletons.

We can see that, while scurvy was recorded in individuals from all five sites, a vastly smaller fraction of each population was thought to have shown signs of the disease than the fraction that did not display any visible skeletal indicators. This is unsurprising; given the difficulty in identifying scurvy in any consistent, reliable way from skeletal remains, it should be expected that the paleopathology data would suggest a low prevalence rate.

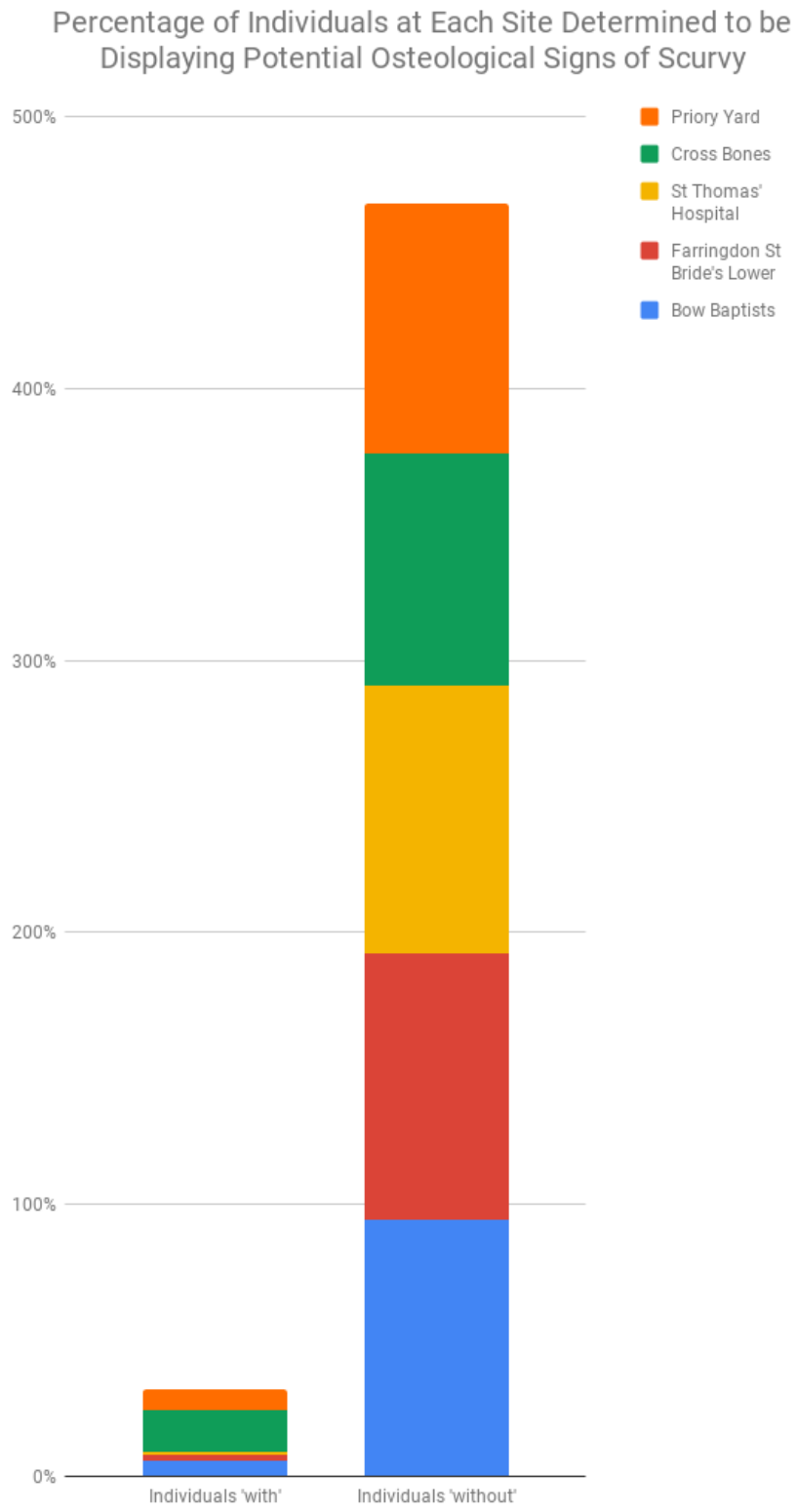


Figure 17: Graph showing the percentage of individuals from each of the five sites osteologically determined to show potential signs of scurvy, versus those who showed no osteological signs of the disease

When comparing the prevalence of scurvy recorded in sub-adult remains with that recorded in adult remains (Figure 18), we can see that this disease appears to be far more common in subadults, having not been recorded at all in adults at three out of our five sites.

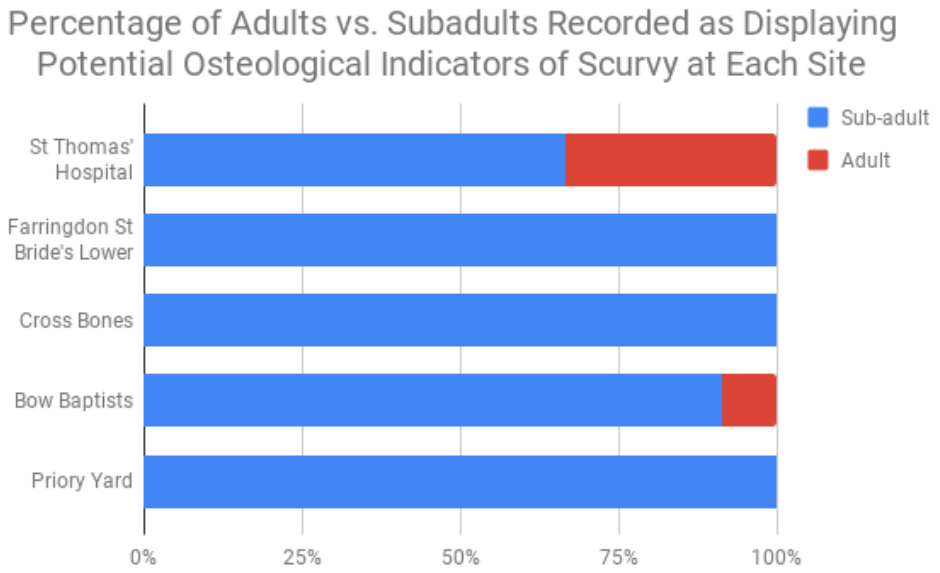


Figure 18: Graph showing the percentage of adults versus subadults osteologically determined to be displaying signs of scurvy at each of the five sites included in this study

However it is worth noting that recent work by Moore and Koon (2017) has suggested that it may be possible to identify scurvy in subadults if the basilar portion of the occipital bone is present and intact, although the same criteria do not appear to be applicable to adult skeletal remains. It has been noted (Pendery & Koon 2013, 62) that subadults are far more likely to display osteological evidence of scurvy, due to the fact that they are still growing, and are therefore laying down new bone at a much more rapid rate that adults. With this information in mind, it is also unsurprising that significantly more potential instances of the disease are observed in

subadults than in adults, and this further reinforces the need for a reliable scurvy biomarker to be established.

Research Contexts of the Five Sites

Figures 19 and 20 provide details of how each site - and the maximum potential Life Period (LP) of the individuals buried at those sites - fits into the wider context of London and Norwich, respectively, during the course of the Long Eighteenth Century.

The ‘Life Period’ reflects the ‘extra’ time before burials at a site began, when people excavated from that site could have been alive. Events occurring during this time would have shaped the lives of some of the individuals interred at the site, and so they are relevant to our considerations. This has been calculated bearing in mind the osteological restriction of not being able to reliably age people over the age of 45 based on skeletal evidence. For example, the earliest burials at Farringdon St Bride’s Lower for our sample set would have taken place in 1770; if any of those individuals buried in that year were osteologically aged to ‘45+’, then we would be interested in a Life Period of 1725 - 1770.

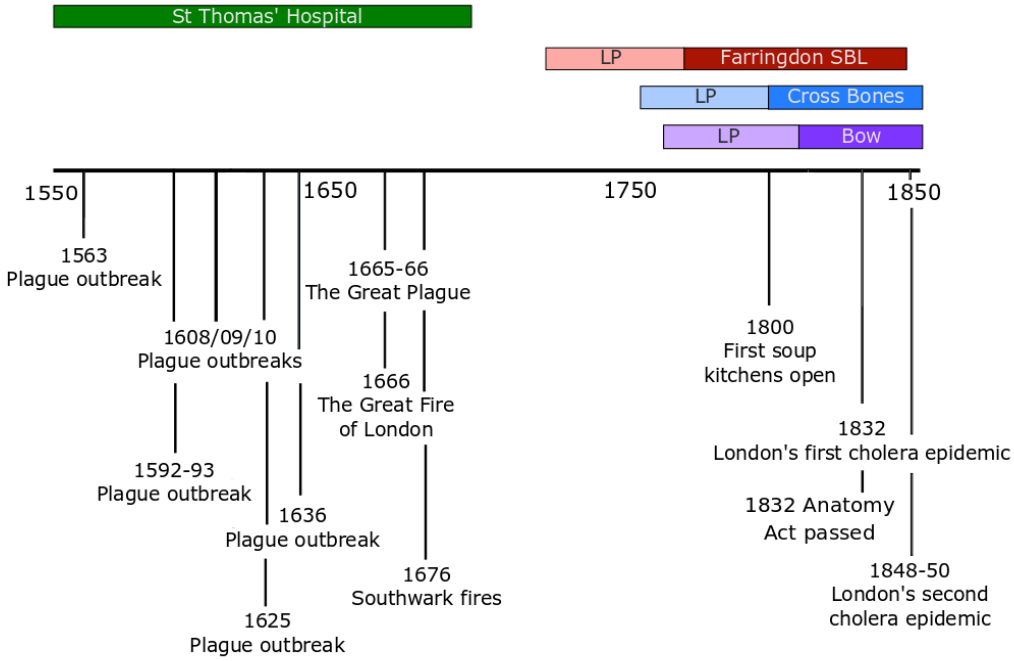


Figure 19: Timeline of London events, 1550 - 1850. The darker coloured bars represent the period of interest of use for each burial ground, with the lighter coloured bars denoting the maximum potential life period (LP) of individuals buried at each site

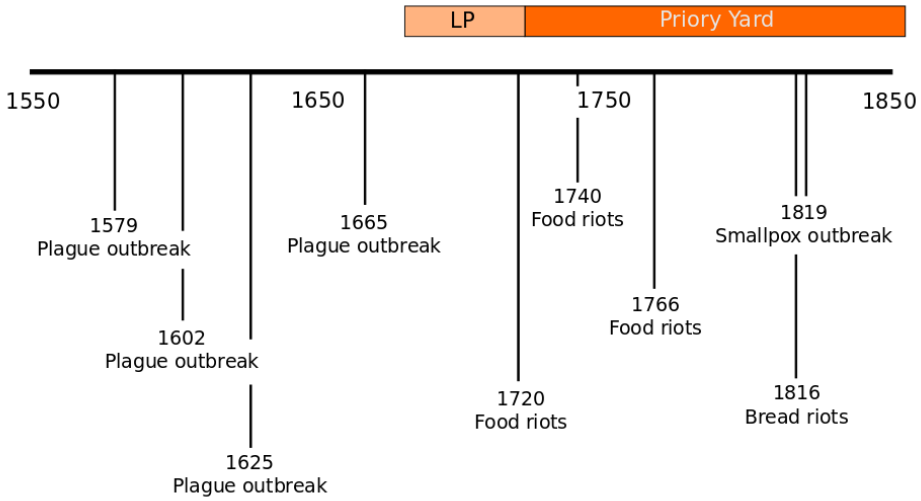


Figure 20: Timeline of Norwich events, 1550 - 1850. The darker coloured bar represents the period of interest of use for the burial ground, with the lighter coloured bar denoting the maximum potential life period (LP) of individuals buried at the site

St Thomas' Hospital

St Thomas' Hospital is the earliest of all the sites included here, with the human skeletal remains (HSR) having been dated, based on the typology of the pottery that was present in the grave cuts, at between 1550 and 1700. One of the pottery sherds was stamped '1579', giving us a *terminus post quem* for the burials (Jones 1991, 13). The burial ground does not appear on the 1746 Rocque map, suggesting that it had gone out of use prior to the major building work that took place around the turn of the eighteenth century (Jones 1991, 13). Evidence for the specific location and size of the hospital and its burial ground is also frustratingly vague; there appear to be no accurate maps or hospital plans from the 17th century still in existence, and the building of London Bridge Railway Station in the late 19th century destroyed much of the archaeological evidence pertaining to street layout, which could have been useful here (Jones 1991, 13).

The excavations carried out at the site in 1991 revealed at least 15 burial groupings in three distinct burial pits (Jones 1991, 28). It has been suggested that these 'body trenches' could have been the burial place of plague victims, due to the seemingly hurried nature of their interment; for example, in some cases, there was no layer of earth placed between one row of bodies and the row that was buried atop them, and in another trench, the individuals were buried with their heads to the east, rather than to the west, as would be traditional in a Christian burial ground (Jones 1991, 31).

Location of the Site

During the 150 year period that the burial ground may have been in use for, St Thomas' Hospital was located on St Thomas' Street, in the parish

of St Thomas', in the north of Southwark. The parish itself was small, covering only eight acres (McInnes 1963, 205). However seventeenth century north Southwark was densely populated and hygienically unpleasant. One reason that this area was so busy and had such a large population during this period is the fact that London Bridge was 'the only bridge over the Thames in the central London area' until Westminster Bridge was built in 1750 (Jones 1991, 9).

At this time there were open sewers, tanneries and tallow smelters throughout the parish (McInnes 1963, 38 & 65; Jones 1991, 11). These crowded, unhygienic living conditions, coupled with a close proximity to animals such as rats, found commonly in this area at this time, meant that disease outbreaks were common - particularly plague, which is recorded to have affected the Southwark population every nine to ten years - and spread easily (Jones 1991, 11).

The residents of the parish were mainly tradesmen, with the local industries being largely focused on brewing and tanning, and by all accounts were more stable than the stereotype (e.g. Payne 2008, 30; Handy 2009, 325). For example, McInnes (1963, 214) notes that they were generally good tenants, excluding the minority of individuals who had a tendency to disappear on rent day.

History of the Hospital and Burial Ground

The original St Thomas' Hospital, dedicated to St. Thomas the Martyr, is believed to have been founded sometime around 1106, as a hospital for the sick and the poor of St Thomas' parish. The hospital then moved to a new site in the 13th century, before being closed down in 1540 as part of Henry VIII's dissolution of the monasteries. Eleven years later, in 1551, the hospital was reopened as one of three Royal hospitals in the city (as opposed to being a voluntary hospital), this time dedicated to St. Thomas

the Apostle, but still with the aim of providing medical treatment for the poor (Pennant 1813, 69; Holmes 1896, 171).

Upon reopening, St Thomas' (along with four other hospitals), was granted some land and properties, which when rented out provided the hospital with a generally steady source of income (http://www.londonlives.org/static/Hospitals.jsp#Voluntary). However, this income was not enough to cover all of the hospital's costs, and so it was still necessary for fees to be charged to the patients.

In 1605, St Thomas' Hospital established the first known Hospital Committee, who were responsible for deciding on issues deemed to be of relevance and importance (Hughes 2002b).

The Privy Council requested on November 11th, 1664, that only servicemen be admitted to St Thomas' for the duration of the war, possibly referring to the Anglo-Dutch war (Hughes 2002b). However, while the hospital did admit them, they could not afford to admit solely them. The Great Plague that struck London in 1665 and the Great Fire of London that occurred in 1666 then caused further financial strain, with many of the properties in the City that were owned by the hospital either catching fire or needing to be blown up as part of the attempts to stop the fire from spreading (McInnes 1963, 53–7; Hughes 2002b). There were further fires in Southwark in 1671 and 1676, which again affected property owned by the hospital without damaging the hospital itself, although it did get worryingly close (McInnes 1963, 53–7; Hughes 2002b).

By the beginning of the eighteenth century, the hospital was in desperate need of repair, and so much of it was rebuilt at this time - in brick, rather than wood: a decision that resulted from the devastation caused by the seventeenth century fires. The Charing Cross Railway Company

purchased the site on which the hospital stood in 1859, in order to build London Bridge Station, resulting in another move, this time to the south bank of the Thames, just south of Westminster Bridge and opposite the Palace of Westminster, where the current hospital stands today.

Jones (1991, 19) concluded that St Thomas' Hospital was originally burying their dead in the graveyards of St. Mary Overie and St. Margaret's. However, those in charge of St Thomas' at some point realised that if they had their own burial ground, they could 'secure the burial fees' for themselves. It was subsequently decided that the hospital would have its own burial ground, but negotiations were had with the canons of St. Mary and the rectors of St. Margaret's, in order to reach a compromise whereby compensation for their losses was paid to them.

Burial at St Thomas' Hospital

Jones (1991, 22) records that the minutes from the governors' meeting on December 13th, 1601, detail that complaints had been made regarding "the churchyard which had become unpleasantly full". This is a potentially confusing point, as elsewhere Jones states that the hospital burial ground is never referred to using the term 'churchyard', and yet the discussion is part of the governors' meeting, suggesting that this is concerning ground used by St Thomas' Hospital. The governors concluded that the minimum depth for burial should be two feet (this was later increased to five feet), but it is not known whether this decision was adhered to (Jones 1991, 22).

From the limited available evidence, it appears that St Thomas' Church and St Thomas' Hospital were very much linked - there are references in the vestry records to the hospital (Dopson 1949, 69), and the Minutes of the Court of Governors for the Hospital discuss at length the rebuilding of the Church in the late 1600s (Anon 1697b; Anon 1699a; Anon 1699b).

This suggests that the burial ground could have been used by both the hospital and the church. However, a letter sent by the St Thomas' Hospital Archivist in 1952 states that there were two burial grounds in use at the area by the end of the 17th century - one used by the hospital and one by the church (McInnes 1952). Frustratingly, Miss McInnes does not detail how she knows this, and there appears to have been no further communication on the matter.

In 1632, further complaints regarding the state of the burial ground were noted, concerning the issue of burial depth and suggesting that burials at the time were so close to the surface that putrefaction could be observed by the living (Jones 1991, 22). The governors' subsequently ordered that the minimum burial depth from then on should be two feet (Jones 1991, 22). In order to investigate whether or not this was actually carried out, research into the depth at which the first burials were uncovered during excavation, along with establishing the change in ground level from the seventeenth century through to the present day, would need to be completed. Unfortunately this was beyond the scope of this project.

The minutes from a meeting of the court of St Thomas' Hospital governors, on August 26th, 1697, detail the decision "that all such Corps be laid and interred in Coffins and not buried without as formerly has bin used" (Anon 1697a). Jones (1991, 30) states that this meeting took place after the burial ground that is being studied here had ceased to be used. However there is no justification given for this, and the fact that a number of coffin nails were discovered during the excavation could suggest that the meeting took place while the burial ground in question was actually still in use. Hughes (2002a) asserts that this decision coincides with another burial ground being opened, but even so, it could still be possible that some individuals were buried in coffins in the burial ground that we are concerned with, following the meeting but prior to the closure of the existing ground.

Patients accepted for treatment at St Thomas' hospital were expected to provide evidence of a guarantor who would cover their burial expenses if they died, which was very often their parish (Anon 1699c). In these cases, a certificate from the parson of the parish was requested, which would confirm that the parish would either receive the individual back if they recovered, or pay the burial fees if they died (Jones 1991, 23). However the parish burial - designed to provide burial when families of the deceased could not afford to cover the costs themselves (Laqueur 1983, 109; Harding 2002, 61; Boulton 2014, 195) - was something that many of the poor would go to great lengths to avoid.

The Plague Pit Theory at St Thomas'

As mentioned earlier in the chapter, it has been suggested that the individuals buried in the three pits being discussed here may have been plague victims (Dawson 2002, 5). However, as Jones (1991, 31) acknowledges, documentary evidence relating to the hospital states that plague victims were not to be admitted, and literature detailing the various burial grounds accepting the plague dead do not make mention of St Thomas' (e.g. Defoe 1722; Harding 1993). It would therefore seem to be an unlikely possibility that the St Thomas' human skeletal remains were plague victims.

One possible way to investigate this would be to compare St Thomas' to a known plague burial ground. Few of these have been analysed, but the Moorfields burial ground was excavated in 2015, which was a known location for the burial of victims of the Great Plague (Hartle 2017, 136–40). Comparisons with this burial population may hint at whether the St Thomas' Hospital individuals could actually have been plague victims, and the body trenches therefore be plague pits. There were 3354 articulated human burials excavated from the Moorfields site, only some

of which relate to the plague burials (Walker 2017, 95). Unfortunately, the way in which the data has been reported, it is not possible to extricate the information regarding plague deaths from 'general' deaths (Walker 2017, 95 & 98), and so a comparison between Moorfields and St Thomas' Hospital is not possible at the present time.

If the St Thomas' Hospital body trenches did contain the bodies of plague victims then they must all date to a particular year, and as we know which years plague outbreaks occurred in London (see Figure 17), this would massively narrow down the window of consideration for this site. However, given the overall lack of supporting evidence for this theory, there will be no further discussion of plague as part of this project.

Farringdon St Bride's Lower

The Farringdon St Bride's Lower site was used for burial from at least 1624 up until 1849. After the Great Fire of London in 1666, the St Bride's Lower site was one of three burial areas in use in the parish during the early modern period, the others being the St Bride's Church main churchyard, and the St Bride's Church crypt (Mant & Roberts 2015, 191; Miles & Conheeney 2005b, 4). The site was known by several names over the duration of its use; primarily as the 'lower graveyard', but also as the 'Shoe Lane ground', 'new churchyard', and 'Fleet Market ground' (Miles & Conheeney 2005b, 1). The specific time period that we are interested in for this site covers roughly 1770 to 1849, which will be discussed further below.

Location of the Site

The St Bride's lower graveyard, as we will refer to it here, was located on Farringdon Street, in the parish of St Bride's, to the west of the modern day City of London.

Miles and Conheeney (2005b, 4) state that in 1800, roughly the middle of our period of interest regarding this particular area of London, there were 881 houses in the parish (830 of which were occupied, with the remaining 51 being unoccupied). They also note that there were “1592 families, 3424 males and 3654 females, giving a total population of 7078” living in the parish in 1800, but there must also have been children living there, meaning that the total population would have been higher than 7078.

According to census data for 1831 to 1851, the population of the parish declined from 6860 men and women in 1831, to 6039 men and women in 1851 (Forbes 1972, 16). However, Forbes (1972, 16) puts this down to the shift in central London during this period away from primarily residential to primarily commercial.

History of the church and burial ground

The lower graveyard was consecrated on 2nd August 1610 by the Bishop of London, the land having been provided by the third Earl of Dorset, Richard Sackville. His reasoning for donating the land to the church was that he wanted to prevent any further burial in the existing burial ground, as it was becoming over-full and unsightly (Miles & Conheeney 2005b, 1). This ground was extended on July 28th, 1611, expressly to be used either for burial or for the relief of the poor (Miles & Conheeney 2005b, 1). However, one of the Minute Books for the Vestry of St Bride’s records that, by 1654, this extra land was being used for neither. For example, it was recorded in the same Minute Book that in April of that year, soldiers were ordered to cease exercising on that ground (Miles & Conheeney 2005b, 2).

As with all of the other London sites being considered as part of this project, the Great Plague in 1665 had a significant impact on the lower churchyard. The nearby St Dunstan's parish was granted use of the lower churchyard at this time to bury their dead who had fallen victim to the infection. This obviously would have put extra pressure on space, and it is recorded that by August that year, the lower churchyard was getting rather full (Miles & Conheeney 2005b, 2). This is not so surprising when considering that the Bills of Mortality for 1665 record 2111 deaths in the St Bride's parish that year, roughly 68% of which were due to plague (Miles & Conheeney 2005a, 25).

Just as Richard Sackville had complained over 150 years earlier about the upper churchyard, on July 17th, 1750, a Mr Hawes and a Mr Frond registered complaints to the Vestry regarding overcrowding in the lower graveyard (Miles & Conheeney 2005b, 3). Over the next few years, these complaints became more and more common as more burials took place. Eventually, on April 26th, 1753, the Vestry ordered that the burial ground be closed immediately and that an alternative be found. The suggestion was made that an area of the original upper churchyard be used, but with the same fees that were being charged for the lower graveyard (Miles & Conheeney 2005b, 3). However, the Vestry Minutes from May 7th, 1806, note the establishment of a committee to investigate the conditions of the lower graveyard, with the aim of providing guidance on the burial of the poor, suggesting that this order may not have been adhered to (Miles & Conheeney 2005b, 3).

In line with the rest of London, the decision was made in 1849 to cease burying the dead within the city, and to instead inter people at the City of London Cemetery that had been established at Manor Park (Miles & Conheeney 2005b, 4). The last burial at St Brides - it is unconfirmed whether this was in the lower graveyard or upper churchyard - took place on June 24th, 1849 (Miles & Conheeney 2005b, 4).

Burial at Farringdon St Bride's lower graveyard

Cauch (1840, 33) recorded the burial fees at St Bride's in the mid-nineteenth century, towards the end of our period of interest (Figure 21). Miles and Conheeney (2005b, 2) note that the churchwardens accounts at St Brides started in 1639, and so should have been long-established by the period that we are interested in here. Within these accounts will be recorded the fees paid for each burial at the site. It has unfortunately not been possible to view and analyse these in order to compare earlier fees - for example from the late 1700s - with those listed by Cauch.

ST. BRIDE'S, FLEET STREET.

	Parishioners.	Non-Parish.
Upper Ground	£.1 10 0	2 12 0
Lower Ditto	0 11 6	0 19 6
South East Vault	3 9 0	5 1 8
New Vault	3 19 0	5 18 0
South West Vault	2 15 0	3 15 4
Chancel and Aisle	4 7 0	7 6 0
Lady Jersey's Vault	4 9 0	6 8 0

Including Desk Service and Bell. Bury at 4 o'Clock every Day.
Early Dues 8s., or Fittings.

Mrs. Good, Sextoness, *Bride Lane.*

Figure 21: Details of the different burial fees at St Bride's in 1840 (Cauch 1840, 33)

However we can see that in 1840, burial in the lower graveyard was cheaper than burial in the upper churchyard, presumably because it was reportedly a less desirable place in which to be buried (Cauch 1840, 33; Miles & Conheeney 2005b, 4). It is worthwhile noting that the cost for non-parishioners to be buried there was higher than the cost for parishioners, but it was still a fairly cheap burial location compared to similar burial grounds in nearby parishes (Miles & Conheeney 2005b, 4).

The ‘Traffic in Corpses’

This brings us on to the consideration of what has been termed the ‘traffic of corpses’, whereby an individual is buried in a different parish to the one in which they lived. With an expanding population, burial space in London became increasingly limited during the long eighteenth century, so for many individuals it was necessary to search out an affordable burial location (Boulton 2014, 182). Legally, no one could be refused burial in their local churchyard; if an individual was deemed to be too poor to be able to afford their own burial fees, they could be granted a parish burial (Harding 2002, 59–61, 135–6, 207, 270–1; Boulton 2014, 193). However, as has already been briefly mentioned in this chapter, a parish burial was something that the vast majority of the population wanted to avoid. A large part of the general horror with which they were regarded seems to have come from the social stigma associated with not being able to pay for your own funeral, the sense of failure that created, and the negative impacts that this would have on the reputations of your surviving relatives (Laqueur 1983, 109 & 120; Roberts 1990, 87; Hurren & King 2005, 322). Therefore if it was cheaper for an individual to be buried in a different parish to the one in which they had lived, that would have been the preferred option.

Financial considerations were not the only reason that someone might be buried in another parish; religion could also play a part in wanting to be buried in a different parish. For example, wanting to be buried in a Baptist burial ground and there not being one in the parish in which you lived. Other possible reasons for an individual being buried in a different parish to the one in which they lived around the time of their death could be wanting to be buried in the same place as family, or wanting to be buried in the parish in which they were born (Schofield 1984, 50–2).

This traffic in corpses is particularly difficult to trace. The parish in which the burial took place would have recorded the individual’s details in their

burial register, but not necessarily as an ‘imported’ burial, and it’s highly uncommon for the parish where the individual lived to have recorded that they died there and were subsequently buried elsewhere (Boulton 2014, 186). Even when the latter is the case, if the location where the individual was eventually buried is not recorded, it becomes an incredibly time consuming task to work this out.

This traffic in corpses, common throughout the long eighteenth century, reminds us that we cannot assume that everyone buried in a particular graveyard lived their whole life nearby; there are many potential reasons why someone would be buried outside of the parish in which they last lived. Unfortunately this is difficult - in some cases impossible - to accurately trace, and will have an affect on what what we can say about a local population based on skeletal data (whether osteological or biomolecular). However, archaeological study of human skeletal remains is still a valid and useful line of investigation, and still sheds light on life in the past in a way that historical documents alone cannot.

Cross Bones

The burial ground at Redcross Way, in the Southwark area of London, is commonly referred to as the ‘Cross Bones’ burial ground. Burial at the site began in the seventeenth century, but the individuals that have been included in this study were interred considerably later, between roughly 1800 and 1853, by which time the site is thought to have been declared a paupers’ burial ground.

Location of the Site

The Cross Bones burial ground was situated on Redcross Way, in the parish of St. Saviour’s in Southwark. The parish was formed in 1540 by

Act of Parliament, and combines the previous parishes of St Mary Magdalen and St Margaret (Brickley et al. 1999, 5).

In 1801 there were 15,596 people recorded as living in the parish of St. Saviour's, but only 2660 houses, and 114 of those were unoccupied (Brickley et al. 1999, 20). This would mean, as a crude average, that there were six people living in each occupied house. By 1838, the population of the area had increased to 31,711 (Brickley et al. 1999, 23). It is hardly surprising then that Chadwick, writing in 1843, labelled the population density in the parish "extreme" (Chadwick 1843, 265).

The ever-increasing size of the population of St Saviour's parish continued to be a problem throughout the period being considered here. Added to this were issues pertaining to old, poor-standard housing and poor hygiene, which resulted in infestations of vermin. The description given of Ewer Street - one of the roads within the parish - in a newspaper cutting from 1852 (anon 1852) is likely somewhat exaggerated, but paints a very vivid picture of the supposed scale of the problem:

"This street is composed almost entirely of very old wooden houses… each house is occupied by several families - indeed, the houses are crammed full of the poorest and most wretched of our inhabitants. The houses being so old, and built of wood, are literally alive with vermin, and the wretched occupiers were actually driven outdoors by the vermin, and at nightfall, for weeks and months, were to be seen the unfortunate poor creatures of both sexes, of all ages, sleeping huddled together on the doorsteps, and on the footway, so as to render the street almost impassible."

It is unknown to the author as to who wrote the text from this newspaper cutting, but it would appear that the situation was exaggerated in order to support the wider rhetoric regarding public health. The quote itself says

nothing about averages: it focuses on a 'shock horror' extreme that may not represent all - or even any - of the people who ended up being buried at the Cross Bones site. However it is easy to see how a description like this can fit with the idea that a burial ground in the same area would have strong ties to impoverishment. An important point emerges here regarding the interplay between archaeological and historical data: the historical account just presented was clearly written with an agenda in mind, and so this is an opportunity for the archaeological evidence to cut through that, and to contribute to an understanding of the real picture.

To return to the consideration of the area surrounding Cross Bones; vermin or not, the problems of overcrowded accommodation, poor hygiene, and spread of disease were exacerbated when someone died, as the body was frequently kept in the house for a number of days before the undertaker came to take it away (Brickley et al. 1999, 22; Richardson 2006, 152). This sometimes resulted in the body of an individual who had died from an infectious disease remaining in the same room that the surviving family would continue to live and sleep in (Brickley et al. 1999, 22). An undertaker by the name of John R Wild recounted a particularly vivid explanation of the problems that can arise when the deceased remain in the home for some time after death, during an interview with Edwin Chadwick, secretary to the Poor Law Commission (1843, 38-9, cited Brickley *et al.* 1999, 22):

"In cases of rapid decomposition of persons dying in full habit there is much liquid; and the coffin is tapped to let it out. I have known them to keep the corpse after the coffin had been tapped twice, which has, of course, produced a disagreeable effluvium. This liquid generates animal life very rapidly; and within six hours after a coffin has been tapped, if the liquid escapes, maggots or a sort of animalculae, are seen crawling about."

Aside from the problems caused by overcrowded accommodation and the treatment of the dead, there were major waste disposal issues all over London at this time, as the Public Health Act did not come into effect until 1848. St. Saviour's parish was no exception, with waste typically being disposed of either directly into the river Thames (the same river that the drinking water was taken from), or into cesspools beneath the houses (Brickley et al. 1999, 21). Brickley *et al.* (1999, 21) even mention excrement being piled up and sold.

It will come as little surprise, then, that the quality of drinking water obtained from the Thames was terrible, and would most certainly have been polluted (Brickley et al. 1999, 21). As this water would have been used for both drinking and for making food (e.g. the weaning food 'pap', which has been discussed in more detail in Chapter Four), it is easy to see how it could have been responsible for helping diseases to spread (Brickley et al. 1999, 21).

Levels of nutrition in the parish at this time are also believed to have been low (Brickley et al. 1999, 21). In children we see this through the fact that they were fed 'pap' and 'panada' during the weaning process (Wood 1955, 475; Stone 1977, 272; Brickley 1997, 106; Stevens et al. 2009, 35). In regards to adults, we know that the St. Saviour's parish committee decided in 1795 to try giving out potatoes and rice to those in need of food, in an attempt to lower the demand for bread (Brickley 1997, 107; Brickley et al. 1999, 21). However this approach did not work, and the demand for bread stayed high (Brickley et al. 1999, 21). This could be for a number of different reasons. Firstly, it could be that the people of Southwark did not trust potatoes and/or rice; again, reasoning behind the distrust of potatoes has been discussed in Chapter Four. Alternatively, it could be that people would have rather had bread because, unlike potatoes and rice, it wouldn't have needed to be cooked. Cooking would

have required extra firewood and coal, which would be an unnecessary expense if bread could be eaten instead.

History of the burial ground

The Cross Bones burial ground had an arguably longer life than it should have, given its size, and it faced constant threat of closure. The burial ground is believed by some to have been established sometime in the seventeenth century as a prostitutes' cemetery, used for the burial of women from the Bankside brothels (Taylor 1833, 141; Holmes 1896, 183; Brickley et al. 1999, 5). Technically, prostitutes would have been refused burial in a Christian cemetery as a result of the 'sinful' lives they had lead, and so the unconsecrated Cross Bones ground could have been deemed an appropriate location for their burial (Concanen & Morgan 1795, 262; Taylor 1833, 141; Brickley et al. 1999, 6).

The nature of use of the burial ground had changed by 1769, however, to that of a paupers' cemetery, and this is how it remained until its closure in 1853, following the Burial Act of the same year (Brickley et al. 1999, 16).

It was recorded in 1831 that the burial ground was nearly full, and then proposed in 1832, when the problem of overcrowding had become even worse, that some graves be secretly moved (Brickley et al. 1999, 9). This did not actually happen, but the burial ground was temporarily closed from 12th April 1832 until 22nd March 1833 (Brickley et al. 1999, 9–10). A further closure took place at some point between 1836 and 1839, but the exact dates of closure are not clear. What we do know is that before the burial ground was re-opened this second time, the 'Irish corner' of the site was cleared of burials (Brickley et al. 1999, 10).There was an unsuccessful attempt made to close the burial ground again in March 1845, and in September 1849 the Board of Health issued an order that it must be closed immediately (Brickley et al. 1999, 11 & 14). Clearly this

order was ignored, as it was reported by the churchwardens on 29th September 1849 that "...a summons had been issued against them for not obeying the Board of Health's order.", and in October of the same year there was a debate over the Board's power to issue orders (Brickley et al. 1999, 14). Following this, the Board relented somewhat on their order for closure, and instead laid out some new conditions for burial at the site (Brickley et al. 1999, 14). A report dated 22nd October 1849 detailed that Cross Bones was in a better condition following the implementation of the new conditions, but there is no archaeological evidence to suggest that the conditions were actually adhered to (Brickley et al. 1999, 15). The next couple of years at the site appear to have been relatively calm, but the Board of Health began receiving complaints again in November 1852 (Brickley et al. 1999, 15). In August 1853, in line with the rest of London, the church wardens received the royal order that the burial ground was to close permanently, with 21st September of that same year being the last date on which burial at the site would be allowed (Brickley et al. 1999, 16). The burial ground officially closed on 24th October 1853.

Burial at 'Cross Bones'

The individuals buried at Cross Bones were buried very simply - plain coffins, often no coffin plates. Brickley and colleagues assign this to the nature of the burials being paid for by the parish authorities (Brickley et al. 1999, 7). As has already been noted in this chapter, a burial under the conditions just described was far from desirable and many of the 'poor' residents of Southwark are believed to have gone to extraordinary lengths to avoid such a thing. For example, an account from the 1840s details:

"The working people would sell their beds from under them sooner than have any parish funerals: it is heart rending to them." (Chadwick 1843, 102-3).

While it is beyond the scope of this project to discuss it in any detail, the practice of grave-robbing, or 'body snatching', may have been one reason why the idea of parish burials in grounds such as Cross Bones were so opposed by many who knew they would likely be interred there. Body snatching involved the illegal exhumation of a recently deceased corpse from its grave, which would then be sold on to a medical school. Prior to the passing of the Anatomy Act in 1832, this practice was a problem affecting burial grounds and graveyards all across England, but it was particularly pertinent to poor or pauper burial grounds (Brickley et al. 1999, 8). Cauch (1840, 40), in his 'The Funeral Guide: Or, a Correct List of the Burial Fees…', recorded the costs for being buried at Cross Bones in the mid-nineteenth century (Figure 22).

ST. SAVIOUR'S, CHURCH STREET, BOROUGH.

				Under 10 years.
Cross Bones Ground, 4-feet deep	£.0	12	10	8s. 2d. & 9s. 2d.
Ditto, with Desk Service.....	1	5	8	
Church Yard, 6-feet deep.....	1	5	10	0 16 2
Ditto, with Desk Service	1	13	10	1 8 0
College, 8-feet deep	2	0	10	1 3 0
Ditto, without Desk Service.....	2	8	10	1 14 4
Great Vault, in Lead.....	3	13	8	2 12 0

Family Vault, 10s. extra. Non-Parishioners Double the above.
Bury at 4 o'Clock every Day. Early Dues 25s. 6d.

Mrs. DREWETT, Sextoness, 2, *York Street*.

Figure 22: Details of burial fees at the Cross Bones burial ground (Cauch 1840, 40)

The fact that any burial fees are recorded for this site shows that it is at least possible that some of the individuals buried there were not subject to a parish funeral. Interestingly, it was more expensive for a Southwark parishioner to be buried at Cross Bones than it was for a St Bride's parishioner to be buried in the St Bride's Lower burial ground. Similarly, it was more expensive for a non-parishioner to be buried at Cross Bones than for a non-parishioner to be buried at Farringdon St Brides Lower. While both sites have been archaeologically associated with the poor, a

stronger impression of poverty is given for the Cross Bones site than for Farringdon St Bride's Lower, which these figures do not appear to support.

Bow Baptists

Following the 1689 Toleration Act, Baptists (and other non-conformist religious groups) were allowed to congregate freely in their own registered places of worship. Here we will explore the history of the Payne Road Baptists, formed nearly 100 years after this Act was passed, to provide a background and context for the osteological and biomolecular data that will be considered in later chapters.

Location of the Site

The 'Bow Baptist' churchyard, as it has become known, was situated on Payne Road, on the western side of the river Lea, in the village of Bow (Lynn 1935, 2–3; Henderson et al. 2013, 33). The village was later incorporated into the greater London area as the city grew and expanded, but when the Baptist Chapel was first founded there in 1785, Bow was a large village in Middlesex (Lynn 1935, 2–3; Powers & Miles 2011, 233–4).

Bow was part of the Stratford St Mary parish, otherwise known as 'Stratford-le-Bow' (Walford 1878; Henderson et al. 2013, 3). During the eighteenth century, Bow was a seemingly pretty village where several people made a living by catering to the needs of travellers going between Essex and London; there was a blacksmith's forge and 'Ye Olde Bowe Taverne', as well as the more locally-relevant trades based at the windmill and nearby farms (Henderson et al. 2013, 33).

During the nineteenth century, however, the area changed beyond all recognition. As industrialisation took hold in east London, the city

expanded and saw Bow change from a village to a London suburb, with those who could afford to moving out of the area, away from the factories, warehouses and hordes of incoming workers, who themselves lived either in the specially-built, very small workers houses, or in the once great mansion houses that were turned into tenements (Lynn 1935, 12).

History of the Church and burial ground

The Bow Baptist Church, as a group, was formed on June 21st, 1785, by pastor John Knott, with a congregation of eight people (Powers & Miles 2011, 236; Henderson et al. 2013, 35). Their meetings were held in an old printing factory that had been converted from an abandoned granary near to the River Lea (Powers & Miles 2011, 233–4; Henderson et al. 2013, 35). In his 'Brief Outline of the Story of Bow Baptist Church', Lynn (1935, 3) describes the crowds of curious spectators who gathered around the Lea to witness the first baptism that was conducted in the river, in July 1786. Lynn provides no references or evidence for this tale, but if true, it could be argued that this was done to garner attention, or to make a statement to the non-Baptists living in Bow at that time.

On September 21st, 1799, the meeting house was closed in favour of a new, dedicated chapel being built (Henderson et al. 2013, 35). This decision was presumably influenced in part by the poor conditions just described. Another contributing factor that we know to have strongly influenced the decision was the increase in congregation size, having grown to 90 members at this point in time (Lynn 1935, 7). Construction of the new building began in July 1800, costing just over £2000, and the New Chapel opened on May 25th, 1801 (Lynn 1935, 7; Henderson et al. 2013, 35). This New Chapel contained a stone baptistry (Henderson et al. 2013, 35), suggesting that, from this point in time, baptisms no longer took place in the River Lea. Therefore, we must ask ourselves what might have changed between 1786 and 1801 to mean that it became preferable

to conduct baptisms indoors, within the Chapel, rather than the way that they had been performed for the previous 15 years.

In 1814, the Bow Baptists acquired some of the land adjoining the New Chapel, to be used as a burial ground (Lynn 1935, 9; Henderson et al. 2013, 35). Burial practices at the site will be discussed further below, but it is important to note at this point the short-lived nature of the Bow Baptist burial ground. Like many of London's burial grounds, the Bow Baptist ground was ordered to close in 1853. After this date, only the occasional burial of individuals who were to be interred in pre-established family plots occurred (Henderson et al. 2013, 37). The burial ground was therefore in use for less than 40 years, which contrasts with some of the other sites discussed here, and provides a much smaller window of focus.

By 1823 there were over 600 members of the Bow Baptist congregation, which exceeded the maximum capacity of the New Chapel, but it was not until 1866 that a new Baptist chapel, with a capacity of nearly 1000, was built (Henderson et al. 2013, 35). By that time, the congregation size had diminished significantly, to less than 300 members (Lynn 1935, 12). The decision to build a new, bigger chapel at a time when congregation size was decreasing has been viewed by some as "a bold venture of blind faith" (Lynn 1935, 12), and it is true that the decision left the Bow Baptists in debt for 27 years after the chapels construction. However, at the time when the decision was made, the situation was similar to that which they had faced in 1799, in that their existing place of worship was in a poor condition and deemed not worthy of having any more money spent on it for repair or upkeep (Lynn 1935, 12).

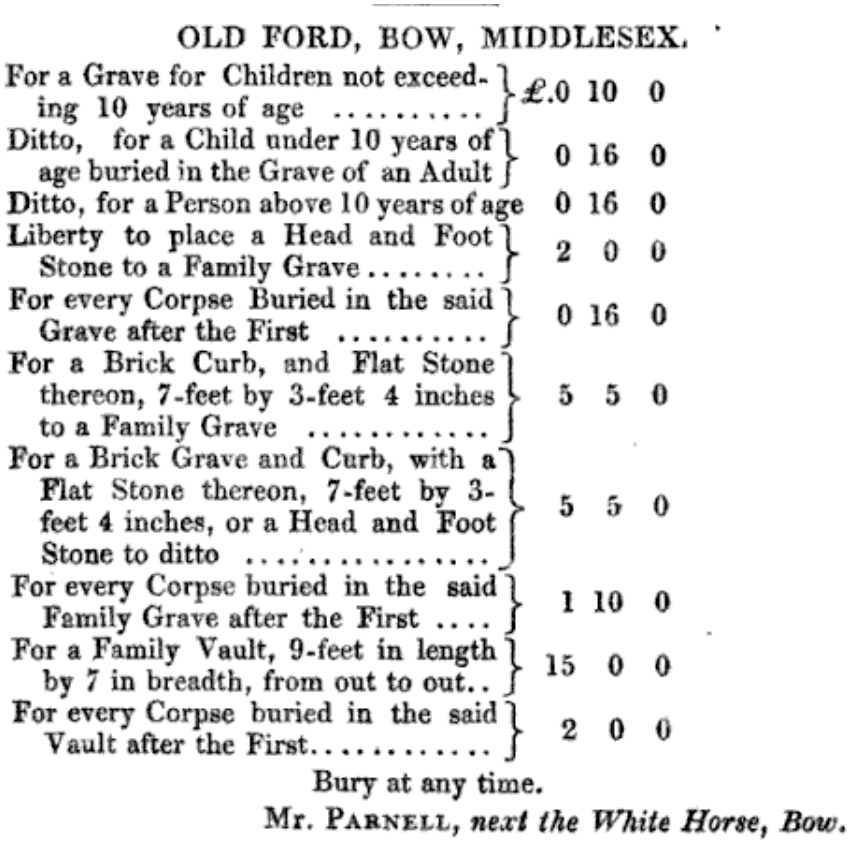
We need to consider whether the 600-strong congregation that gathered in the Baptist chapel in 1823 all lived in Bow; either way this has particular implications. If the entire congregation did live in Bow, how many of those individuals were born there and how many had moved as a result of their

faith? If there were some members of the congregation who did not live in Bow, how far did they travel in order to attend services? Having the necessary means to either move or to travel regularly to a particular place on the basis of following your faith would not imply living a life of what we today would term poverty.

The 1821 census records that 2,349 individuals were living in Bow (Anon n.d.). By 1861, just five years before the new chapel was completed, the population of Bow had risen to 11,590. This would seem to suggest that the burial ground population of the Bow Baptist chapel is not necessarily representative of the population living in the immediate area, as congregation numbers were falling at the same time that population numbers were rising.

Burial in the Bow Baptist burial ground

There appears to be only one surviving burial register for the congregation, covering the years 1816 to 1837. A letter in the Tower Hamlets Local History Library and Archives, from a H. A. Arnold, suggests that there were at least a further 12 volumes of records regarding the Bow Baptists (for example the minutes of meetings), but that these were destroyed by a World War Two bombing in September 1940 (Henderson et al. 2013, 39). However, information regarding burial at the site can be gathered from other sources. For example, Cauch's 1840 book, *The Funeral Guide*, details the cost of burial in the Bow Baptist ground (Figure 23).



OLD FORD, BOW, MIDDLESEX.

	£.		
For a Grave for Children not exceeding 10 years of age	0	10	0
Ditto, for a Child under 10 years of age buried in the Grave of an Adult	0	16	0
Ditto, for a Person above 10 years of age	0	16	0
Liberty to place a Head and Foot Stone to a Family Grave	2	0	0
For every Corpse Buried in the said Grave after the First	0	16	0
For a Brick Curb, and Flat Stone thereon, 7-feet by 3-feet 4 inches to a Family Grave	5	5	0
For a Brick Grave and Curb, with a Flat Stone thereon, 7-feet by 3-feet 4 inches, or a Head and Foot Stone to ditto	5	5	0
For every Corpse buried in the said Family Grave after the First	1	10	0
For a Family Vault, 9-feet in length by 7 in breadth, from out to out..	15	0	0
For every Corpse buried in the said Vault after the First.....	2	0	0

Bury at any time.

Mr. PARNELL, *next the White Horse, Bow.*

Figure 23: Details of the different burial fees in the Bow Baptist burial ground (Cauch 1840, 52)

The figures show that burial in the Bow Baptist ground was cheaper than at either Farringdon St Bride's Lower or Cross Bones. However this is largely unsurprising, as the burial ground at Bow was located outside of the city.

Individuals buried at the site were generally buried in single wooden coffins, most of which had name plates (Henderson et al. 2013, 41 & 73). However, these name plates were most often made from tin-dipped iron and so had decayed to the point where legibility had been significantly reduced in almost all cases. Lead breastplates survived much better, but were in the minority at the site (Henderson et al. 2013, 74).

Existing burial records suggest that there were family plots at the site, and it appears that there were also separate areas for the burial of children (Henderson et al. 2013, 69).

There are contemporary records to suggest that corpses were kept in the home for long periods after the individual's death, with a strong implication that this was causing the living to contract illnesses from the deceased due to the living and dead sharing such a small space while decomposition of the corpse was starting to occur (Chadwick 1843, 34–36; Henderson et al. 2013, 67–68). Establishing how long exactly the body of the deceased would have remained in the home - particularly in regards to Baptist communities - requires further investigation, but the contemporary site of New Bunhill Fields - also largely nonconformist - saw burial delays of between one and 18 days (Henderson et al. 2013, 68).

Priory Yard, Norwich

Similar to the site in Bow just discussed, the Priory Yard site in the post-medieval period was a Baptist place of worship. In the medieval period the site was known as Whitefriars, as the Carmelite friary of the Whitefriars had at that time stood on the same site, being demolished following the Dissolution of the Monasteries in 1542 (Penn 2001, 4 & 6).

When excavations were carried out in 2002, both medieval and post-medieval burials were exhumed. For the purposes of this project, we will only focus on the post-medieval individuals, potentially buried between 1697 and 1854, but most likely between 1726 and 1854 (Caffell & Clarke 2011, 253).

Location of the Site

The Priory Yard site is located in Norwich, Norfolk, some 120 miles north-east of modern day London (Figure 24). The long eighteenth century was a period of massive change for the city; at the start of this period, Norwich was the 'Second city' of England, with a booming textile economy (James 1857, 259; Corfield 2000, 41; Corfield 2004, 140; Wilson 2004, 219). However by the end of the period it had fallen to be only the fourteenth largest city in England and Wales, with many inhabitants leaving in order to find work in the emerging industrial areas of the north, and more remaining behind to face unemployment and possible subsequent poverty (Corfield 2000, 47; Clark 2004, 386; Corfield 2004, 161; Knights 2004, 187; Wilson 2004, 231).

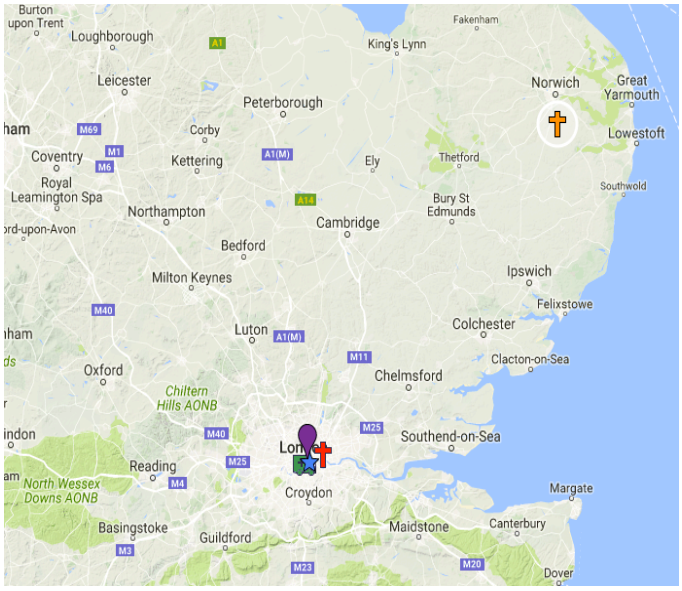


Figure 24: Map showing the location of the four London-based sites and the Priory Yard site in Norwich (marker shown in orange)

Archaeologists have described that the Priory Yard Baptist Chapel was located in one of the poorest areas of the city (Rutledge n.d., 2; Caffell & Clarke 2011, 253). Further evidence that seems to have been used to support the archaeological conclusion that this site was essentially exclusively for the burial of the poor or impoverished comes from the

Priory Yard Chapel births register (Anon 1821-1836; Caffell & Clarke 2011, 255). A total of 42% of births recorded in the register between 1821 and 1836 occurred in the following yards, all of which have been said to have been known ‘slums’ (this being another term where scholars have called for tighter definitions for its use (e.g. Mayne 2011)):

- Thompson’s Yard
- White Lion Yard
- Tilyards Yard
- Mr Orton’s Yard
- Hinds Yard
- Pastons Yard
- Kyal’s Yard

However, the remaining 58% of births seemingly did not come from areas associated with poverty. If births were registered from more or less affluent areas of the city, it is surely possible that the same could be true for deaths and therefore that individuals interred in the burial ground could have been from any economic background?

Also, as was the case with the Bow Baptist Chapel, it was a choice - based on religious preference - for individuals to worship at the Priory Yard Chapel, and a choice for them to be buried there. It has been noted that there is evidence of wealthier members of the Norwich population having worshipped at the Priory Yard Baptist Chapel (Rutledge n.d., 2-3; Caffell & Clarke 2011, 251), and so even if the site was located in a notably poor area, this supports the fact that that does not necessarily mean only poor people would have been buried in the graveyard.

History of the Church and Burial Ground

A Baptist chapel was first established at Priory Yard in 1697 by Thomas Grantham, with an accompanying piece of land (recorded as 41 x 30 feet)

being used as a burial ground (Caffell & Clarke 2011, 251). In 1726, a further 27 feet of land was added to the burial ground by Thomas Grantham's grandson, Grantham Killingworth, and it is this newer ground that saw the interment of the individuals included in this study, with the final burial taking place in 1854 (Caffell & Clarke 2011, 253). Shortly after this, in 1855, a non-denominational cemetery was opened in the city, which could explain why burial at the Priory Yard site had ceased.

Burial in the Priory Yard burial ground

There is no surviving burial register for the site, but we know from osteological evidence that both adults and non-adults were buried in the Priory Yard burial ground. Excavation at the site revealed that most, if not all, individuals were buried in coffins, which is somewhat unexpected for a site in a particularly poor area of the city, associated with poverty.

Archaeological Links to Poverty

Each of the sites included in this project have been classed by archaeologists as having links to the poor or to poverty. As a final point of consideration, having examined the general history related to these sites, we will investigate and attempt to evaluate the reasons for this.

Regarding the St Thomas' Hospital site, Jones (1991, 28) noted that "the body trenches have the appearance of pauper's graves". Beyond this, there seems to be no discernable reason why this site would be classed as linking with poverty. As a hospital burial ground, patients who died and were subsequently interred there could feasibly have come from all walks of life. The residents of the parish, as has been discussed, were mainly tradesmen ((e.g. Payne 2008, 30; Handy 2009, 325)). They were therefore not the most affluent members of society, but equally were likely not the poorest either. Ultimately, while it was clearly not a sought

after burial location chosen by wealthier individuals and families, we cannot reliably conclude on the basis of the available evidence that the St Thomas' Hospital burial ground was solely used by the poor or impoverished.

In discussing the Farringdon St Brides lower graveyard, Miles and Conheeney (Miles & Conheeney 2005b, 1), cited a text that referred to "the poor of the said parish" being buried there. There are a number of issues to be considered here: first, the text being quoted is indenture terms for the graveyard, which date to 1611. While this tells us that in 1611 there were deemed to be poor individuals living in the area, it does not mean that this was still the case almost 150 years later, when the burial ground was closed in 1849. Second, and a theme that we will continue to see over the course of this project, is the point that language changes and evolves over time - 'poor' as used here in 1611 does not necessarily mean the same thing that we would use it to mean today. Finally, this text may have referred to there being 'poor' individuals living within that parish, but that certainly does not mean that it was an entirely 'poor' area, or that only 'poor' individuals were buried in the graveyard.

Archaeological literature on the Cross Bones burial ground is limited, despite the site having been excavated over 20 years ago. However one study by Brickley (1997, 103) stated that "It soon became clear, even from the limited area excavated, that it had been a burial ground used by the poorest members of society" and that "... the form of burial suggested extreme poverty". These statements appear to be based on the fact that the coffins uncovered at the site were of particularly low quality. Following the plague outbreaks in the 17th Century, the concept of the 'parish coffin' previously used for pauper funerals had largely gone out of use, it being believed that it was unsanitary to reuse such a thing (Hayes 2017). Instead, cheap, basic quality coffins with no adornments were offered at the expense of the parish to those who could not afford to cover the expense of their own funeral or that of a loved one (Rugg 2000, 269;

Hurren & King 2005, 327; Mihailovic 2011, 114). However, while the coffin evidence uncovered at Cross Bones may have been of low quality, we have no way of knowing whether these were paid for by the parish, or simply the only option that the individuals buried at the site could afford. It would seem that the area in which Cross Bones was located was notably poor during our period of interest, and it could be the case that a number of the individuals buried at the site were poor or impoverished. However, due to a variety of factors discussed over the course of this chapter, those labels cannot necessarily be applied to the burial ground population as a whole.

Caffell and Holst (2007, 46) record that the proportion of non-adult individuals observed at the Priory Yard site (also known as Whitefriars) is higher than that seen at sites typically perceived as being wealthier, and note that "This suggests that the Whitefriars Anabaptist population may have been among the poorer sections of society". It is true to say that some of the population may have been some of the poorer inhabitants of the city at that time, but - as was also the case at the Bow Baptist burial ground, also dubiously categorised as a 'poor' burial ground - it was a choice to be buried there, and would have been based more on religious preference than on finances.

Labelling these sites as having been 'poor' burial grounds, or solely - even primarily - for the burial of those 'living in poverty' is an incorrect oversimplification arguably common in archaeological research. To use the words 'poor' and 'poverty' in such a way is a notably loose application of them, which generalises the history of the site, the area in which it operated, and the lives of the people who were buried there. Very often, the assignation of the term 'poverty' to an archaeological population is based on the location of the site and the oversimplified idea of 'poor' areas and 'wealthy' areas. It is considerably more straightforward to assign these terms (as long as they are well-defined) to an

archaeological site of residential nature (e.g. Hungate in York (Richardson 1959, 72)), although care should still be taken.

The application of the words ‘poor’ and ‘poverty’ is fraught with difficulties relating to the ways in which language evolves and changes over time. For example, Defoe (1709, 142) professed that people could be sorted into seven classes:

“1. The Great, who live profusely.
2. The Rich, who live plentifully.
3. The middle Sort, who live well.
4. The working Trades, who labour hard but feel no want.
5. The Country People, Farmers, etc. who fare indifferently.
6. The Poor that fare hard.
7. The Miserable, that really pinch and suffer want.”

Defoe does not mention the term ‘poverty’ - we could speculate that what we would mean by that today, Defoe refers to as ‘The Miserable’, but we cannot be sure. The terms ‘poverty’ and ‘miserable’ are not interchangeable in today’s use of the terms - one can be miserable without living in poverty, and vice versa. Even where the terms ‘poor’ and ‘poverty’ are used, these concepts are relative - they can only be defined in relation to overall affluence (Ravallion 2011, 11). Hindle (2004, 38) notes that policy makers in the late 16th century distinguished vagabonds from the labouring poor from the impotent poor: how do we know which of these we would class as having been poor and which as having lived in poverty?

As scholars, we need to ensure that the terminology that we choose and the ways in which we use it is more robust; we can’t just say ‘poor’ or ‘poverty’ if it doesn’t really mean anything.

The sites chosen for inclusion in this project were selected based on their reported links with poverty, however the benefit of hindsight, along with

knowledge gained over the course of the research process, now shows that this was a selection criteria with arguably little grounding. Even if the language issue just discussed is ignored and assumed to not be a problem, it needs to be remembered that, although the concepts of poverty and nutritional deficiency are often linked, one does not necessarily equal the other: while it would be unsurprising for individuals living in poverty, or around the poverty line, to be suffering from nutritional deficiency, this is not a given. Similarly, an individual can suffer from a nutritional deficiency without living in poverty or around the poverty line - for example three sub-adults from Chelsea Old Church, and five from St Marylebone - both categorised as being 'wealthy' sites - were identified as showing the osteological signs believed to indicate scurvy (Bekvalac & Kausmally 2009; Powers & Walker 2009).

Thinking specifically about scurvy in relation to poverty, while what we would today term scurvy has been seen as a disease of poverty (Hirschmann & Raugi 1999, 900; May 2013, 100; Kirby 2015; Brickley et al. 2016, 92; Barber 2018, 230), we cannot assume that anyone who suffered this disease in the past was therefore automatically living below or around the poverty line. For one thing, we know that antiscorbutic 'remedies' were being sold in London during the long eighteenth century (as discussed in Chapter Four), and surely if only those living in poverty required them then there would be no point in selling them as those individuals would not have been able to afford to buy them.

Easily applied labels of 'poor' or 'poverty' are not the only language issue important to this project; the term 'post-medieval' is wide-ranging in its scope and can lead to a lack of specificity in what can usefully be learned. For example, the sites studied here are all classed as being 'post-medieval', but St Thomas' covers a vastly long time period of 150 years, and has no overlap with any of the other sites, with the last burial there taking place at least 26 years before the first burials at any of the other sites. Quite clearly, the individuals buried at St Thomas' will have had

very different events impacting and affecting their lives to those experienced by individuals buried at the other four sites included here. In that sense, despite the fact that all of the sites included here are technically from the same designated 'post-medieval' time period, it is not necessarily meaningful to compare the contextual information or scientific data from St Thomas' with any of the other sites, as in reality there would have been a great deal of difference between London in the time of St Thomas' and London up to 200 years later. When carrying out archaeological research and when writing up reports on this, we therefore need to not only be more robust in our language choices, but also to be more specific about chronological precision. However, the decision was made to include this site in the project to enable the possibility of tracing dietary and pathological change over the whole of the long eighteenth century.

Conclusions

This chapter aimed to consider the relevant history of the five sites included in this project, in order to provide contextual information for the osteological and biomolecular data for the human skeletal remains studied. The nature of the sites included in this project - mosty city-based, located in 'poor' areas - supposedly makes it more likely that scurvy will be identified than when considering a wealthier population. However, we've seen through evidence presented in both this chapter and in preceding chapters that this isn't necessarily the case. Caution should therefore be exercised not to assume that the socioeconomic status of an individual - if ascertainable - is directly linked to their nutritional status.

Care should also be taken when using the terms 'poor' or 'poverty', particularly when assigning them to a burial ground or cemetary population, in order to assume that a simplistic generalisation is not being

made. This point touches on that discussed in more detail in Chapter Four, namely the change in use of language over time, and reiterates the need to make justifiable, robustly applied choices in terminology.

One of the main aims of this project was to investigate the biological processes by which we might identify scurvy in human skeletal populations. This will be detailed in the next chapter (Chapter Six: The Search for a Scurvy Biomarker), but it is worth noting here that where the skeletal populations come from (i.e. the locations of the sites) is not really of importance - as long as we can be confident that we aren't looking at individuals who were known to be eating diets rich in fruits and vegetables, they are relevant to this work. The historical information pertaining to the economic status of individuals buried at these sites is ambiguous and cannot tell us for certain that we are working with impoverished communities. Crucially for this work, however, the success or failure of the search for a scurvy biomarker is not based on how the burial ground populations were formed.

Chapter Six: The Search for a Scurvy Biomarker

The biomolecular analysis work detailed in this chapter was undertaken in collaboration with Dr Simon Hickinbotham (SH). KR sourced and sampled the human skeletal remains studied here, undertook the laboratory work and performed the MALDI-TOF-MS analyses. LC-MS/MS analysis was carried out at the University of Copenhagen by Meaghan Mackie (MM), with the data being processed by both KR and MM. KR determined the direction of the data analyses undertaken on both the MALDI-TOF-MS and LC-MS/MS data, in consultation with Professor Matthew Collins and SH, and in line with the project's research aims. KR and SH collaborated on data analysis, with KR providing data to and feedback on SH's code.

The initial aim of including biomolecular work in this project was to test a potential biomarker identified during an earlier research project; peptides with the mass 2291 had been present in some of the human samples tested as part of that project but not in others, leading to the perceived possibility that that peptide could be related to scurvy (Rowsell 2011). A subsequent aim was to establish whether the same systematic approach taken to test the potential biomarker could be used to identify an alternative scurvy biomarker, should 2291 turn out to be invalid.

A further aim was to compare the peptides visible in MALDI-TOF-MS spectra when slightly different methods - detailed in Chapter Three - have been used to extract collagen from sub-samples of the same bones. This is particularly important when looking for a biomarker for scurvy, as it needs to be able to be justified whether a peptide being absent is the result of disease or of the method being employed to obtain the data.

The preferred way to investigate this would have been to test individuals from a human skeletal remains collection known to have either died from, or to have been severely affected around the time of death by, scurvy, such as the Dutch whalers briefly discussed in Chapter Four. However, a continual barrier faced by bioarchaeological researchers is access to appropriate samples, and it was unfortunately not possible for the author to gain access to such a collection. The approach that was chosen here was to compare data from individuals osteologically determined to have had scurvy with that of individuals where it would have been highly unlikely for them to have had scurvy; namely individuals from the Priory Yard site already detailed in Chapter Five, and individuals from a site in Southern Italy. Another alternative is to compare human data with data from an animal that cannot develop scurvy, such as a cow or a sheep, which we will discuss more later in this chapter.

Overall, the goal was to be able to establish an accurate, reliable, non- or minimally-destructive biomarker for scurvy, and to apply this to the human rib samples from the five post-medieval sites discussed in Chapter Five.

Establishing the Peptide Possibilities

When beginning the biomolecular analysis work for this project, the first step was to know which of all the peaks that are produced by MALDI-TOF-MS can be explained, either as part of the collagen sequence or as a contaminant. This is a level of detail not previously seen in bioarchaeological research involving MALDI-TOF-MS analyses. Typically, this method is used for Zooarchaeology by Mass Spectrometry (ZooMS) investigations, where only 11 peptides are used to discriminate most mammals; the rest of the peptides in the spectra are largely ignored as they are typically common sequences, and not necessary for discriminating between species.

The MALDI-TOF-MS spectra produced as part of this project were analysed in the first instance using mMass (Strohalm et al. 2008; Strohalm et al. 2010; Niedermeyer & Strohalm 2012), according to the following settings:

- Signal/noise threshold: 3.0
- Absolute intensity threshold: 0.0
- Relative intensity threshold: 1.0
- Picking height: 75
- Apply baseline: Yes
- Apply smoothing: Yes
- Apply deisotoping: Yes
- Remove should peaks: Yes

Figure 25 shows an example spectrum produced by MALDI-TOF-MS carried out on an archaeological bone sample: the peaks that are seen are strong and are exactly what we would like to see in terms of their shape and distribution. However, it is necessary to understand the amino acid composition of each of these in order to establish which may be useful in the search for a scurvy biomarker.

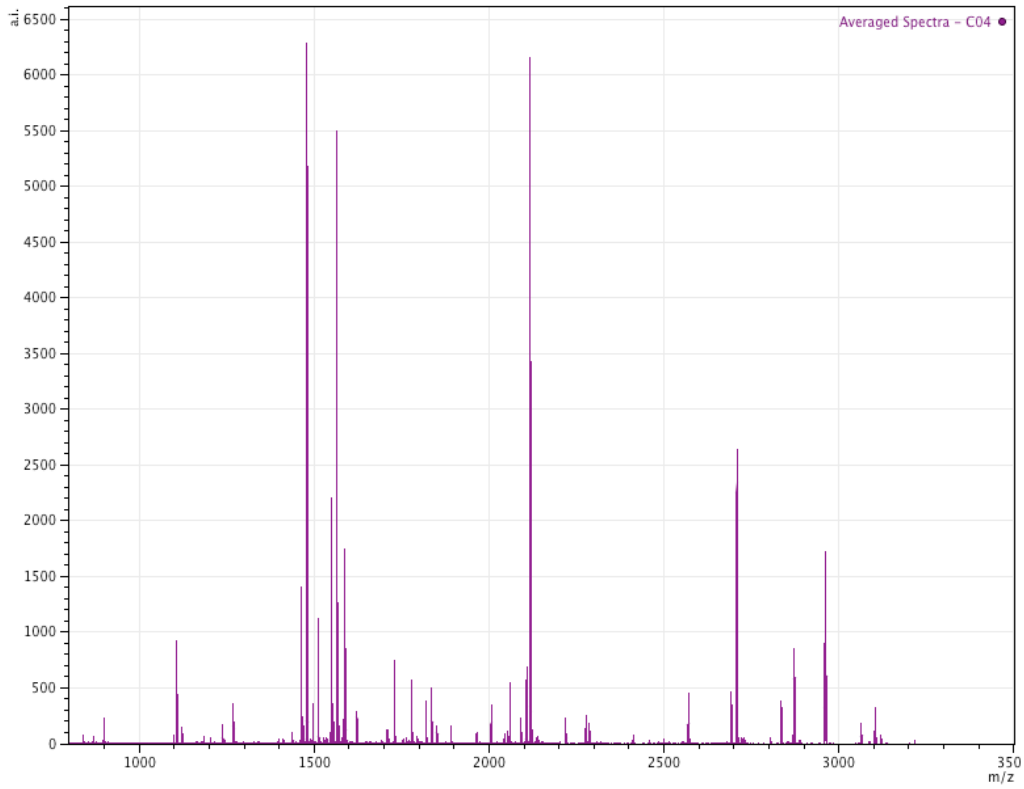


Figure 25: Sample spectrum produced using MALDI-TOF-MS on archaeological human collagen

The first task in explaining all of the peptide peaks observed in the MALDI spectra was to determine all of the peptides that could possibly be observed. This involved using a known human collagen sequence for both the COL1A1 and COL1A2 chains, and establishing all of the possible peptides that can be produced when digesting with trypsin, which is known to cleave at lysine (K) and arginine (R) (see Olsen et al. 2004). However, not all of the possibly occurring peptides will 'fly' (be well ionised and therefore detected) when carrying out MALDI-TOF-MS analysis in positive ion mode. Some peptides have masses that fall outside of the MALDI range (i.e. a mass of less than 800 or more than 3,500 daltons (Da)): below 800 Da, there are so many peptides that it becomes impossible to assign a peak to a particular sequence, and above 3,500, the precision on the mass becomes too questionable for reliable alignment with expected masses. A further hindrance to

observing all of these peptides is the fact that this project deals with archaeological material, which will have been subject to some natural degradation, likely to have modified the collagen chains.

A list of 99 possible peptides with masses between 800 and 3,500 Da was established. However, this list did not account for two commonly occuring post-translational modifications; deamidation and hydroxylation. The masses for the various combinations of deamidations and/or hydroxylations for each fragment were subsequently calculated, which took the total number of possibly occurring peptides up to 872. Hydroxylation - the most directly relevant of these two processes to the study of scurvy - affects the imino acids proline (P) and lysine (K), and is defined as the addition of a hydroxyl (-OH) group to a molecule. However, the process also involves the removal of a single hydrogen (H), and so the overall mass change to the molecule is +15.9994 Da (the mass of a single oxygen atom). Deamidation is a degradation reaction whereby asparagine and/or glutamine are converted to aspartic acid or glutamic acid, respectively. A fully deamidated asparagine or glutamine will have a mass one Da heavier than a non-deamidated version, due to the replacement of an NH_2 by an OH group.

Filtering the Peptide List

Once these modified masses had been added to the original list of peptides, the spectra from the second phase of the extraction technique development work (detailed in Chapter Three) were compared to the theoretical possibilities. Each sample was run in triplicate, and so the aim was to match each of the three spectra for each peptide observed to a peptide from the calculated theoretical sequence (Figure 26).

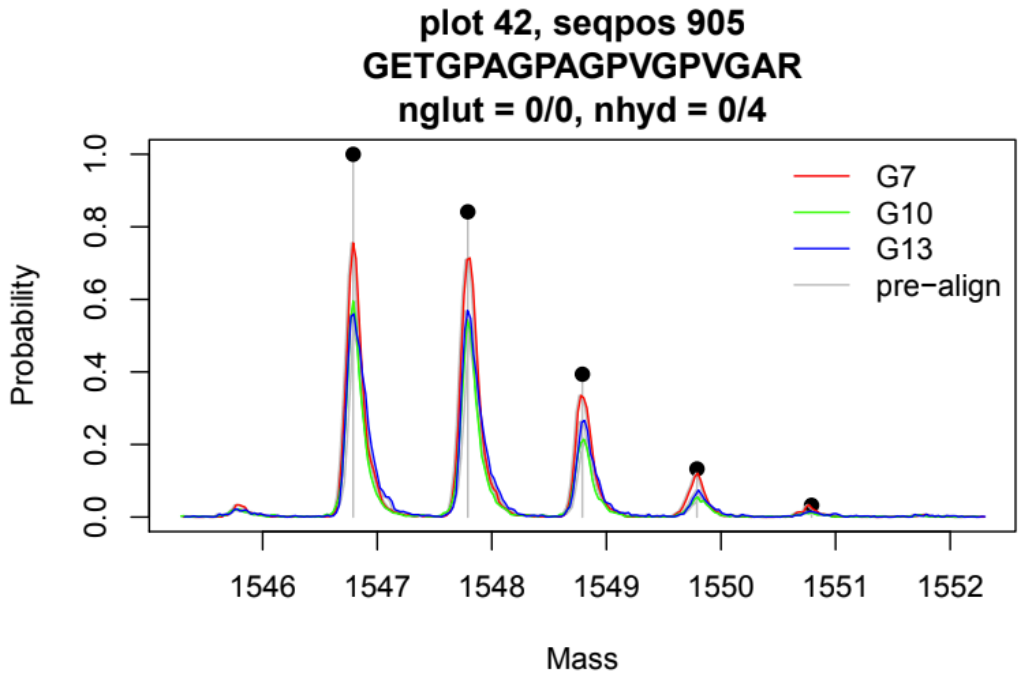


Figure 26: Attempting to match an observed peptide from triplicate spectra (the red, green and blue lines) with a theoretical peptide (the black pinheads) - this plot shows a good match

Sample data was deemed significant and taken forward if a peptide showed an intensity of greater than 1/10 of the maximum observed intensity: this left us with 89 peptides. Even at this early stage in the analysis, the potential biomarker '2291' had been ruled out. It was also at this point that we established that one observed peptide can be covered by more than one theoretical peptide, ultimately meaning that LC-MS/MS further along the process became an inevitability in order to resolve this issue.

Applying the Peptide List to Archaeological Data

These 89 peptides were then again considered in relation to the five different extraction methods tested in Phase Two of the collagen extraction experiments discussed in Chapter Three, but in a different way. This was in order to determine whether there was a difference in

the results produced by different methods; ultimately, a peptide that ‘flies’ regardless of the extraction method used would be the ideal candidate for a biomarker of any kind, but it was also important to establish whether different extraction methods routinely produced different peptide ‘sets’.

The results of this confirmed that Methods B and N favour the lower masses and perform fairly poorly with the higher masses, whereas C, K and I perform better with the higher masses and less so with the lower masses (Figure 27). The potential reasoning for this has been discussed in Chapter Three, and so won’t be revisited here.

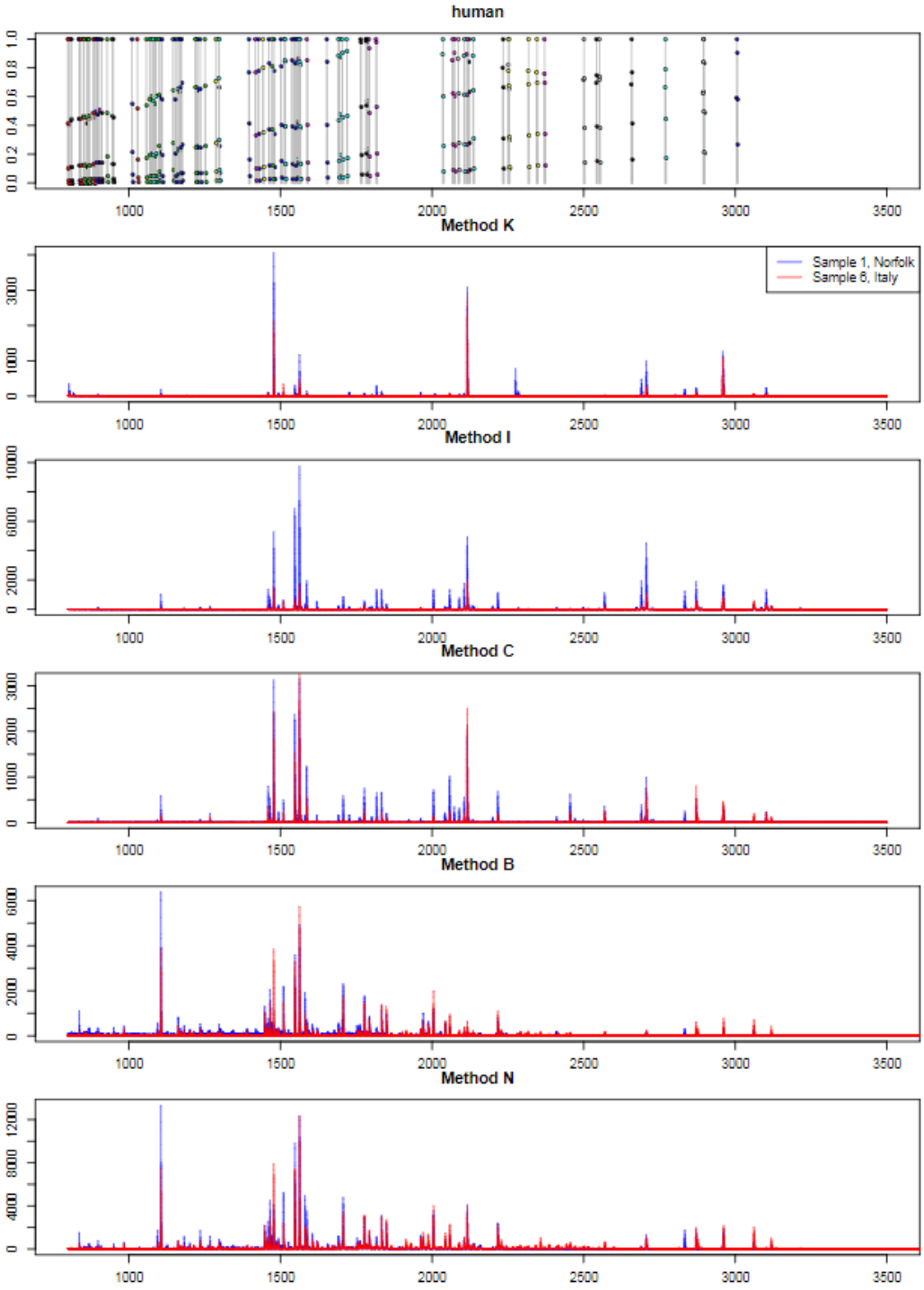


Figure 27: The theoretical human peptides that could be detected (top) and the actual peptides that were detected through using MALDI-TOF-MS, separated by extraction technique

This could be significant in regards to the search for a scurvy biomarker, as higher masses are more likely to have higher levels of hydroxylation,

and so methods which allow peptides with higher masses to fly could be more likely to work for this purpose.

At this point it was decided to continue with only Methods C and N for the remainder of the analyses; Method C because it is the standard destructive method employed for collagen extraction for proteomic analysis, and it proved to produce higher quality spectra with a greater number of peptides than Methods I or K (the two other destructive methods included up until this point), and Method N because it proved to be the most successful non-destructive method tested here, as was detailed in Chapter Three. Having just pointed out that methods favouring higher masses would likely be the best option for identifying a scurvy biomarker, it may seem that only the destructive Method C should have been continued with at this stage. However, as can be seen from Figure 25, Method N does produce the higher molecular weight peaks, and it should also be remembered that an aim of this project was to find a non- or minimally-destructive biomolecular method for reliably detecting scurvy in human skeletal remains, as this would both avoid the moral dilemmas that can surround the sampling of human skeletal remains, and enable the disease to be investigated without negatively impacting future analyses (whether osteological or biomolecular).

Given that the previously widely accepted theory (that there is at least one proline or lysine in the human collagen chain that always has to be hydroxylated, and without that hydroxylation you have scurvy), has not yet been demonstrated successfully - indeed Montgomery et al (2012, 5899) concluded that there was considerable variation in hydroxylation locations - it was decided to take a different approach to establishing the likelihood of hydroxylation occurring in different peptides. Hydroxylation probability levels for each of the 89 peptides in our established list were calculated by SH based on a binomial distribution, and assuming 5% hydroxylation at the X-position and 90% at the Y-position; where the GPP

sequence was present, 90% hydroxylation at the X-position was assumed, and 100% at the Y-position. Knowing the various hydroxylation probabilities is important, as the presence or absence of hydroxylation affects the overall peptide mass, and so determines both the most likely masses that will be observed, and which could be linked to scurvy.

Following this step, the observed peaks from the C1 and N1 samples that had passed the 'lag test' were checked against both the theoretical human collagen sequence and the global proteome machine (GPM) data to determine the most likely sequence that explained each peptide. The lag test involved plotting the masses observed for each sample using MALDI-TOF-MS, and filtering those that had been detected as having a mass either half a Da more or less than they should have had, according to the theoretical masses (Figure 28).

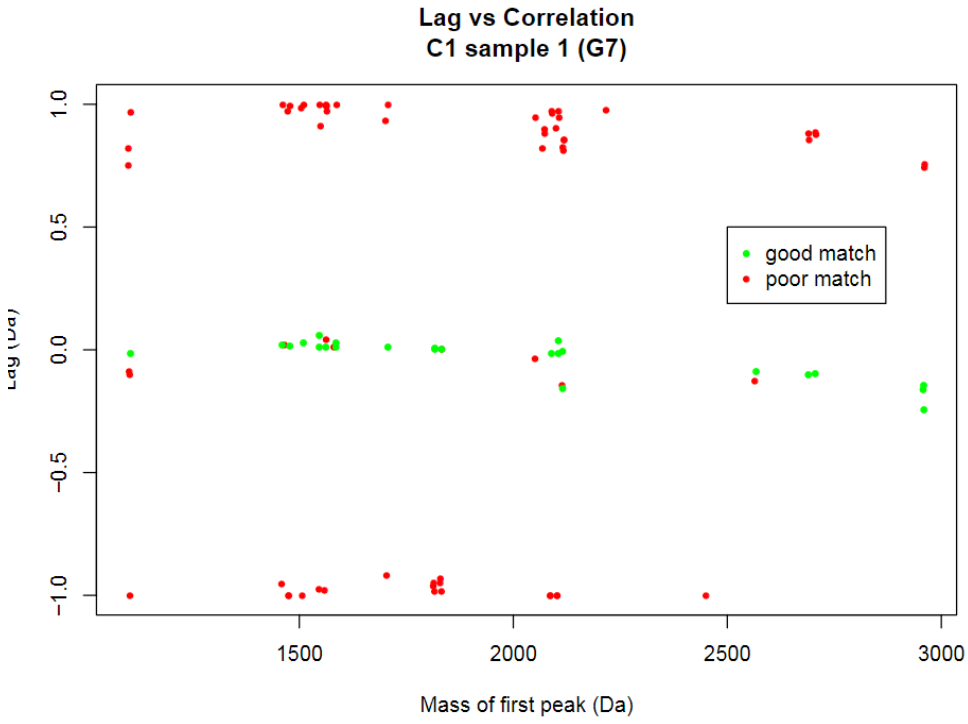


Figure 28: Graph showing 'lag test' filtering of the MALDI-TOF-MS data

In some, simple, cases there was only one sequence option that could explain the peak. However, in some cases there were two or three

options. In those instances, the option that appeared in both the theoretical sequence and the GPM data, with the highest hydroxylation probability, was chosen as the most likely explanation. It should be noted that it cannot be guaranteed that the most statistically probable peptide is the one being observed. However in order to move the process forward a consistent methodological decision needed to be made, and in the absence of definitive information, wherever the most statistically probable option appeared in both the theoretical sequence and the GPM data, this was the peptide that was chosen for inclusion in the list. This point will be revisited later in this chapter, in the 'Method N and a Scurvy Biomarker' subsection.

Once this stage was complete, we had established a list of 59 peptides that were to be checked and plotted for the samples from the sites in Norwich and Italy.

Figure 29 compares the data from Method C with that from Method N, and the Norwich samples (green) with the Italian samples (red). When trying to look for hydroxylation differences that might be related to scurvy, we needed to think about the number of hydroxylations (x-axis) but also the ion counts (y-axis). The reasoning for this was that the ion counts provide a ratio if there are multiple hydroxylation levels for the same peptide, which we hypothesised could be used as a measure for the development of scurvy.

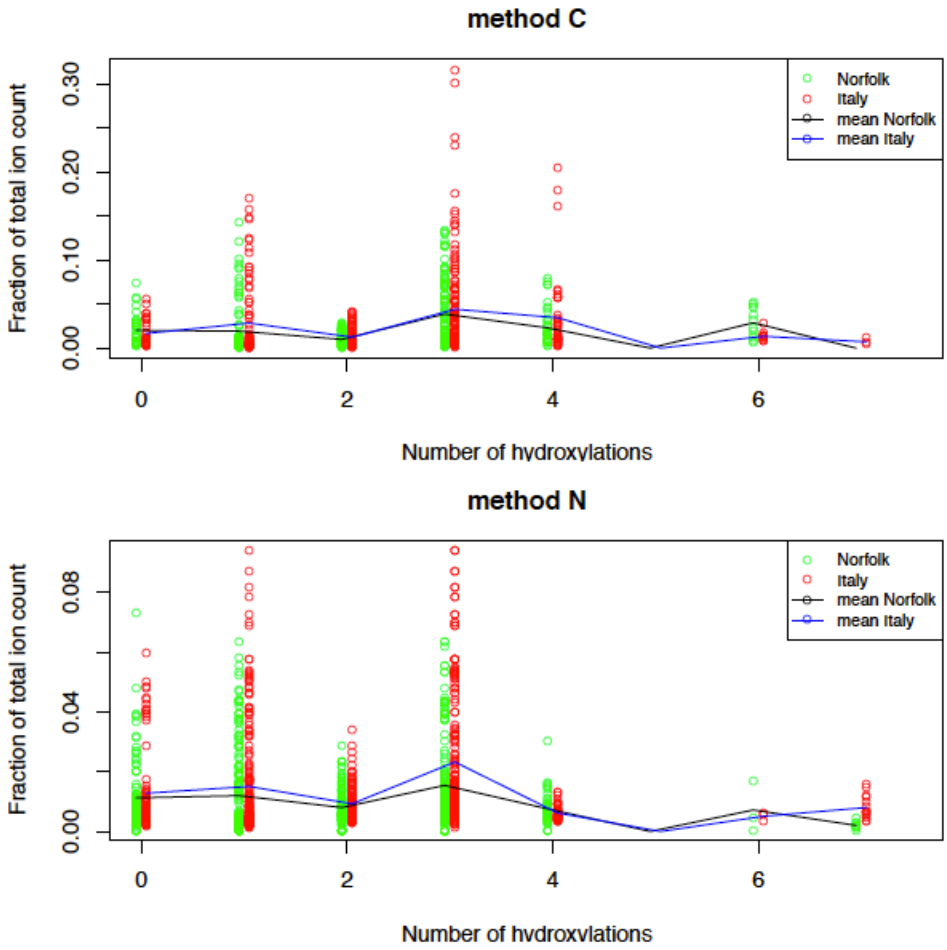


Figure 29: Split graph comparing the number of hydroxylations observed in the Method C data with the number number observed in the Method N data, while also considering the ion counts, and in both cases also comparing the Norwich samples (green) with the Italian samples (red)

However, these plots were based on averages of all of the individuals from each of the two sites, rather than using the data for each individual, meaning that subtle differences at the individual level, which could be important, might get missed.

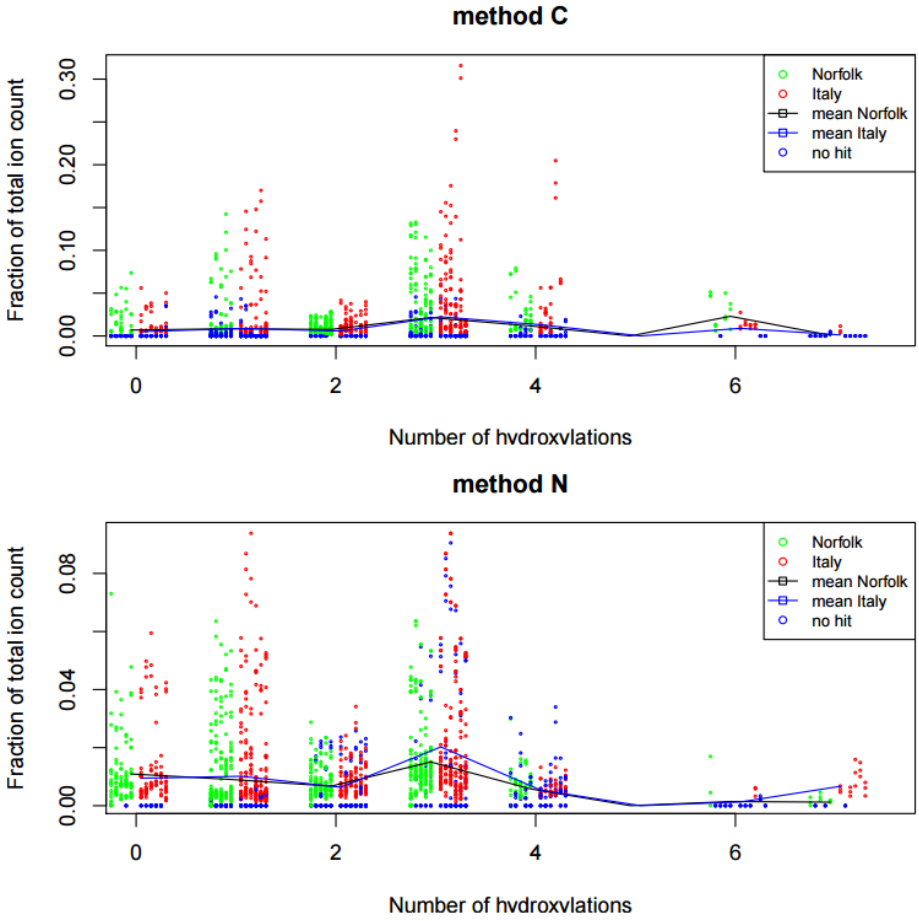


Figure 30: Split graph comparing he same variables as Figure 29, but plotting individuals rather than group data, in order to have the opportunity to investigate changes at the individual level

When the plots were reproduced (Figure 30), the data was separated by individual in order to investigate this. It was also decided that theoretical peptides from the established list which weren't detected in the sample data should still be represented in the plots in some way, as their absence could be relevant. These 'no hit' peptides are shown in blue in the graph above, and created some interest in the plot for the Method N data around the zero hydroxylations area. However, there is obviously a difference in the data being produced by the two different methods, as the same results are not seen with Method C. It would also be important to know which peptides each of the graph points were representing, in order to establish whether the results were consistent, and therefore of

potential importance, or not. However, this was deemed to be infeasible, as considering every datapoint included in the graph would essentially take the research back to square one again. Furthermore, this figure does not show the ratio of observed hydroxylation level against expected level, which is key to establishing a peptide's usefulness in relation to identifying incidences of scurvy: for example, if a peptide has six prolines but only four hydroxylations, that tells us a different story to a peptide that has four prolines and four hydroxylations, as the former could be missing a vital hydroxylation, whereas the latter is obviously not.

As this line of investigation ultimately proved to be inconclusive, we needed to establish whether there was a different way to determine which peptide(s) - if any - could be potential scurvy biomarkers. It was decided to investigate the peptides that displayed variable levels of hydroxylation. However before this was carried out, four samples were sent for LC-MS/MS analysis, in order to ensure that all of the peptides in our established list were appearing in both Method C and Method N data. This also enabled us to confirm the positions of prolines and hydroxyprolines in any peptides of interest.

LC-MS/MS Analysis - Round One

The collagen extractions sent for LC-MS/MS analysis were chosen from the extraction experiments performed as part of the method development work detailed in Chapter Three. The two samples that produced the best spectra using Method C, and the two that produced the best spectra using Method N, were chosen for this (see Table 7 for details).

Extraction Experiment Code	Sample ID	Extraction Method	Skeletal Element
C1	13329	Eppendorf - Method C	Cranial fragment
C7	15726	Eppendorf - Method C	Rib
N1	13329	Eppendorf - Method N	Cranial fragment
N6	15735	Eppendorf - Method N	Rib

Table 7: Details of the four samples included in the first round of LC-MS/MS analyses.

The LC-MS/MS analyses data were plotted by peptide against the corresponding MALDI-TOF-MS data for each sample (Figure 31).

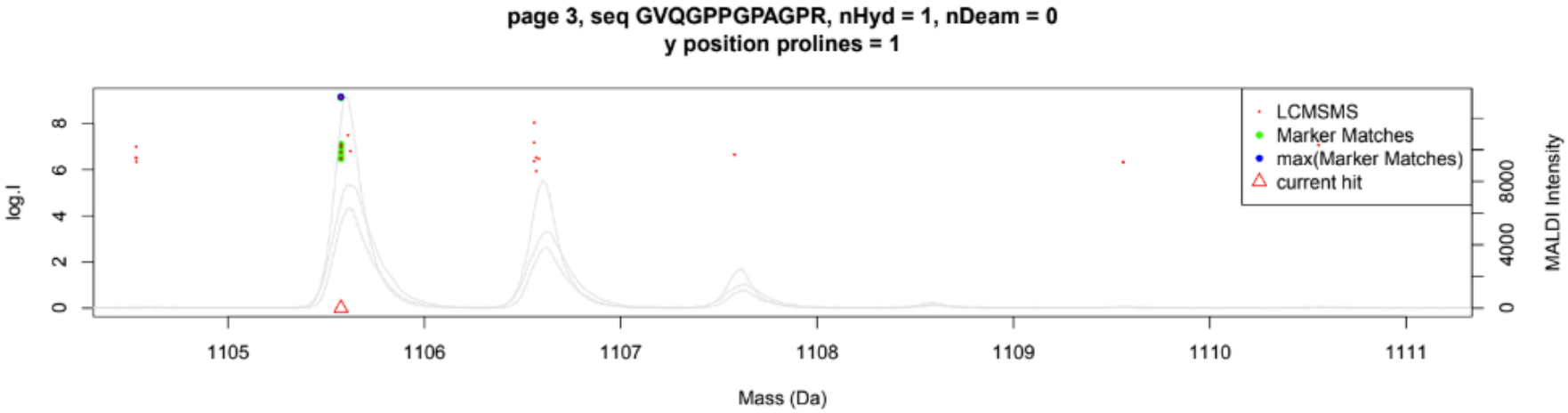


Figure 31: Graph showing the MALDI-TOF-MS spectra for a particular sample (in grey), with the LC-MS/MS data (in red) plotted over the top. LC-MS/MS peptides that match the marker list are shown in green, wit the maximum intensity match plotted in blue

Analysis of all of the LC-MS/MS data in this way, for each of the four samples, revealed consistent matches with 48 of the 59 peptides in our list. When proceeding to consider the variable levels of hydroxylation, this work only included these 48 peptides that had been observed through both MALDI-TOF-MS and LC-MS/MS.

Eppendorfs vs Falcon Tubes

When the various different extraction methods detailed in Chapter Three were tested, all methods involved the use of eppendorf tubes. This was to ensure that the test conditions for each method were kept as similar as possible, so that any variation in results between the different methods was caused by the designed variables. However, this meant that the 'non-destructive' Method N was actually being tested in a destructive way, as subsamples had to be taken in order for them to fit into the eppendorf tubes. It was assumed that using the same method but scaled up - simply placing the entire bone fragment in a falcon tube, so that it isn't broken up - would have no effect on the relative collagen yield.

However this study has revealed that, when carried out using falcon tubes, Method N ('Method N (FT)') did not show the same results as when carried out using eppendorf tubes; the lower molecular weight masses are seen (albeit with much more noise than in the eppendorf spectra), but higher molecular weight masses didn't seem to fly (Figures 32 and 33).

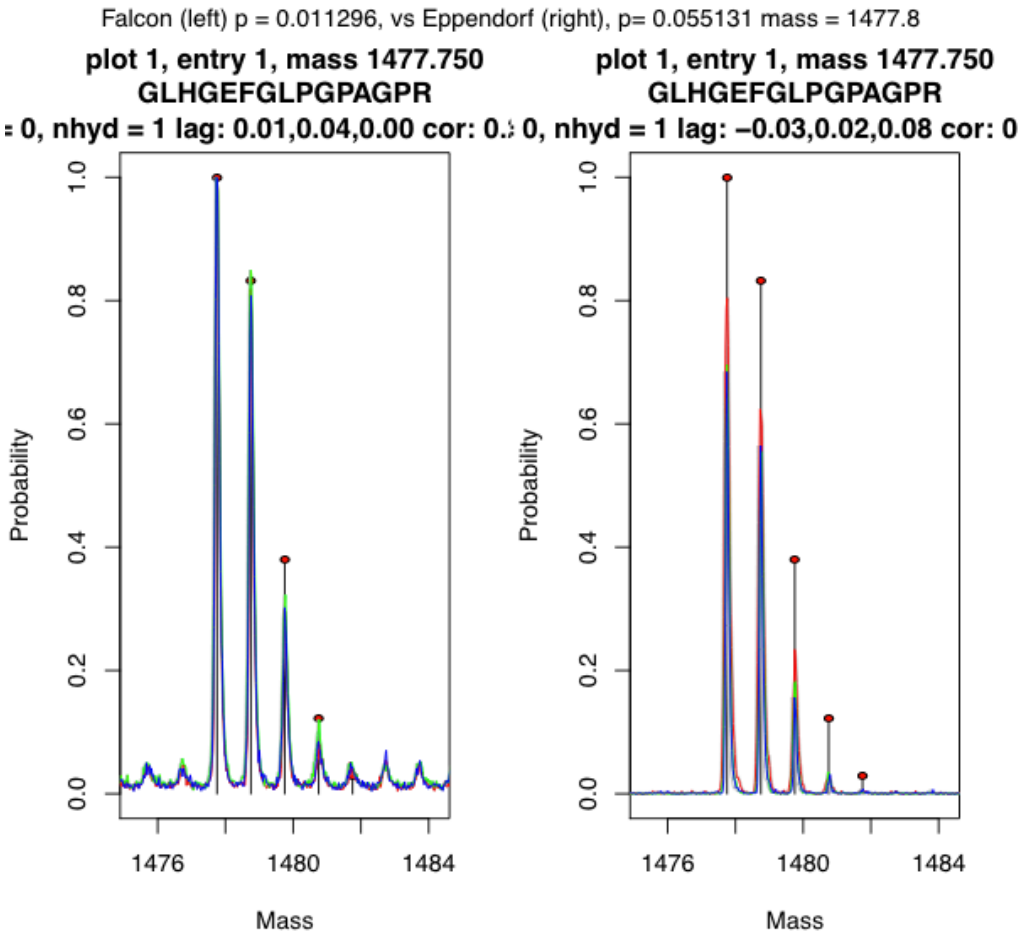


Figure 32: Graphs illustrating that both the falcon tube (left) and eppendorf tube (right) 'versions' of Method N successfully extract the lower molecular weight peptides, although the falcon tube data is noisier

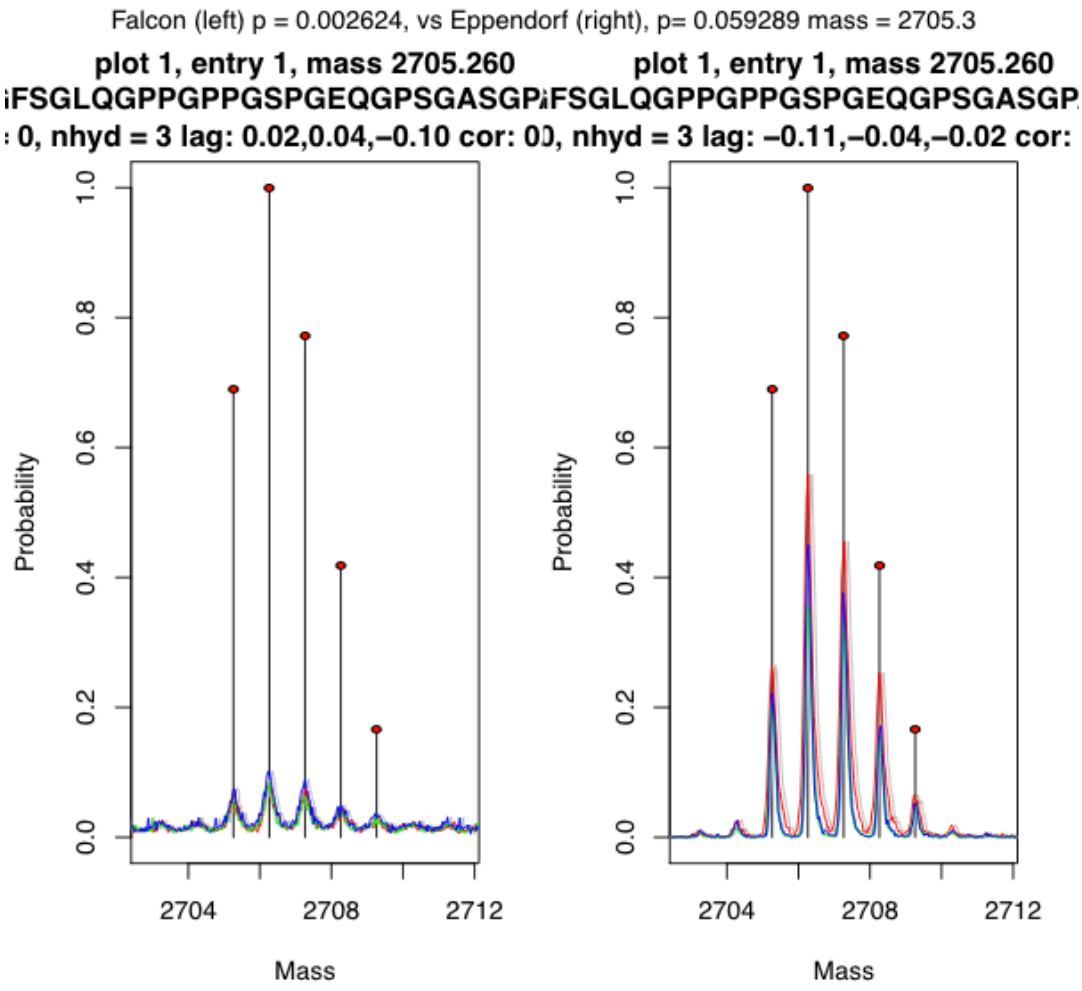


Figure 33: Graphs illustrating that the eppendorf tube version of Method N (right) tends to more successfully extract the higher molecular weight peptides than the falcon tube version (left)

Further work needs to be done here to ascertain whether (i) a difference in the material used to create the falcon tubes, or (ii) the available surface area for binding in the falcon tubes, compared with eppendorf tubes, is responsible for the problem. Alternatively there is the possibility that introducing a freeze-drying step has caused the changes in the distribution (although this is doubtful as freeze-drying large volumes of collagen-containing buffer is standard practice in stable isotope analysis), or finally the possibility that the issue arises from an as yet unknown cause.

Method N and a Scurvy Biomarker

The data from the eppendorf tests using Method N showed potential promise in regards to a scurvy biomarker: from hundreds of theoretical peptides from the known human collagen sequence, narrowed down based on which peptides are routinely detected using LC-MS/MS and by what flies using MALDI-TOF-MS, we ended up with six peptides that routinely fly and can possibly provide information on hydroxylation (Table 8). It should be noted that these peptides were observed in both the Method N and Method C samples.

Peptide number	Mass	Sequence
1	1689.77	DGEAGAQGPPGPAGPAGER
2	1693.75	GEPGSPGENGAPGQMGPR
3	1783.87	GPPGPMGPPGLAGPPGESGR
4	2072.00	GSPGADGPAGAPGTPGPQGIAGQR
5	2656.26	GFSGLQGPPGPPGSPGEQGPSGASGPAGPR
6	2836.41	GLTGPIGPPGPAGAPGDKGESGPSGPAGPTGAR

Table 8: The six peptides that fly routinely using Method N, which also contain Y-position prolines.

Having established such a large number of hydroxylation level possibilities earlier in the process, we had initially expected to observe a large amount of hydroxylation variability. However these six peptides were the only peptides from the list of 48 that was established where variable hydroxylation was observed; 36 peptides had no observable

variation in the level of hydroxylation, and were subsequently deemed to be of no use to this project.

At this point it is important to return to the process by which the peptide list was formed and applied to archaeological data. Earlier in this chapter it was detailed that, where two or three peptide options existed to explain a peak, the option that appeared in both the theoretical sequence and the GPM data, with the highest hydroxylation probability, was chosen as the most likely explanation. None of these six peptides had alternatives that could have been chosen, and so there is no potential selection error to be considered and accounted for.

Of the six peptides identified, three were deemed likely to be of most use: Peptides One, Three and Five. We will now look at the testing of each peptide in more detail, to understand the reasons why this was concluded to be the case.

Peptide One: DGEAGAQGPPGPAGPAGER

1689.77 is present in all samples, with either no hydroxylations, or with one. No deamidated variants were observed. The isotope distribution of the observed MALDI-TOF-MS spectra matched well with the theoretical peak and the LC-MS/MS data (Figure 34).

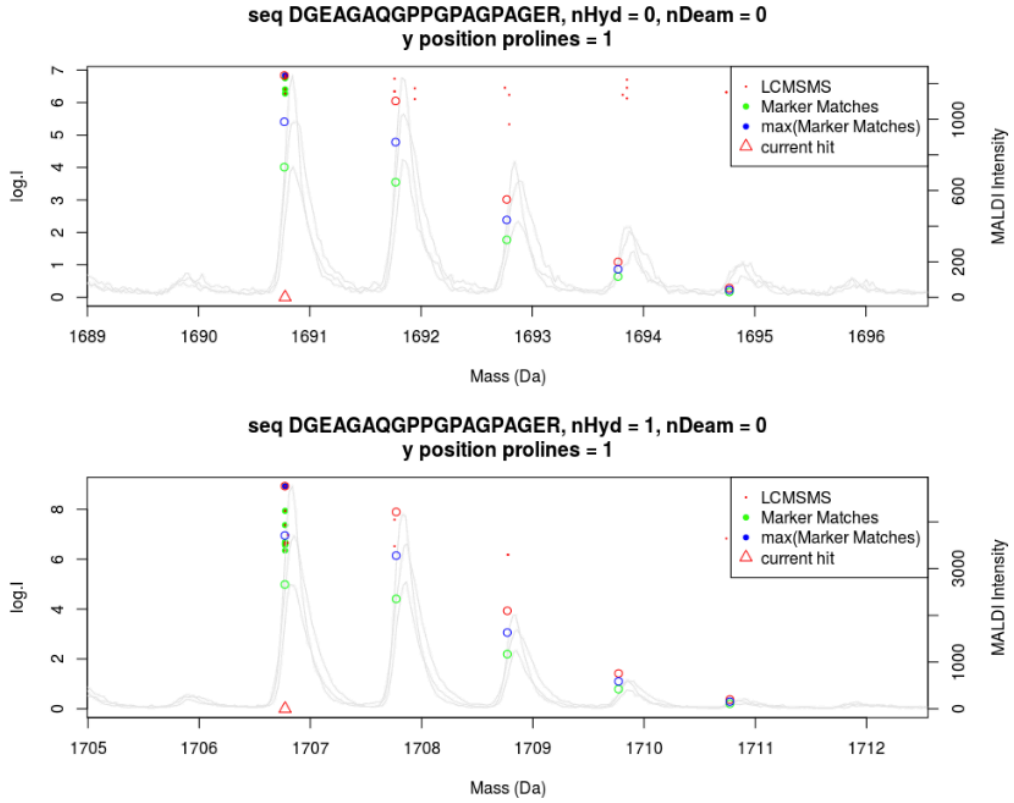


Figure 34: Graph showing the MALDI-TOF-MS spectra (in grey), with the LC-MS/MS data (in red) plotted over the top, for sequence DGEAGAQGPPGPAGPAGER. LC-MS/MS peptides that match the marker list are shown in green, with the maximum intensity match plotted in blue. The observed MALDI peaks correlate well with the potential marker and the LC-MS/MS data

The ratio of zero hydroxylations to one hydroxylation for this peptide in each of the different human samples was then calculated and plotted, and this was compared to five sheep control samples (Figure 35). The red bar present for each group reflects the group mean.

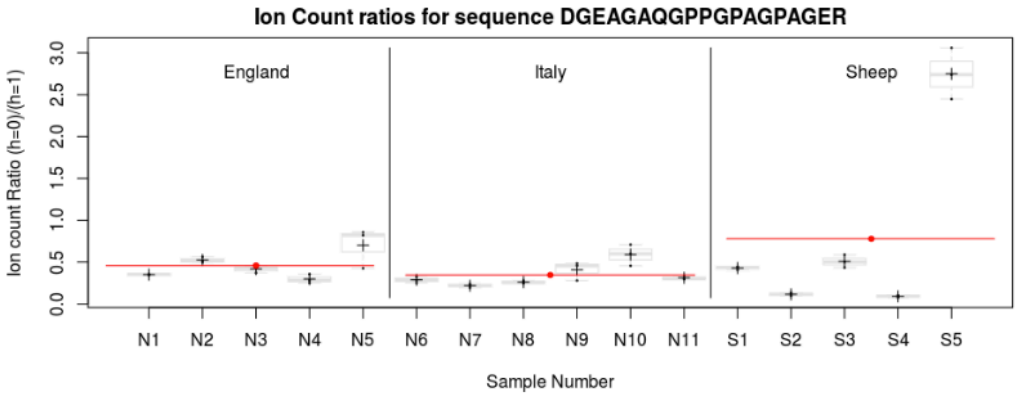


Figure 35: Graph showing the ion count ratios for sequence DGEAGAQGPPGPAGPAGER - the ratio value for sheep was different to what should have been observed, but this could be due to two overlapping peptides and so the sheep ratio data can be ignored

The ratios for the samples from Norwich are on average slightly higher than those for the Italian samples, meaning that - if this potential biomarker is a true marker for scurvy - they appear to be slightly more scorbutic. This is what we would expect to see and so is a potentially good result. The sheep data, used as a control due to the fact that sheep cannot develop scurvy, initially raises concern in that the sheep look to be the most scorbutic group of the three. However, there is an issue with the sheep data in that there appear to be two overlapping peptides around this point with similar masses, and so the sheep data should be discounted in this instance.

Peptide Two: GEPGSPGENGAPGQMGPR

1693.75 was observed in all samples with either two hydroxylations, or with four (although only with four hydroxylations in the Method N data). There was no evidence of deamidation. However, this peptide was dismissed as a potential scurvy biomarker on the basis that it was a poor match to the observed peaks (Figure 36), and also because the sequence includes a methionine.

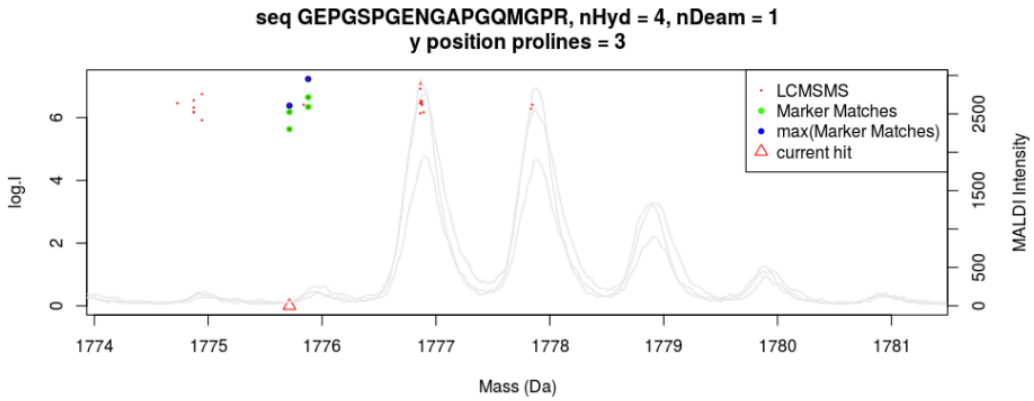


Figure 36: Graph showing the MALDI-TOF-MS spectra (in grey), with the LC-MS/MS data (in red) plotted over the top. LC-MS/MS peptides that match the marker list are shown in green, with the maximum intensity match plotted in blue. The observed MALDI peaks do not correlate with the potential marker or LC-MS/MS data, and so this peptide was dismissed as a potential scurvy biomarker

Peptide Three: GPPGPMGPPGLAGPPGESGR

1783.87 is present in all samples, with either two hydroxylations, or with three. The isotope distribution of the observed MALDI-TOF-MS spectra matched well with the theoretical peak and the LC-MS/MS data (Figure 37).

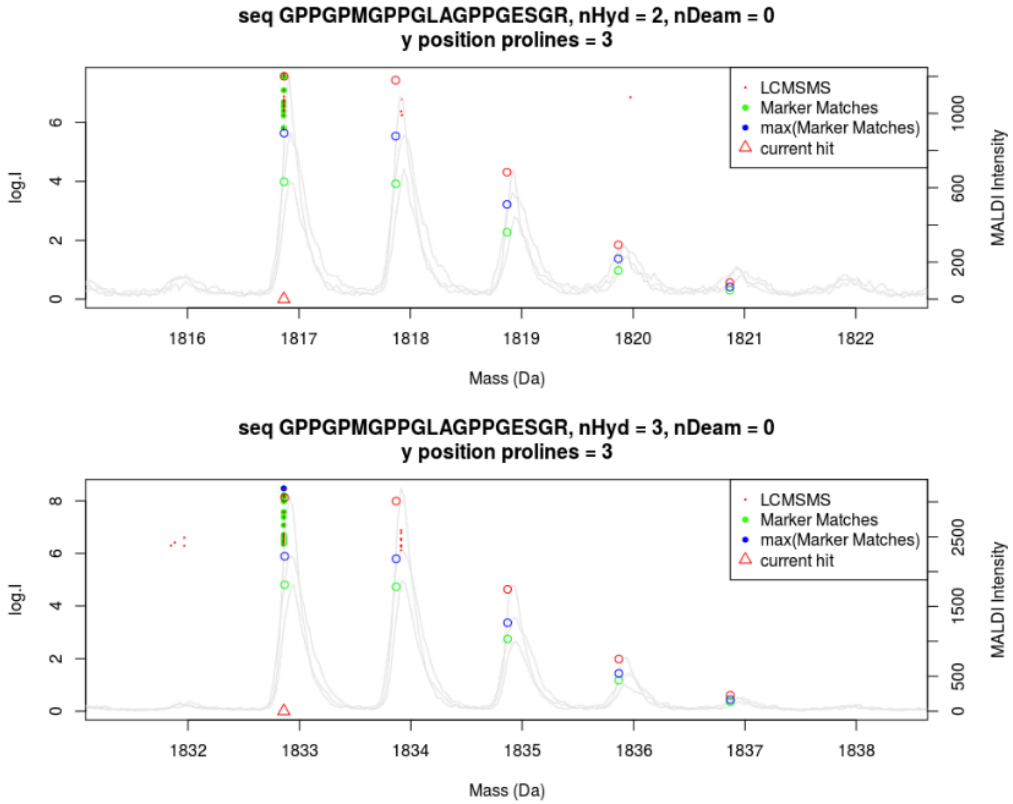


Figure 37: Graph showing the MALDI-TOF-MS spectra (in grey), with the LC-MS/MS data (in red) plotted over the top, for sequence GPPGPMGPPGLAGPPGESGR. LC-MS/MS peptides that match the marker list are shown in green, with the maximum intensity match plotted in blue. The observed MALDI peaks correlate well with the potential marker and the LC-MS/MS data

Unfortunately though, this peptide also contains a methionine: methionine can be oxidised, which, while not linked to scurvy, would still cause the same mass shift of +15.9994 Da that occurs with hydroxylation. This makes this peptide unsuitable for inclusion in this study, as MALDI-TOF-MS only provides the overall peptide mass, with no way to determine the composition of that mass. However, it is still possible that this peptide could be useful in relation to biomolecular studies of scurvy. Subsequently it would be recommended for any future work that methionine oxidation (or reduction) is performed prior to trypsin cleavage; that way, one could be sure that any mass shifts of +15.9994 Da is linked to hydroxylation.

Peptide Four: GSPGADGPAGAPGTPGPQGIAGQR

2072.00 was present in all samples, observed with either one or two hydroxylations. The isotope distribution of the observed MALDI-TOF-MS spectra matched well with the theoretical peak and the LC-MS/MS data (Figure 38).

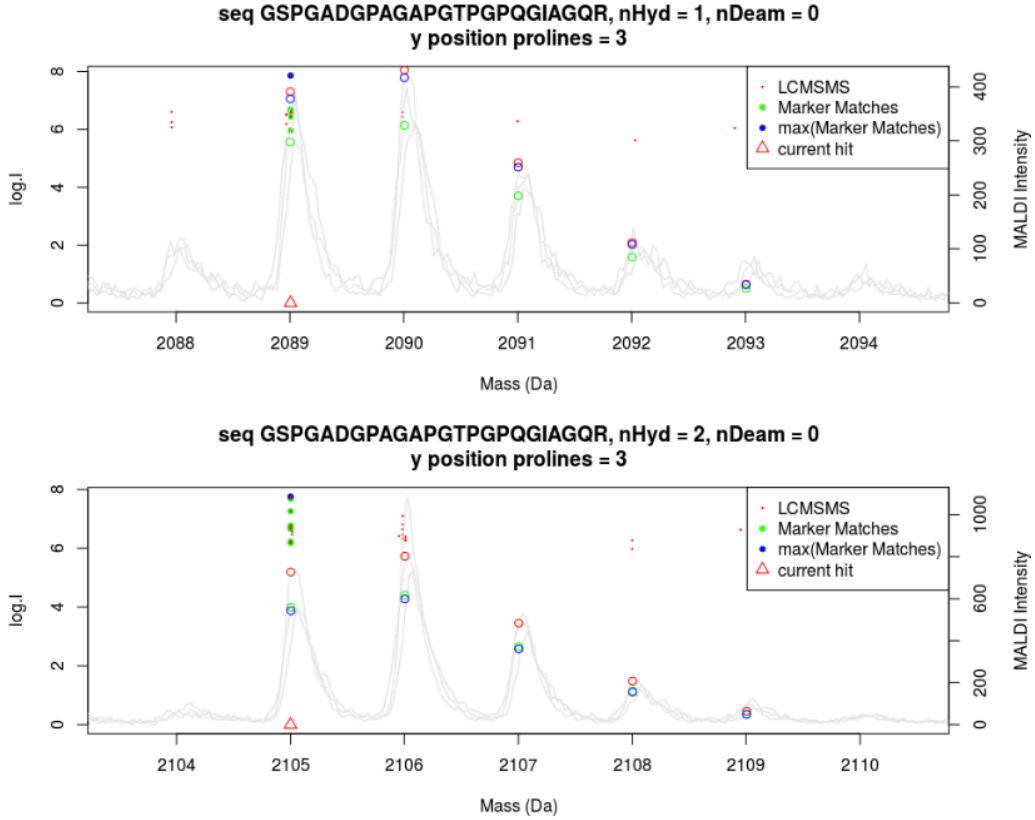


Figure 38: Graph showing the MALDI-TOF-MS spectra (in grey), with the LC-MS/MS data (in red) plotted over the top, for sequence GSPGADGPAGAPGTPGPQGIAGQR. LC-MS/MS peptides that match the marker list are shown in green, with the maximum intensity match plotted in blue. The observed MALDI peaks correlate well with the potential marker and the LC-MS/MS data

This peptide was dismissed because, in checking the hydroxylation ratios, it became clear that something was wrong with the sheep data (Figure 39): the ratios are unexpectedly high - much higher than is seen

in either the Norwich or Italian groups, when the sheep should be the lowest, as it is impossible for them to develop scurvy. Until this issue is resolved, the peptide cannot be considered as a potential scurvy biomarker.

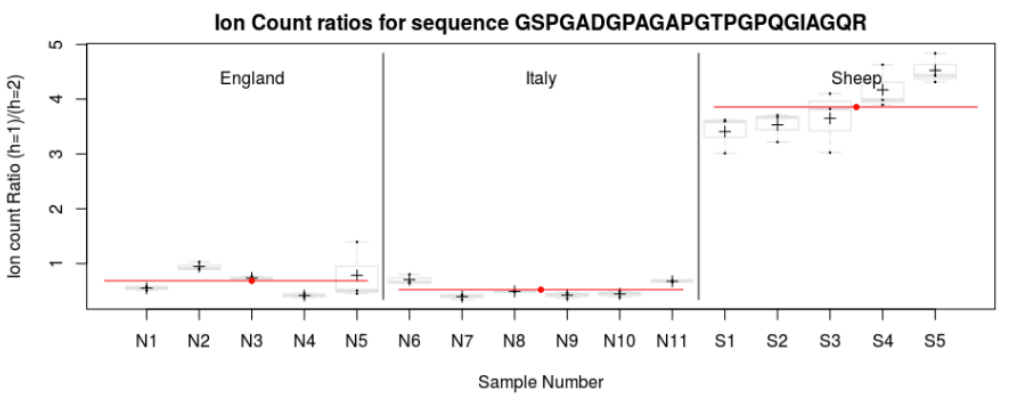


Figure 39: Graph showing the ion count ratios for sequence GSPGADGPAGAPGTPGPQGIAGQR - the ratio value for sheep was significantly different to what should have been observed, and so this peptide was taken out of consideration as a potential scurvy biomarker

Peptide Five: GFSGLQGPPGPPGSPGEQGPSGASGPAGPR

2656.26 is present in all samples, with either two hydroxylations, or with three. No deamidated variants were observed. The isotope distribution of the observed MALDI-TOF-MS spectra matched well with the theoretical peak and the LC-MS/MS data (Figure 40).

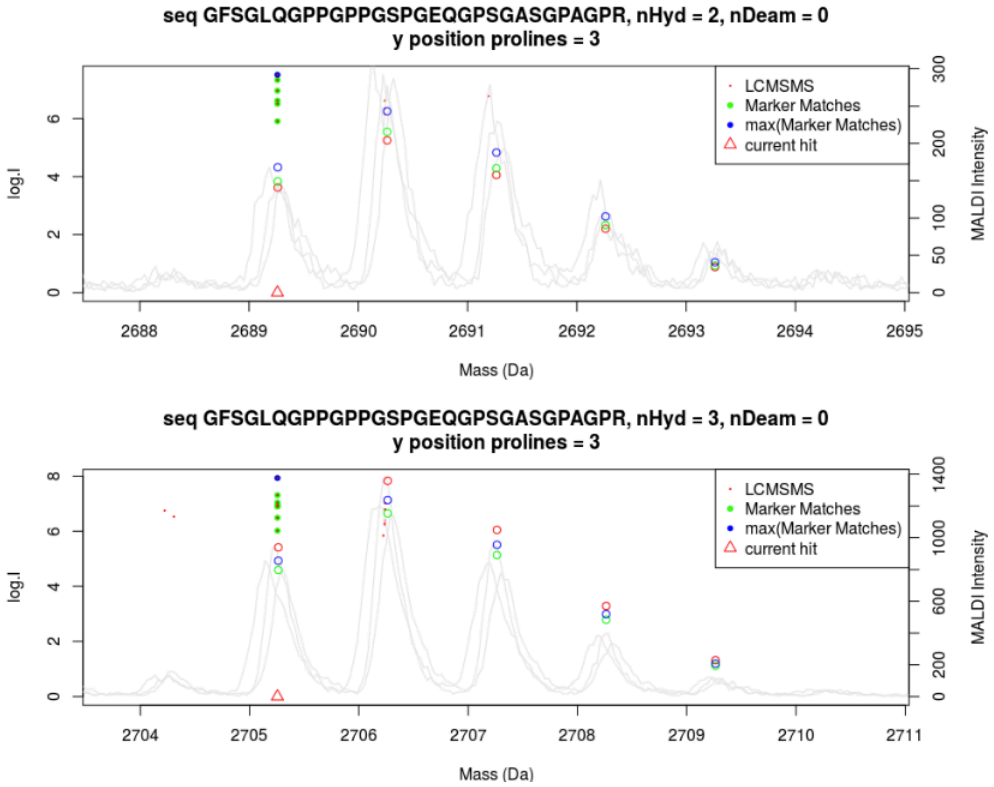


Figure 40: Graph showing the MALDI-TOF-MS spectra (in grey), with the LC-MS/MS data (in red) plotted over the top, for sequence GFSGLQGPPGPPGSPGEQGPSGASGPAGPR. LC-MS/MS peptides that match the marker list are shown in green, with the maximum intensity match plotted in blue. The observed MALDI peaks correlate well with the potential marker and the LC-MS/MS data

The ratio of two hydroxylations to three hydroxylations for this peptide in each of the different human samples was then calculated and plotted, and this was compared to the five sheep control samples (Figure 41).

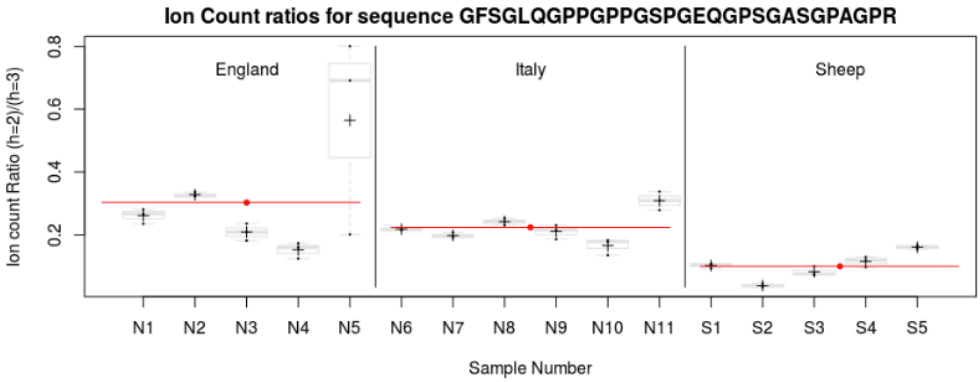


Figure 41: Graph showing the ion count ratios for sequence GFSGLQGPPGPPGSPGEQGPSGASGPAGPR. If a true marker for scurvy, the ratios for this peptide would suggest that the Norwich samples were the most scorbutic, which is what would be expected

The Norwich samples display the highest ratio, which could suggest (if this peptide is proven to be a true biomarker for scurvy) that they were the most scorbutic of the three groups. The sheep - who cannot develop scurvy - display the lowest ratio, implying that they are the least scorbutic group. This is what we would expect to see, and is therefore a promising result.

Peptide Six: GLTGPIGPPGPAGAPGDKGESGPSGPAGPTGAR

2836.41 was present in all samples with either two or three hydroxylations. The isotope distribution of the observed MALDI-TOF-MS spectra matched well with the theoretical peak and the LC-MS/MS data (Figure 42).

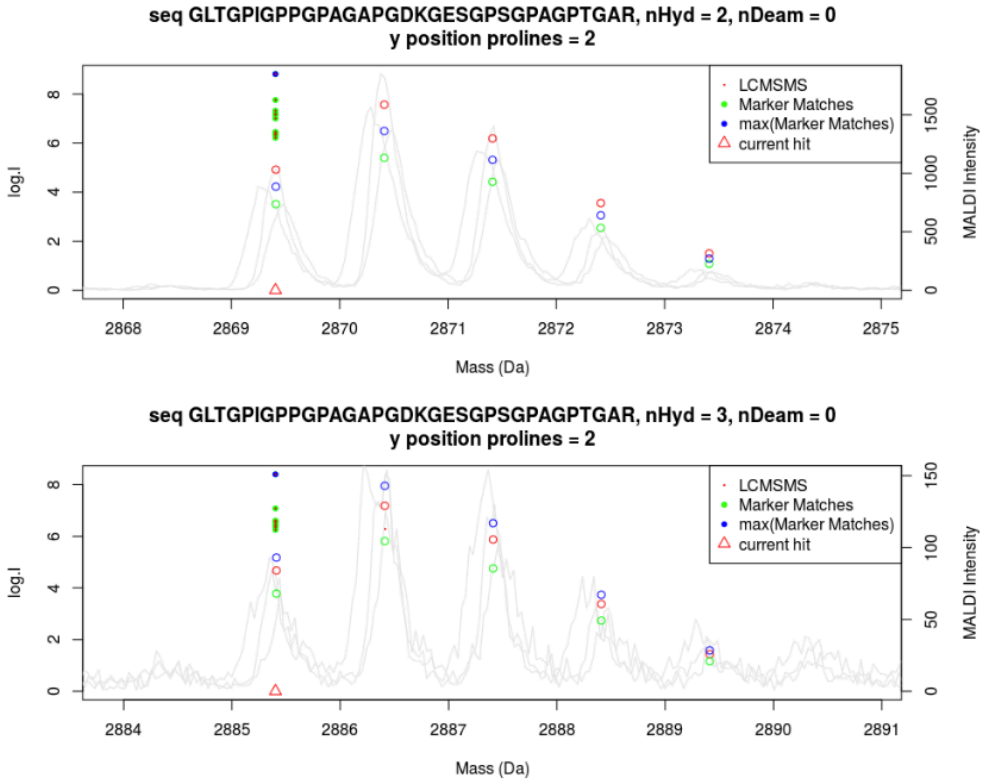


Figure 42: Graph showing the MALDI-TOF-MS spectra (in grey), with the LC-MS/MS data (in red) plotted over the top, for sequence GLTGPIGPPGPAGAPGDKGESGPSGPAGPTGAR. LC-MS/MS peptides that match the marker list are shown in green, with the maximum intensity match plotted in blue. The observed MALDI peaks correlate well with the potential marker and the LC-MS/MS data

This peptide was dismissed for two reasons. First, the hydroxylation ratios were not as expected, ultimately meaning that further testing is required to explain these results: based on Figure 43, the Italian individuals would appear to be slightly more scorbutic than the English individuals, which goes against the initial hypothesis for this work, and against what we've seen with the other peptides. Second, confusion may be being caused by the presence of a lysine in the sequence, which can also be hydroxylated. While lysine hydroxylation could be linked to scurvy, our focus was on prolines and hydroxyprolines, and so the potential effects of the lysine or hydroxylysine could not be considered and accounted for at this stage.

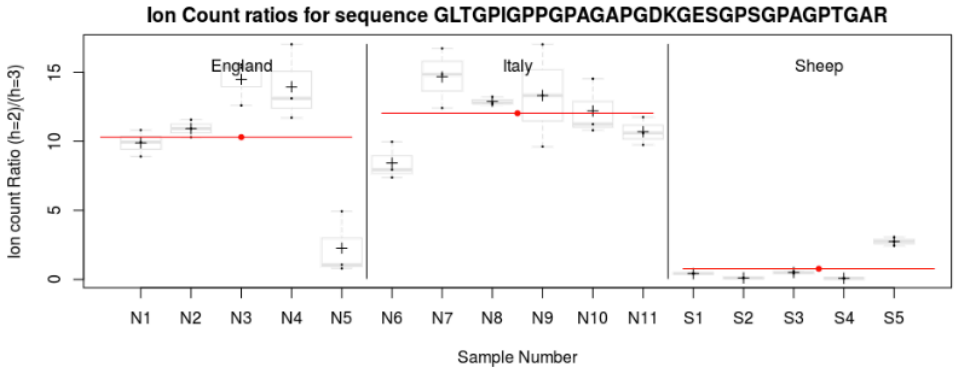


Figure 43: Graph showing the ion count ratios for sequence GLTGPIGPPGPAGAPGDKGESGPSGPAGPTGAR - the Italian samples are displaying a much higher ratio than would be anticipated, which contributed to this peptide being removed from consideration as a potential scurvy biomarker

Peptides One and Five show a good correlation between the MALDI-TOF-MS data, the LC-MS/MS data, and the theoretical peptides. They also display the ratio trend that it was hoped would be seen, and have no issues resulting from methionine or lysine presence. Peptide Three shows the variable hydroxylation that we were interested in, but unfortunately this peptide also has an issue - albeit an explainable one - that currently prevents it from being considered useful as part of this research. Specifically, the inclusion of methionine in the sequence would need to be accounted for in order to test this peptide further. Peptide Two similarly shows variable hydroxylation and also contains a methionine, but has the added complication that there was not a good correlation between the MALDI-TOF-MS data, the LC-MS/MS data, and the theoretical peptides. Peptides Four and Six show variable hydroxylation, and a good correlation between the MALDI-TOF-MS data, the LC-MS/MS data, and the theoretical peptides. However, they don't follow the ratio trend that we would expect to see, and Peptide Six also has the added complication of the inclusion of a lysine in the sequence. The overall conclusion on the six potential scurvy biomarkers is that Peptides One and Five currently appear to be the most useful candidates.

Interestingly, Peptides One, Three, Five and Six all contained GPP in their sequences. As was briefly discussed in Chapter Two, it has been widely believed for a long time that prolines can occur in either the X- or Y-position, while hydroxyprolines can only occur in the Y-position. The data produced as part of this research largely conforms to this, with prolines occurring in both the X- and the Y-positions, and hydroxyproline usually only occurring in the Y-position. However, in the six peptides just detailed, it was observed that when hydroxyproline was present in the Y-position, it was sometimes also present in the X-position. Both Weis and colleagues (2010, p.2583) and Eyre and colleagues (2011, p.7733) similarly observed hydroxyproline in the X-position when it was also present in an adjacent Y-position hydroxyproline, although this was in rat and chicken tendon collagen, respectively, rather than human. However Montgomery et al (2012, 5900) also reported this observation, and they were studying humans. The samples being analysed were breast tissue, rather than bone, but they were studying Type I collagen, which is present in both. These findings may therefore support the findings from the research presented here.

Applying the Potential Biomarkers to Post-Medieval Sites

The final stage in the process was to apply the potential biomarkers to the data from the five sites studied as part of this project (detailed in Chapter Five). This data was all gathered using Method N (FT), to ensure that the bones could be returned at the end of the project.

Expectations

Despite the potential problems outlined above regarding the compatibility between falcon tube data and eppendorf tube data, and despite the fact that the potential biomarkers discussed have not been conclusively proven to be true markers for scurvy, the data from the five sites provides a large sample size from which to see if anything meaningful can be deduced.

Having looked into the backgrounds of each of the different sites included here, in an attempt to establish how these five burial ground populations came to be formed, it was found that there were many factors which influenced this; it was not simply a case of 'poor' people being buried in a 'poor' burial ground. This subsequently makes it quite difficult to hypothesise what results might be seen in regards to the presence or absence of scurvy at each site. For example, in the cases of Bow and Priory Yard, religion seems to have been the driving force behind the decision to be buried there, and there is archaeological evidence - such as the presence of coffin plates - that implies not everyone interred at the site was 'poor' or 'living in poverty'. St Thomas' Hospital burial ground was used for the interment of hospital patients (although possibly not exclusively), which would lead us to expect a higher overall incidence of ill-health among the burial ground population compared to the general population, but not necessarily a higher incidence of scurvy in particular. Farringdon St Brides and Cross Bones were probably the two sites included here where the burial ground population will most closely resemble the general population, but as we have previously determined (in Chapters Five and Six) that scurvy is not solely a disease of poverty, even if everyone who was buried there had been proven to be 'poor' or 'living in poverty', that doesn't necessarily mean that we would expect to see high incidences of scurvy.

Results

Figure 44 shows the results for the Bow Baptists, Cross Bones, and Priory Yard.

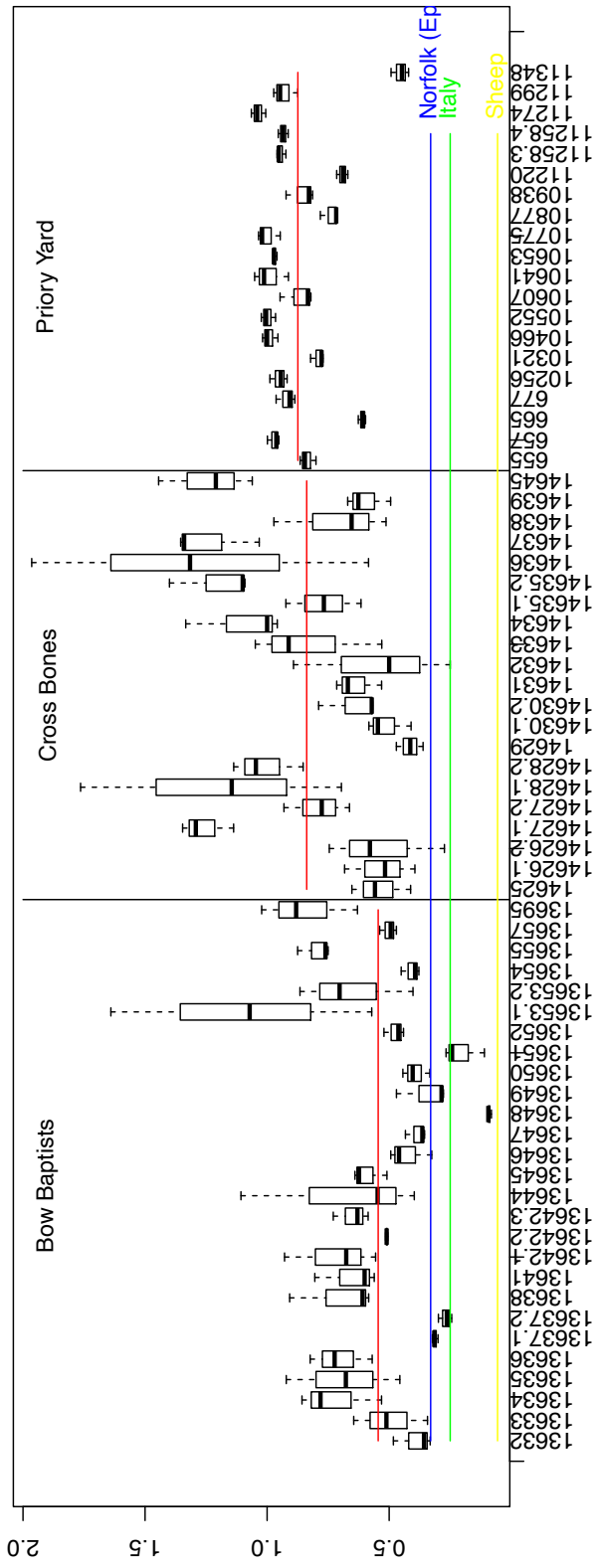


Figure 44: Graph showing the application of the potential scurvy biomarkers to the data from three of the five sites included in this project

The first thing to note is that the data produced from the Priory Yard samples appears to be much less variable than that produced from the two London populations. This could be reflective of different post-excavation cleaning processes being applied to the different collections; while this does not appear to have been specifically documented, there was physical evidence observed during the sampling process for this project that seems to suggest that this was the case. Where the skeletons from the London sites had been cleaned using water, this was obviously not the case with most of the Norwich individuals included here, as many still had soil adhering to the bone surface. If bones were cleaned with water (or worse, soaked in water) then Method N may be compromised as a technique and unable to produce the same quality of results that can be achieved when bones have either not been cleaned or have been dry-brushed. It is becoming increasingly clear that minimal post-excavation cleaning is the best option for enabling biomolecular work to be carried out in the future.

The Farringdon St Bride's Lower data and St Thomas' Hospital data were not included here, as it was noticed that the signal strength for the Bow Baptists and Cross Bones were very low - essentially little more than noise. This was an unexpected finding, and two potential explanations are hypothesised here. First, that the low yield is the result of water-based post-excavation processing with the London samples. Second, that there is a difference in yield when using eppendorf tubes versus using falcon tubes.

A potential point of concern was raised when comparing the general Priory Yard data with the Priory Yard samples chosen specifically because they had been osteologically identified as having scurvy: the general population appeared to have had higher levels of scurvy than the control samples, which shouldn't be possible. It is important to note that

the four ‘control’ samples were taken from various skeletal elements, but none of them ribs, whereas the general population samples were all ribs. It is possible that this somehow resulted in different yields, and that rib data is therefore not compatible with data from other skeletal elements. An alternative explanation is again the possibility of differing yields from eppendorf tubes and falcon tubes, which could mean that eppendorf tube data is incompatible with falcon tube data.

In order to try and resolve these issues, a second LC-MS/MS run was carried out on five different collagen extractions.

LC-MS/MS Analysis - Round Two

The details of samples included in the second LC-MS/MS run, as well as a reminder of those included in the first, can be seen in Table 9.

LC-MS/MS code	Sample ID	Skeletal Element	Collagen Extraction Method	LC-MS/MS Run no.
HCR	15726	Rib	Eppendorf - Method C	1
HCS	13329	Cranial fragment	Eppendorf - Method C	1
HDR	13329	Rib	Falcon tube dilution	2
HFR1	13329	Rib	Falcon tube	2
HFR2	15726	Rib	Falcon tube	2
HFR3	15735	Rib	Falcon tube	2
HNR1	15735	Rib	Eppendorf - Method N	1
HNR2	13329	Rib	Eppendorf - Method N	2
HNS	13329	Cranial fragment	Eppendorf - Method N	1

Table 9: Details of the sample ID, skeletal element and extraction method of samples sent for LC-MS/MS analysis

The author is aware that this is a very small sample set, and that it would be preferable to carry out further LC-MS/MS runs on a larger number of samples to increase reliability. Due to financial constraints, this was not possible as part of this project. However the data obtained from the analyses that were undertaken give an indication as to whether or not

collagen from different bones is comparable, and whether there is a difference in the data produced using eppendorfs versus falcon tubes.

LC-MS/MS analysis was also carried out on a known sheep sample as a control; as mentioned at the start of this chapter, it is impossible for sheep to get scurvy. Table 10 details the three extraction methods that were carried out on sub-samples of the same sheep bone.

Sheep LC-MS/MS Code	Collagen Extraction Method
OCR	Eppendorf - Method C
ONR	Eppendorf - Method N
OFR	Falcon tube

Table 10: The different extraction methods used on sub-samples from the same sheep bone (used as a control sample), the collagen from which underwent LC-MS/MS analysis

The total number of peptides detected for each sample from these LC-MS/MS runs can be seen in Figure 45.

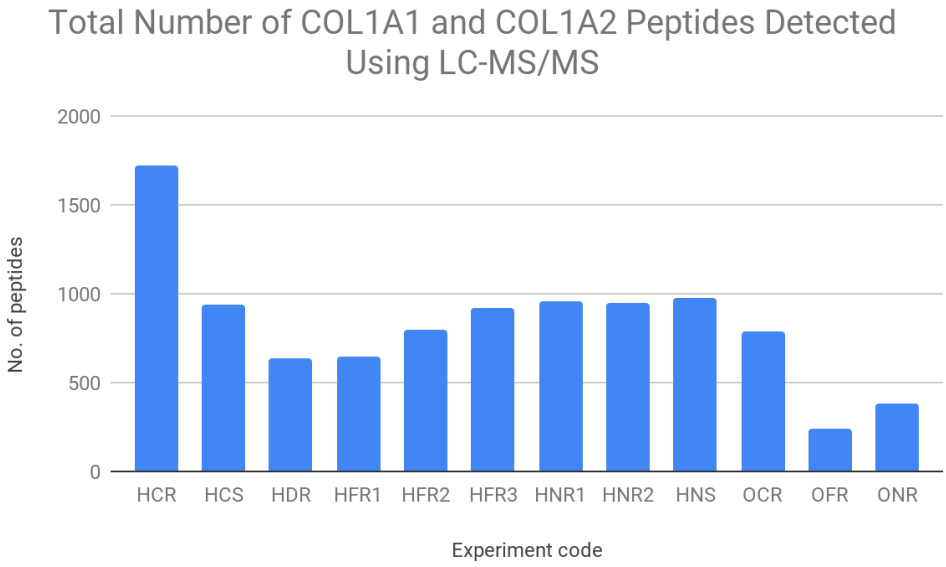


Figure 45: Chart showing the number of peptides detected for each sample using LC-MS/MS analysis

Starting with a comparison of extractions HCR and HCS - both human, both extracted using the traditional, destructive 'Method C' - the results for these samples would appear to suggest that using a rib sample produces a far higher number of peptides than is produced using a cranial sample. This could potentially relate to the higher concentration of cortical bone found in the cranium than in the ribs; it is easier to break down the collagen structure in a rib sample that contains a higher proportion of weak trabecular bone, than to have to break down the dense cortical bone that makes up the cranium. However it should be noted that these two samples are from two different skeletons, albeit from the same site, and so this difference could be a result of differential preservation between individuals. If a real difference does exist, and rib samples produce more peptides than a cranial sample, this could be very beneficial in regards to obtaining samples, as it is much more likely for approval to be granted to sample ribs from a human skeletal remains collection, than for cranial samples.

However, looking at extractions HNS and HNR2 - a cranial and a rib sample, respectively, taken from the same individual as HCS (13329),

but extracted using Method N (eppendorf variation) - we see very little difference in the number of peptides detected. These results therefore do not support the idea that different skeletal elements could produce different amounts of data. Further tests need to be carried out to investigate this potential difference in more detail before any firm conclusions can be drawn.

Samples HFR1 and HNR2 are the same rib from the same individual (13329), both extracted using Method N, but using the falcon tube and eppendorf variations, respectively. The data from these samples would suggest that the eppendorf method enables a higher number of peptides to be detected (HFR1 = 642, HNR2 = 952). Samples HFR3 and HNR1 are also from the same individual (15735), and have also been extracted in the same ways as HFR1 and HNR2. However, while HNR1 does produce more peptides than HFR3 (HNR1 = 956, HFR3 = 922), the difference is nowhere near as pronounced as that with the 13329 samples.

LC-MS/MS Analysis and the Six Potential Scurvy Biomarkers

All six of the potential scurvy biomarkers listed in Table 8 were found in all of the human collagen extractions run using LC-MS/MS.

Discussion and Conclusions

Perhaps the most important point to note is that at this stage we unfortunately cannot prove whether any of the individuals studied here did or did not have scurvy. However, we *can* show how the distribution of hydroxylation levels in proteins can be deduced and studied in MALDI data, with the surprising result that remarkably few peptides have variable hydroxylation levels. This is previously unknown information,

which may - on the basis that scurvy is linked to hydroxylation levels - help archaeologists be able to identify scurvy in human skeletal remains. Currently we can only determine the relative chances than an individual had scurvy, compared with a population of similar samples.

The potential scurvy biomarker identified during earlier research - a peptide with the mass 2291 - was proven through a systematic analytical approach to be invalid. However alternative peptides have been established as potential biomarkers through this same approach. Further work is needed to test these, however this has been beyond the scope of this project. Ideally, this would be carried out on both a human skeletal population known to have definitely suffered from scurvy around the time of death and one known to have definitely not.

This leads us on to an important point in relation to the search for a scurvy biomarker: if the further work just suggested is carried out and a biomarker is established and proven, this will only tell us that an individual suffered from scurvy, we still won't know when in their life this occurred, how long for, or if it contributed to their death. Subsequently, in terms of studying human life in the past, a biomarker is potentially of limited use. On the other hand, a reliable, accurate biomarker would enable archaeologists to finally be able to determine the true scale of the disease in the past, which would in turn help us to understand more about historical diets. Applying this information to enable inferences about poverty is made complicated by two particular issues that have been discussed throughout this thesis; that of the changing use of language over time (what does 'poverty' really mean and how is this defined in a useful way?), and the fact that where a person is buried is not necessarily where they died is not necessarily where they lived - and if they did live there, then it wasn't necessarily for the whole of their life. This does not mean that a scurvy biomarker would have no use - it would solve the identification problem that has plagued osteoarchaeological research

throughout the subdiscipline's history - just that we have to remember the potential limitations of the data.

An important point that follows on from this is that there is currently very little published work which considers bone turnover rates, and those that do report a high amount of variability between individuals – partly due to traceable influences such as age and sex, but also as a result of individual variability, which is essentially impossible to account for. While it could be both interesting and informative to be able to compare data for different age and sex groups (for example, adult males with adult females, and adults with subadults. From there, we could try to compare adult females at different ages, adult males at different ages, and subadults at different stages of growth and development), the current lack of concrete understanding regarding bone collagen turnover rates in humans leaves this out of our reach. If a comprehensive, reliable study that established the turnover rate for each of the different bones in the human skeleton was undertaken, this could potentially be very useful in resolving this, but in the meantime, it is important to simply be aware that variability exists when analysing data and attempting to draw meaningful conclusions from it.

We have seen in this chapter, as was the case in Chapter Three, that different collagen extraction techniques produce different data. However, of key importance to this project is the fact that both Method N and Method C are able to detect the potential scurvy biomarker peptides.

The limited LC-MS/MS analysis undertaken as part of this project has shown that there may be a difference in the data produced when using eppendorfs versus falcon tubes, with eppendorfs potentially enabling a greater number of peptides to be detected. However, as has been mentioned, further work on a larger number of samples would need to be carried out before this could be said with any certainty.

Another avenue for further work involving the LC-MS/MS data would be to run the data produced through the STRING database, in order to map the various protein-protein interactions that are observed (Szklarczyk et al. 2017, p.D362). This could help to further understand the extraction capabilities of the different methods used here.

Overall, the analyses undertaken here in the attempt to establish a scurvy biomarker have revealed three important considerations for studies concerning the archaeology of health and disease: first, the way(s) in which scurvy affects the human skeleton is more complicated than traditionally thought - there does not appear to be one point in the human collagen chain where hydroxylation should always occur and if it does not, then you have scurvy. It is possible that we are looking for variants in the collagen sequence that never made it into the bone: if the collagen being produced by a scorbutic individual was unstable, it may not have been properly incorporated into the bone, making it potentially impossible to identify. Second, we need to be cautious in the way that bones are treated post-excavation, and it would be particularly helpful if the treatment that they do receive was documented in some way. Traditionally, bones were washed using, or soaked in, cold water, to remove dirt and make them easier to study from an osteological perspective. However it would now seem that this approach is destroying some of the biomolecular information that could be gained. If dry brushing was carried out to enable osteological analyses, this could help to preserve the biomolecular information; if non-destructive biomolecular techniques - such as Method N - are then carried out, this would mean that both biomolecular and osteological work can be undertaken on the same bones without negatively impacting on one another. Finally, we need to remember that identifying the presence of a disease is only one piece of the puzzle - we also want to try and understand when in an

individual's life they suffered from that disease, how long for, and whether that caused or contributed to their death.

Chapter Seven: Research Context - Using Dental Calculus to Reveal Evidence of Past Diets

Human dental calculus offers the opportunity to study direct evidence of diet in the past, as food particles become trapped and preserved, surviving long after death. Archaeologists mainly rely on stable isotope analysis in order to analyse past diets (e.g. Beaumont & Montgomery 2016), but while this can give a general overview of dietary trends, it cannot provide any direct information regarding the specific foods that were consumed. Microscopic analysis of dental calculus, on the other hand, can provide direct evidence of some of the foods that individuals were consuming. This chapter will provide information on the existing work that has been carried out on human dental calculus in order to learn about the foods consumed by people living in different time periods and geographical locations. Coupled with the information from Chapter Four (Scurvy and Diet in England), this will set the scene for the analyses detailed in Chapter Eight (Method: A Study of Dental Calculus from Farringdon St Bride's Lower Churchyard).

It is important to note from the outset that dental calculus can be studied for more than just dietary evidence (e.g. see Blatt et al. 2011). However, as diet is the most relevant factor to this project, the other areas of information will not be discussed here in any great detail.

In regards to dental calculus, diet and this project, it is unknown when the potato was first introduced to England, when it was first eaten by people here, or when it was first adopted as a staple part of the English diet. However, it *is* known that at the start of my period of interest the potato was not being consumed, whereas by the end of the period it had very much become an everyday food, particularly for the poor. Therefore, studying the dental calculus of individuals who lived during this period

could potentially reveal evidence that contributes to the debate surrounding the issue of potato adoption in England.

What is Dental Calculus & How Does it Form?

Dental calculus is the term given to mineralised plaque found on the surface of teeth (Hillson 1996, 255; Lieverse et al. 2007, 331; Hardy et al. 2013, 194). Plaque, a biofilm that occurs naturally in the mouth, exists in all humans as the 'pellicle', a thin layer that coats the surface of the teeth (Hillson 1996, 254; Lieverse 1999, 221; Jin & Yip 2002, 427). If the pellicle is not regularly removed (as it is generally thought not to have been for much of human history), enzymes in saliva will cause it to become mineralised and harden (Hillson 1979, 156; Driessens & Verbeeck 1989, cited Hillson 1996, 255 (also 259); White 1997, 509; Hardy et al. 2013, 194; Najeeb et al. 2016, 3; Radini et al. 2017, 72). However, this is a particularly simplified version of events and there are many factors that affect salivary secretion, plaque composition and calculus formation. Here, we must consider both dietary and non-dietary influences.

Arguably the most widely discussed factor affecting the formation of dental calculus is the relative proportion of protein versus carbohydrate in an individual's diet. Traditionally it has been believed that a high-protein diet is more likely to cause dental calculus deposits to form, as it leads to a more alkaline oral environment, which results in increased precipitation of salivary minerals (Hillson 1979, 150; Lieverse 1999, 219), with high-carbohydrate diets being more likely to cause dental caries (Hillson 1979, 150; Meiklejohn & Zvelebil 1991, 132; Lillie & Richards 2000, 969; Humphrey et al. 2014, 954). Indeed some studies have gone as far as concluding the relative makeup of a person's diet based on the presence or absence of calculus and caries (e.g. Lillie 1996, 140; Keenleyside 2008, 275). While there may be evidence to suggest that

the ‘high-protein - calculus / high-carbohydrate - caries’ theory is true, it would seem a little risky to use this as the basis for dietary assumptions about individuals living in the past, especially given that oral hygiene was generally so poor.

A further dietary factor to be aware of in regards to plaque deposition is that of tea-drinking. A modern study involving 35 adult volunteers showed that rinsing the mouth with an oolong tea extract solution before and after meals, along with once at the end of the day, significantly *reduced* the amount of plaque being laid down by the participants (Ooshima et al. 1994, 147–8). This could suggest that tea-drinking results in fewer dental calculus deposits, although one point to be aware of is that, while plaque deposits are required for the formation of calculus, there is no proven correlation between the amount of plaque occurring in the mouth and the size of the resulting calculus deposits, and the rate at which calculus deposits form is unknown (Hillson 1996, 259; Henry & Piperno 2008/7, 1944). Another study looked at the effect of tea-drinking on salivary amylase (amylase being an enzyme present in saliva that is responsible for the initial stage of starch digestion), comparing black and green teas (Zhang & Kashket 1998, 234). The results suggested that both kinds of tea, but especially black tea, inhibited salivary amylase (Zhang & Kashket 1998, 237). This could mean that starch is more likely to survive in the calculus of tea-drinkers, in those instances where calculus does form, than in non-tea-drinkers. However, it should be noted that both of these studies involved the participants rinsing their mouths with a small volume of tea that had been prepared to a particular concentration, and that this might have a different effect to drinking tea, especially if the tea is prepared in a different way and/or to a different concentration. While it may very well be possible to ascertain how tea was commonly prepared in the past, it is impossible to know the concentration of tea in every cup that was made or how much any individual was actually consuming. Finally the importance of the nature of the starch granules themselves

and the effects of processing / cooking on the speed of action of amalyse should be considered (Sarikaya et al. 2000). Waxy starches are generally more resiliant, cooked starches are less likely to persist relative to uncooked starches, unless retrograded, (which follows from cooling or partially swollen starches) which makes them perculiarly resilient (Fredriksson et al. 2000). The results of these studies, therefore, serve as a useful guide, but they do not provide us with directly applicable evidence.

More recently it has been recognised that things are actually more complicated than being purely based on diet, and are certainly more complex than purely whether an individual consumes a high-protein or a high-carbohydrate diet (Radini et al. 2017, 73); there are other, non-dietary, factors that also need to be considered.

One obvious consideration is that of oral hygiene: the toothbrush as we would recognise it today was not invented until 1938, when 'Dr West's Miracle Tuft' toothbrush began to be produced by the Weco Products company (González et al. 2015, 211; Anon n.d.), but prior to this there were boar bristle toothbrushes (first introduced in China in 1498), and before that there were 'chew sticks', which have been referenced in literature as far back as 3000BC. However, these items were relatively less sophisticated than the oral hygiene products available today, and they were not necessarily widely adopted, whether that be due to lack of availability or the cost of such items (Roberts & Cox 2003, 327).

Another important factor is that of salivary flow rate (SFR). As has been mentioned, saliva contains enzymes that mineralise plaque on the teeth, resulting in dental calculus deposits. A change in the rate that this saliva is produced and moves through the mouth could therefore have an effect on the amount of dental calculus build-up. However, SFR can be

influenced by a number of factors, such as dehydration, which slows it down (Dawes 1970, 1264; Dawes 1987, 653; Walsh et al. 2004, 153).

One notable factor that also slows down SFR is smoking tobacco: a factor definitely relevant to life in England during the long-eighteenth century for members of all socioeconomic classes (Stewart 1967, 243; Nash 1982; Goodman 1994, 68). One study conducted on 100 long-term smokers and 100 non-tobacco users showed that smokers have a significantly slower SFR and a significantly higher rate of dental calculus than non-smokers (Rad et al. 2010, 3 – 4). This finding was supported by a more recent study by Kanwar et al. (2013, 299), who also found the same effect was caused by chewing tobacco as by smoking tobacco, which could again be relevant to studies of life in long-eighteenth century England. Two studies regarding the effects of long-term smoking on salivary secretion mention that SFR actually increases upon taking up smoking, but then slows over time (Khan et al. 2003, 37; Khan et al. 2005, 1), however there seems to be little if any reliable evidence to support this claim. Regardless, as the formation of dental calculus deposits is a lifelong process, short periods of smoking that temporarily either increase or decrease the SFR are unlikely to have a significant effect. Further support for these findings comes from Jan Bergström's two studies, which looked at the effects of smoking tobacco on dental calculus deposits, although with a less specific focus on SFR, as she also found in both cases that smokers had a significantly higher amount of calculus present (Bergström 1999, 543; Bergström 2005, 83).

There are potentially other factors also affecting the formation of dental calculus, such as age, genetics and disease (Henry & Piperno 2008/7, 1944; Horrocks et al. 2014, 29), but research in these areas is limited and there appears, to date, to be no conclusive evidence.

Added to this, there are different types of calculus, namely supra-gingival (found on the tooth crown, above the gumline) and sub-gingival (found on the tooth root, below the gumline) (Dobney et al. 1987, 8; Hillson 1996, 256; White 1997, 509; Lieverse 1999, 220). Sub-gingival calculus generally survives better in the archaeological record than supra-gingival, as supra-gingival calculus can become detached from the tooth crown(s) either as a result of taphonomic processes, during excavation, or during post-excavation processing and/or storage (Dobney & Brothwell 1986, 55; Dobney et al. 1987, 11; Hillson 1996, 256–7; Freeth 2000, 228). It should also be noted that if the gum line recedes - possibly due to poor oral hygiene - then sub-gingival calculus can essentially become supra-gingival, and subsequently becomes more susceptible to be lost.

With so many different factors potentially affecting the formation of dental calculus and the possible entrapment of dietary debris within that calculus, it is important to question at this point what can be said about a population from dental calculus microscopy work without studying the entire population. Furthermore, even if the entire population is analysed, we can never be completely sure that the information gained is the whole story.

How Does Material Become Trapped in Dental Calculus?

When eating food, small particles can attach to naturally-occurring plaque on the teeth. Salivary enzymes should break down food particles in the mouth, but there are some that manage to escape this process. These particles, if not then removed through oral hygiene processes, will eventually become permanently trapped when the plaque mineralises and becomes calculus (Marcotte & Lavoie 1998, 77; Blatt et al. 2011, 669). The rough nature of the surface of dental calculus attracts further

deposits, which again will mineralise and harden, gradually forming a layered structure (Whittaker et al. 1998, 941; Lieverse 1999; Hardy 2009, cited Hardy et al. 2013, 194). However, as will be discussed later on in this chapter, these layers are not identified or separated for microscopic analyses due to the nature of the sampling and analysis processes used.

For the purposes of this project, we are mainly concerned with the way in which starch granules become trapped in dental calculus (Blatt et al. 2011, 669). As with other food debris, they adhere to plaque but polysaccharides that occur as part of the plaque matrix have been found to protect them from the destructive effects of amylase (Hardy 2009, cited Hardy et al. 2013, 191).

Existing Archaeological Work Involving Dental Calculus

The idea of studying dental calculus for evidence of dietary behaviours was first mentioned by Don Brothwell and Keith Dobney in the 1980s (e.g. Dobney & Brothwell 1986; Dobney et al. 1987, 6). This work was initially based on SEM analyses of calculus samples, with light microscopy being introduced later. However, this was sometimes carried out in a very different way to the light microscopy undertaken today, in that the dental calculus was not always removed from the teeth of the specimen being studied (e.g. Dobney et al. 1987, 19). Despite these differences, this early work recognised the need for and potential difficulties with cleaning dental calculus samples to ensure that there is no contamination (Dobney & Brothwell 1986, 61; Dobney et al. 1987, 17). While the processes we use today are slightly different to the methods being trialled then - it is now common to use a weak hydrochloric acid as opposed to alcohol or airbrushing (Dobney et al. 1987, 17) - the aim of

removing inevitable contaminants from the burial environment while preserving as much of the sample as possible is still the same.

These early studies of dental calculus aimed to establish what, if anything, could be identified within the calculus of both humans and animals, with a primary focus on dietary evidence. This initially involved work that focussed on phytoliths (e.g. Fox et al. 1996; Juan-Tresserras et al. 1997), and then expanded to include the study of starches.

The first archaeological study that aimed to investigate dietary evidence of starches trapped in human dental calculus was published in 2009, and was born of the desire to obtain direct evidence of foods being consumed, as microwear analysis of artefacts such as stone tools cannot confirm whether or not particular foods were actually being eaten (Hardy et al. 2009, 248). Twenty-five samples from twenty-three individuals, excavated from three different sites from different areas of the world, were studied using light microscopy for evidence of starch (Hardy et al. 2009, 251). The authors' report that starches were identified in nearly all of the samples studied, proving that starch can survive in dental calculus, and can subsequently be used to provide direct evidence of consumption (Hardy et al. 2009, 254). However, as is acknowledged in the concluding remarks of the paper, identifying these preserved starches to species level is not always possible, and even when it is, there is still the difficult question of how representative of an individual's diet they are (Hardy et al. 2009, 254).

Since the publication of the paper just discussed, the vast majority of research regarding archaeological dental calculus has focussed on prehistoric samples (e.g. Mercader 2009; Li et al. 2010; Hardy et al. 2012; Henry et al. 2012; Hardy et al. 2013; Henry et al. 2014; Hardy et al. n.d.; Power et al. 2015/8; Cristiani et al. 2016). This raises a number of questions, primarily whether or not this focus is born of a belief that, for

historical periods, reliable information on diet can be gleaned from historical texts, with little to be added by microscopic analyses.

Whilst discussing these prehistoric studies in any great detail here would be largely unhelpful, as the findings would have little impact on the current study of diet and poverty in post-medieval England, there are some interesting and important points raised through them regarding the overall concept of dental calculus light microscopy and what it can achieve. For example, Hardy and colleagues studied one sample of dental calculus from two individuals excavated from the Neolithic site of Çatalhöyük, with the aim of establishing whether or not non-domesticated starchy plants were consumed by people living at the site, and starch granules were found in both (Hardy et al. 2013, 191 & 195). However, the authors do not confirm whether it was domesticated or non-domesticated starch that was observed, and so the published results of the study do not enable us to know whether or not the aim of the research was achieved. Almost regardless of this, it needs to be remembered that this was a study involving two samples - each individual human has the potential to have 32 adult teeth, and so the potential for 32 different calculus deposits. Due to the varied nature of calculus formation, as discussed above, two samples from one individual could tell very different stories. We must also remember that absence of evidence is not evidence of absence: it is possible for foods consumed to not become trapped in an individual's dental calculus. Understandably, then, it is unlikely that these two samples were fully representative of the diets of the two individuals that they came from, let alone of the entire community that those individuals were a part of. A further point of consideration in this case is the site's long period of use - the Neolithic East Mound area of the site, where the two individuals studied were uncovered, dates to c.7400 - c.6000 BC (Hodder 2013, 1). It could reasonably be assumed that over a roughly 600 year period of occupation, consumption patterns at the site may change. Therefore, finding evidence that non-domestic

starchy foods were consumed by two individuals would not help with establishing when they began to be eaten, whether they were widely adopted as a staple, or why. One final consideration is that the samples appear to have only been studied for evidence of starch, in line with the authors' research questions. As has already been mentioned, dental calculus can be studied for both dietary and non-dietary debris, and so a wealth of information could have been lost by only looking for evidence of starch; an arguably important consideration when working with such ancient and limited sample material.

This theme of trying to trace the move towards domesticated plant consumption through dental calculus microscopy work is relatively common. For example, Piperno and Dillehay (2008, 19625) used dental calculus evidence to establish that farming must have been taking place in the Peruvian Andes by roughly 10,000 years ago, some of the earliest evidence for that region. Another example is that of Cristiani and colleagues (2016, 10298 & 10302), who discovered evidence of starch in dental calculus that pushed back the date at which farming was established in the Balkans by roughly 400 years, from 6200 cal. BC to 6600 cal. BC. These examples both serve to confirm the value of studying prehistoric dental calculus samples in order to better understand diet in periods where there are no written records. However, dental calculus - as is the case with any archaeological evidence - is a limited resource, and so we need to be sure that the questions we want answers to are both important enough to warrant its destruction, and are actually likely to be answered by this kind of research.

A recent paper by Hardy and colleagues details the microscopic analysis of one sub-sample of dental calculus dated to 1.2 million years ago (Hardy et al. 2017, 1–2). The authors report the earliest direct dietary evidence for the genus *Homo*, and they consider both dietary and non-dietary findings from the analysis, but given that only part of one sample

was studied, they arguably overstate their concluding claim that an entire population must have "had a detailed understanding of its surroundings and a broad diet." (Hardy et al. 2017, 1, 2 & 4).

A further example of this is the study of 30 dental calculus samples from 27 individuals from Easter Island, where researchers hypothesised that direct evidence of sweet potato, yam, taro, and/or banana might be found. The island was colonised around AD 1200 but dating of human teeth found at the site (only some of which were the same as those selected for the dental calculus microscopy study) placed them around AD 1330, while obsidian hydration dates of artefacts found buried with some of the human skeletons date to between AD 1680 and AD 1880, ultimately giving a potential 550-year period of interest (Tromp & Dudgeon 2015, 56). Existing sedimentary and palaeoecological evidence from the site confirmed that the hypothesised foodstuffs had been present, and written records following European contact in 1722 detail that they were commonly consumed by the island's inhabitants, so it is largely unsurprising that evidence of sweet potato was found in the calculus (Tromp & Dudgeon 2015, 55 & 60). The results of this study do not reveal anything that was not previously known, and serve only to confirm quite sound existing knowledge, which raises the question of how useful and appropriate the undertaking of dental calculus microscopy work was in this instance.

One exception to this seeming trend towards prehistoric samples is the recent study by Radini, Nikita and Shillito (2016), which looked at human dental calculus from 14 individuals from the medieval Parish of St Michael in Leicester. The authors state that they are studying the dental calculus as it could "provide a novel source of information about past diet and living conditions" (Radini et al. 2016, 298), but they don't detail what dietary information is already known for the area during the 200 years that the cemetery site was in use, from AD 1250 - 1450, or mention any

significant recorded dietary shifts or events that could potentially be confirmed or better understood as a result of microscopic analysis of the calculus. The results of this study, in regards to dietary evidence, did not reveal anything unusual or unexpected (Radini et al. 2016, 305 – 306). These findings could be used to question the benefit of studying dental calculus from historical periods for dietary information. On the other hand, non-dietary evidence was also recovered, which could provide information on aspects of life not well-documented, and if the calculus is already being studied then considering dietary evidence would seem to make sense.

When considering the possibility and potential of studying dental calculus from historical periods, it is important be aware of the likely availability of sample material. Roberts and Cox, in their seminal book 'Health and Disease in Britain', declare an average dental calculus prevalence of 21% for the post-medieval period (defined by them as lasting from roughly 1550 to roughly 1850) (Roberts & Cox 2003, 324). This average is based on dental calculus information from four archaeological human skeletal remains collections, the majority of which are reported to have been crypt sites, and are therefore most likely to inform us only about the middle or upper classes, thus excluding a large portion of the population (Caffell & Clarke 2011, 261). A further problem is that four sites, from four different areas of the country, spread throughout a roughly 300-year period of focus, cannot possibly give an average to which any other site can be usefully or meaningfully compared. To highlight this point further, if we consider three of the London-based sites that feature in this project, all dated to between roughly 1550 and 1853, the average dental calculus prevalence is 33% (see Table 11): notably different to the published average that is not based on any more information (658 individuals from four sites compared to 885 individuals from three) (Roberts & Cox 2003, 327). We must conclude from this that the published average needs much more data from a variety of locations across the country in order to be deemed both useful and reliable.

Site	Total	%	Adults	%	Sub-adults	%
Farringdon	213/544	39.15	184/369	49.87	29/175	16.57
St Thomas'	56/193	29.02	47/160	29.38	9/33	27.27
Cross Bones	44/148	29.73	34/44	77.27	10/104	9.62

Table 11: Dental calculus prevalence for three of the London sites featured in this project.

Limitations of Dental Calculus Studies in Archaeology

While the study of dental calculus using light microscopy can provide us with direct evidence of diet in the past, there are a number of issues surrounding the technique that limits its potential usefulness.

In many cases, we still cannot determine the particular species of starch that is being observed. If studying an individual or population where documentary sources detailing dietary information exist, this limitation may mean that the destruction of archaeological evidence is less justifiable. On the other hand, it should be remembered that combining documentary sources with evidence from light microscopy may help in narrowing down the list of potential species that the starch could be, by providing information as to which species were present in that area at the time.

Even when starch is found in dental calculus, and if the species of that starch can be identified, there are still questions raised that cannot be

answered: did this individual eat this food regularly, or only try it once?; When in their life did they eat this particular food?; If they ate it regularly, what proportion of the diet did it make up?; Did they consume that food in the area nearby to where they were eventually buried or elsewhere?

A further problem that was mentioned briefly above is the issue that calculus forms in layers, but these layers do not form at a set rate - meaning that it is impossible to know which stage of life they represent - and furthermore they are destroyed as part of the process of preparing the calculus for study under a microscope (Radini et al. 2016, 309). Even if we could sample and study the calculus in layers, we would not be able to say for certain that different foodstuffs occurring in different layers meant a dietary shift, as it could simply be that only some of the foods being eaten became trapped. Also, given that people did not always stay in the same place throughout their lives, food debris that has become trapped in the calculus may not be reflective of the food that was available and being eaten in the area local to where that individual was buried (Radini et al. 2016, 309).

This leads on to the common problem in archaeological research of rarely having precisely dated material: when it comes to human skeletal collections relating to historical periods, especially, these are usually taken from graveyards or burial grounds that were used for at least a hundred years, if not longer. This can make it even harder to know whether evidence found in dental calculus samples using light microscopy is significant or not in terms of tracing dietary change.

Another point for consideration is that some things will have been deliberately placed in the mouth, but not actually consumed. For example, Hardy (2008, 275) details how women in Papua New Guinea commonly re-moisten bast fibres by chewing them in order to make string. In most cases, especially when studying individuals from post-

medieval England, it should be quite obvious which phytoliths or starches relate to diet and which do not, but it is not necessarily possible to be 100% sure 100% of the time.

One of the biggest limitations of dental calculus analysis is how representative the results can be considered to be. Can one sample from one tooth accurately represent an individual's overall diet? How many people's calculus has to be analysed to be deemed representative of a community or population? People who live in the same geographical area today have very varied diets and dietary habits, and while there would undoubtedly have been less variation in the past, there would still presumably have been some. On that basis, one or two individuals cannot represent an entire population. On the other hand, a common limitation within archaeological research is small sample sizes. In cases where only a limited number of samples are available for study, this does not necessarily mean that those samples should not be studied, but that we as researchers must be careful not to overstate what those samples can tell us.

Chapter Conclusions

In this chapter we have considered the basic biology of dental calculus, how it forms and how debris that could be of interest to archaeologists becomes trapped within it. There are a significant number of factors that influence the formation process and just the presence or absence of dental calculus in an individual is tied to all of these, making it difficult to really compare one person with another, even within the same population.

Light microscopy analysis of dental calculus can provide us with direct information regarding the diets and general lives of people living in the

past, but the limitations associated with its study - which we must not only be aware of but also try to account for - can severely limit its usefulness, particularly in historical periods, where so much is already known. The questions that we hope to answer through the analysis of dental calculus must be clear and it should be established even before sampling takes place whether or not it will be realistically possible to answer them given the restrictions discussed above.

Chapter Eight: Microscopic analysis of dental calculus from Farringdon St Bride's Lower

As discussed in Chapter Seven, research using dental calculus has allowed scholars to identify a wide variety of both dietary and non-dietary microfossils, and thus to make genuinely novel reconstructions of life across many periods of human history. These discoveries lead to the development of one of the key aims of this project; trying to match biomolecular data and dental calculus-derived information about diet, in order to better understand the lives of individuals residing in eighteenth century England.

This chapter will detail the light microscopy analysis of dental calculus samples from individuals buried in the Farringdon St Bride's Lower burial ground. The aim of studying these samples was to track potato consumption through evidence of tuber starch that had become trapped in a calculus deposit during the course of an individual's lifetime. As was discussed in Chapter Four, potatoes are a surprisingly good source of Vitamin C, and were present in England during the long eighteenth century. A number of researchers (e.g. Cheadle 1878, cited ; Poynton 1935; Rajakumar 2001, 2; Pimentel 2003, 329; Brickley & Ives 2006, 171; Magiorkinis et al. 2011, 148; Geber & Murphy 2012, 512–3) have suggested that the inclusion of potatoes in the diet may have reduced levels of scurvy. Furthermore, finding evidence of potato starch in dental calculus could help us to establish when the potato began to be eaten in England - as we know from Chapters Four and Seven, the potato was distrusted when it first arrived in England, and that it was not until the end of the eighteenth century that it was considered a staple part of the diet.

Starch granules are a major component of tubers (Hardy et al. 2009a, 248), and so eating potatoes would put a high number of starch granules

into the oral environment of the consumer. It is likely that some of them would then have become trapped and preserved in the calculus. As will be discussed further below, potato starch granules are particularly distinctive and so should be easily identifiable, with very little chance of mis-identification.

It was hoped that by studying samples from individuals buried in the Farringdon St Bride's Lower burial ground, the dental calculus findings could be compared to the scurvy biomarker findings. This, in theory, would allow a systematic comparison of the presence/absence of potatoes in dental calculus and the presence/absence of scurvy by individual and within this burial ground population. By linking two different kinds of source, it was possible that new material could be found about the changing incidence of scurvy and its relation to diet.

Materials and Methods

Samples

In total, 18 samples from the Farringdon St Bride's Lower cemetery population, currently housed by the Museum of London Centre for Human Bioarchaeology, were made available for analysis using light microscopy. The particular samples used for the microscopy work were leftover material from a proteomic study also undertaken at York, and so were essentially sub-samples of the original larger samples. This is important to note; as discussed in Chapter Seven, if we were only looking at a fraction of the total amount of dental calculus deposited in the mouth, then that means that we were not looking at the full and complete picture of what was preserved in the calculus of each individual. It is possible that there could have been different microfossils trapped in a sample from a different tooth from the same individual, which could tell us a different

story about that individual's diet. The information that we can glean from sub-samples is still valid and potentially of use in learning about the different foods being consumed in the past, as long as we recognise and remember this limitation.

For all 18 calculus samples, there were rib samples available from the same individuals, which were analysed as part of the scurvy biomarker aspect of this project (as detailed in Chapter Six), subsequently making comparisons between the biomarker data and any dental calculus data possible.

Sample Processing and Slide Preparation

Cold hydrochloric acid extraction has been employed by a number of archaeological researchers carrying out microscopy work on dental calculus samples (Hardy et al. 2009b; Hardy et al. 2012; Buckley et al. 2014; Warinner et al. 2014a; Tromp & Dudgeon 2015; Hardy et al. 2016). A slightly modified method of this process was applied here.

The samples were first weighed into eppendorf tubes and the weights recorded. They were then washed with ultrapure water and placed on a roller-rocker for a period of seven days. This removed most of the surface contamination from the samples, whether that originated from the burial environment, storage or handling.

Following this initial washing, the surface of each sample was cleaned more accurately with a fine needle and cold 0.6M hydrochloric acid, to ensure the complete removal of any contaminants. All samples were then suspended in 0.6M hydrochloric acid to decalcify, in order release the trapped microfossils for analysis (Radini et al. 2017, 74). The volume of acid used was dependent on the mass of the calculus sample.

Once decalcification was complete, one drop of the resulting calculus solution was placed on a microscopy slide and a coverslip placed on

top. The coverslip was sealed at the corners using clear nail polish and labelled; by securing only the corners, rehydration of the slide was made possible, although this was not found to be necessary. Slides were analysed immediately and any remaining sample liquid was stored in the original eppendorf tube at 4℃.

Contamination Risks

There have been many studies published on the potential post-excavation contamination risks in dental calculus studies. These have had an important influence on standard procedure for light microscopy analyses. Ultrapure water is the only kind of water used in cleaning the samples, food is not allowed into laboratories, and non-powdered gloves are used as standard (Loy & Barton 2006; Messner 2011). Archaeological and modern samples are prepared in different locations (Loy et al. 1992; Pearsall et al. 2004; Wesolowski et al. 2010), single-use consumables - such as eppendorf tubes - are used wherever practical (Allen & Ussher 2013), and non-disposable items are sonicated and cleaned with alcohol between different samples (Li et al. 2010; Yang & Jiang 2010).

Throughout this process, all of these precautions were taken to ensure that contamination risks were minimised. However, Warriner and colleagues (2014b, 60 – 61) still identify three contamination concerns specific to dental calculus research, which it was important to account for here: first, that there may be food particles on the hands of the researcher when processing the sample or preparing the slides; second, that there may be airborne pollutants or dust that become incorporated into the sample, again either during processing or when preparing slides; finally, there is the potential for soil from the burial environment to have worked its way into micro-cracks in the calculus.

To address the first concern, on days when samples were being processed or slides prepared and analysed, no starchy foods that could potentially be found in the calculus were handled, prepared or consumed. Hands were washed thoroughly, powder-free nitrile gloves worn, and the clothes worn were noted for the potential benefit of future researchers, in case of fibre contamination. The second concern was addressed by regular cleaning of the laboratories where samples were prepared and analysed (two at the Kings Manor, and one at the BioArCh facility, both at the University of York), and by working on fresh aluminium foil for each sample. One aspect of laboratory sterility that could not be controlled was the fact that neither of the laboratories at the Kings Manor were purpose-built for dental calculus work and both had previously been used for other types of research. However, they are both deep-cleaned regularly and all surfaces are wiped down daily, so the contamination risk from any previous work should be negligible. In regard to the third contamination risk, when the samples are cleaned using a needle and 0.6M HCl, this is done under a microscope, to give the best possible chance of removing all contaminants that would not be visible to the naked eye.

Classification of Findings

The different categories of debris that were observed in the dental calculus were recorded according to a presence-absence scale of 0 - 3, where 0 = absent and 3 = abundant. This is quicker than counting how many of each phytolith or starch is observed (which would have been impracticable considering the sheer amount of grit that was seen in every slide for all samples), but is not exact and is therefore subject to interobserver error. Also one individual's opinion on what classes as '1', '2' or '3' might change as more and more samples are analysed.

Identification of Starches

Starch grains grow from a point termed the hilium (see Figure 46) (Gott et al. 2006, 35; Tromp & Dudgeon 2015, 55).

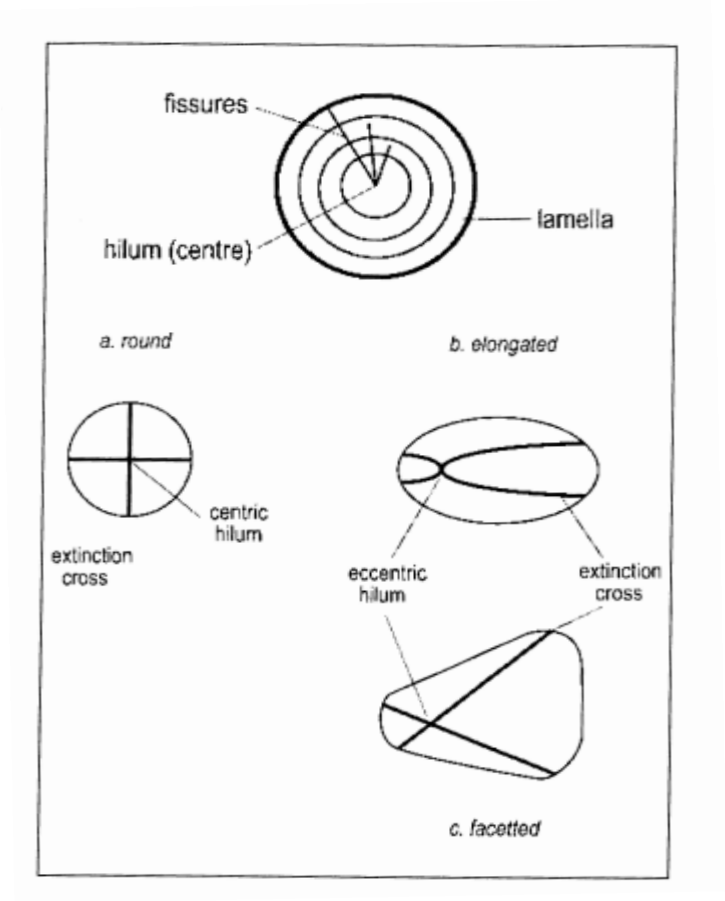


Figure 46: Starch granule characteristics (image by Fiona Roberts, in Gott et al. 2006, 40)

The size, shape and position of the hilium, along with the birefringent cross, visible as a white cross against a dark background under cross-polarised light (Figure 47), are the two most important factors in identifying starches in dental calculus (Hardy et al. 2009b, 249; Tromp & Dudgeon 2015, 55).

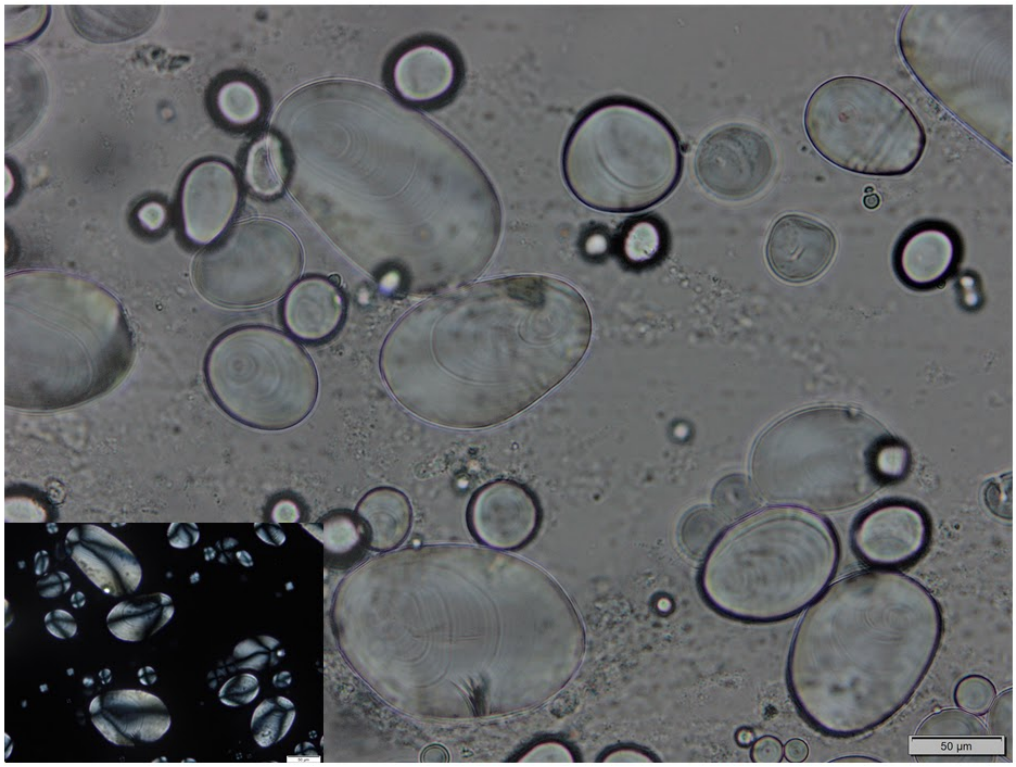


Figure 47: Reference image of modern potato starch, showing its appearance under both standard (main image) and cross-polarised (inset, bottom left) light (credit: Dr Anita Radini, University of York)

When attempting to distinguish between different types of starch, the location of the hilium can be important; whilst generally quite central, in some species - such as yams and potatoes - it is eccentric and so is located towards one end of the granule (Gott et al. 2006, 40).

Torrence and Barton (2016, 121–122) propose the use of iodine-potassium-iodide staining to be sure that starch is present in a dental calculus sample, however this alters the sample in such a way that anything else that has been preserved will not be able to be properly seen and analysed. Hardy and colleagues (2009b, 249) go further than this, stating that the only definitive way to prove that starch is present in a calculus sample is to use the enzyme alpha amylase, as this is specific to starch, but again this would effectively destroy the sample, and so would prevent any future work being undertaken. It could be argued that

the processing and slide preparation method used here on the Farringdon St Bride's Lower samples made it more challenging to accurately identify any potential potato starch evidence. However this decision was justified by the fact that the method chosen ensures that there is a possibility of future work being undertaken by other researchers.

Results

While 18 samples were made available for analysis, these were not all studied here, as the decision was taken to terminate this part of the project. This was because, on average, each sample produced a minimum of 20 slides, and each slide took four hours to fully scan. The value of the data produced was deemed insufficient to justify the time needed to complete analysis of the remaining 14 samples, particularly given the demands of other, more vital areas of the project. The implications of this will be considered in the 'Discussion' section of this chapter.

Of the four samples fully analysed as part of this project, Sk2296 was the only one where starch was identified (Figure 48). Frustratingly, none of the granules observed showed the characteristic signs of potato starch described above, and the species was not identified - reasoning for this will be discussed further below.

Figure 48: Starch granules observed in dental calculus from sample Sk2296, seen under cross-polarised light

The remaining 14 samples were processed as part of this project, but analysed by Dr Anita Radini. Two of those samples - Sk1215 and Sk2134 - making up 11% of the total number of calculus samples available for analysis, contained what appears to be potato starch. Microscopy evidence of this for one of these samples (Sk2134) can be seen in Figure 49.

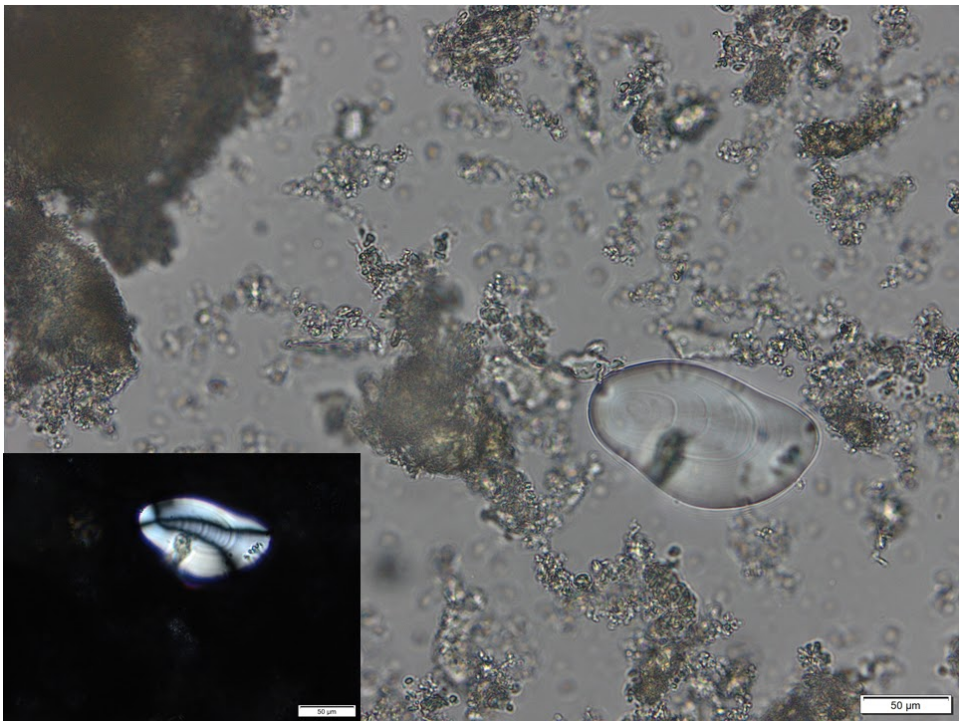


Figure 49: Potato starch granule observed in dental calculus from Sk2134, seen under both standard (main image) and cross-polarised (inset, bottom left) light (credit: Dr Anita Radini, University of York)

To briefly revisit the potential issue of contamination; Crowther and colleagues (2014, 100–101) note that consumables such as laboratory gloves can contain starch contaminants. While this is true, the distinct lack of starch in the vast majority of slides analysed here suggests that this was not a problem in this case.

Discussion

The microscopic analysis of dental calculus samples detailed in this chapter was undertaken both in an attempt to identify potato consumption in England during the long eighteenth century, and in the hope of subsequently relating the results to scurvy biomarker data for the same individuals.

When thinking about the low levels of starch observed, we know that ‘absence of evidence is not evidence of absence’ - the fact that potato starch was not identified in Sk2296 or any of the other three individuals analysed does not mean that those individuals never ate potatoes: they may have eaten them without starch becoming trapped in the dental calculus. Moreover, it should be remembered that the samples available for analysis as part of this project were sub-samples from the deposits on one tooth. They may not include micro-debris trapped in other parts of the calculus - whether from the same teeth or different ones.

Having two individuals in the sample set where potato starch was present in their dental calculus proves that they consumed potato at some point in their lives, however it does not enable us to say whether those individuals were regularly eating potatoes or if they just ate one once. We also don’t know when in their lives they consumed it (or them); the nature of the method used to process dental calculus samples inevitably means that the different layers of the calculus deposit in a single sample get mixed together. It may be possible in future to refine the method so that different layers are analysed separately, but as dental calculus forms at different rates in different individuals, there would still be no way of linking specific layers to specific periods of an individual’s life. With that fact in mind, along with the knowledge that not everything consumed will get trapped in the calculus, it may not be worth the extra time to sample and analyse the different layers separately, as it would not actually enable a meaningful tracking of dietary consistency or change throughout an individual’s lifetime.

It was hoped that light microscope analysis of dental calculus samples from the Farringdon St Brides Lower individuals would enable us to gain direct evidence of diet in that area of London, albeit over a long timespan (the burial ground was in use for roughly 225 years), and that this would help to answer questions that have so far gone unanswered by other

archaeological and historical methods. However, as was discussed in Chapter Five, research as part of this project has confirmed that there is no guarantee that where someone was buried was where they lived; they may have been buried in a different area due to costs, or religious preference. Also important to note is that people did move around during this period, especially from rural areas into cities such as London. It is therefore possible that the individuals who displayed evidence of potato starch in their calculus could have lived either in a completely different area of London - or even outside of London altogether - when that food was consumed, and moved to London afterwards, ultimately telling us nothing meaningful about diet in that area of London.

Overall, the Farringdon St Brides Lower site has too long a period of use with no means of accurate dating of individuals in order to produce results of significant interest to the research questions involved with this project. It is possible that the individuals whose dental calculus showed evidence of potato starch lived during the latter part of the long eighteenth century, by which time we know that many people were regularly consuming potatoes; if this is the case, then it tells us nothing new. Ideally, a site with a short date range, relating to the mid-1600s would have been better for potentially contributing to the still-unanswered question of when people started eating potatoes in England. However, as is often an issue in archaeological research, and as has already been mentioned in previous chapters, it is rarely possible for us to choose our ideal sample set. It was hoped that we would discover some individuals showing biomolecular signs of scurvy and no evidence of potato in their calculus, and others who showed no biomolecular signs of scurvy and an abundance of potato starch in their calculus; had these been the findings, then this could have been a very worthwhile avenue of research, as we would have found a correlation between consumption of potato and presence or absence of scurvy. However, as with all research, we cannot know the outcome from the beginning, and this was unfortunately not the case.

Conclusion

Four samples from the 18 available were analysed by the author in full using the method detailed here. It was then decided that it was not viable to continue with the microscopy work due to the large amount of time that this type of analysis work takes, especially when compared with the value of the information produced in this particular study. Specifically, the long period of use at the site - roughly 225 years, spanning the whole of the long eighteenth century in England - means that the samples could date to a time when it is already known that potatoes were being consumed. As there are no named individuals included in the study, and no way of knowing exactly when each individual lived, died and was buried at the site, it would not have been justifiable to continue with the work, as the aims of the research could not be met, nor the related research questions answered.

This leads us to one more consider the important point that much of the work undertaken within bioarchaeology as a discipline is reactive; we have to work with the samples that are available, we cannot design the perfect site or the perfect sample set.

As was detailed in Chapter Seven, dental calculus analysis using light microscopy has been proven to be a useful tool in bioarchaeological research. However, the work detailed in this chapter shows that we should question whether this is a good approach for the reconstruction of aspects of diet which might affect levels of deficiency disease.

Chapter Nine: Discussion and Conclusions

In this chapter, we will discuss the key findings of the project, their significance, and how they relate to current archaeological and historical research contexts. We will also revisit the research questions, aims and objectives of the project to assess what has been achieved in relation to these. Finally, we will consider the limitations of the project and discuss possible directions for recommended future work, before concluding on the project overall.

Key Findings and How They Relate to Current Archaeological and Historical Research Contexts

In setting the scene for the central research undertaken for this project, a number of key findings were established. For example, the collagen extraction experiments conducted as part of this project showed that the current standard method for collagen extraction from archaeological bone (here referred to as 'Method C') is not necessarily the most effective method to use, depending on the area of the collagen sequence or particular peptides that you want to study. A new, minimally-destructive method (here referred to as 'Method N') was established, which the extraction experiments showed to be arguably as successful, if not more-so, than the standard method. The significance of this result relates mainly to the difficulties that can be faced by bioarchaeologists in trying to obtain samples for research. Understandably, museum and collection curators are hesitant to allow destructive sampling of human skeletal remains, in part because they are a finite resource and in part because this can negatively impact on future osteological research; there are also moral implications to be considered. The development of a minimally-destructive method for the extraction of collagen, which leaves the bones

visually unchanged from how they were prior to the work being carried out, and which enables the same sample to be used in future for other biomolecular work, will hopefully enable greater access to human skeletal remains for bioarchaeological research in the future.

Building the research contexts regarding diet and the dietary deficiency disease, scurvy, it became clear that there are a number of unanswered questions about mortality and nutrition levels in long eighteenth century England, which have been posed by historians but are rarely, if ever, mentioned by archaeologists. For example, the controversy surrounding the McKeown thesis, and the debates regarding average calorie intake during this period. Data deficiencies relating to the currently available historical documentary sources in these areas may mean that some of these questions will never be answered conclusively. However, the questions that these debates aim to address are relevant to the questions posed in this project, and knowledge of them can still help to shape the ways in which we approach research regarding nutritional health in the past.

The five sites included in this project had all been archaeologically classified as being ‘poor’ or as having ties to ‘poverty’. However, historical research into the ways in which burial ground populations were formed during the long eighteenth century in England has revealed that these classifications are an oversimplification of life at this time. This finding has provided further support for the integrated approach of combining archaeological and historical research methods to further our understanding of the past.

Of key importance to one of the project’s central questions, the proposed scurvy biomarker from a previous research project was proved to be invalid. This was done through a systematic analysis of human collagen, in which six other potential scurvy biomarkers were revealed. Additional

work focusing on these has lead to the conclusion that three of the six should undergo further testing and analysis to conclusively prove or disprove their potential links with scurvy. If any or all of these potential biomarkers are shown to be successful in identifying the presence or absence of scurvy in archaeological human skeletal remains, this will have a significant impact on our understanding of the true scale of scurvy in the past. Importantly, the six potential biomarkers identified were all detected in both LC-MS/MS and MALDI-TOF-MS data, using both Method C and Method N extraction techniques. This is significant because it could mean that the presence or absence of scurvy can be bioarchaeologically tested without needing to destroy bone samples.

The final finding of note for this project concerned the light microscopy analysis of human dental calculus samples from the Farringdon St Bride's Lower site. This method can be a useful tool for reconstructing past diets, however during the course of the project it became apparent that the diet-based questions that were of particular importance here could not be answered using this technique. The findings of this project would suggest that, while diet-focused dental calculus microscopy work is particularly useful when applied to pre-historic contexts, where relatively little is known about diet and consumption patterns, it may not be as useful in historic populations.

Revisiting the Research Questions, Project Aims and Objectives

Research Questions

'Is the potential scurvy biomarker identified during a previous research project a true marker for scurvy in human skeletal remains?'

As has been mentioned, the systematic analysis of human collagen - carried out with the assistance of Simon Hickinbotham - proved that the potential biomarker identified previously cannot be used as a marker for scurvy in human skeletal remains. The work that has been detailed in Chapter Six of this thesis was the first systematic analysis of human collagen MALDI data undertaken with the aim of identifying evidence of dietary deficiency. The findings from this work may suggest that scurvy affects collagen through changes in relative ratio levels of hydroxylation, as opposed to their being one (or more) point(s) along the collagen chain that should always be hydroxylated, but aren't when suffering from prolonged Vitamin C deficiency, as was previously thought.

'Can we track potato consumption (a good source of Vitamin C) during the 17th - 19th centuries through evidence of potato starch granules in human dental calculus?'

Evidence of potato consumption was found in the dental calculus of two individuals from the Farringdon St Bride's Lower site, but we need to question how useful this is to our overall understanding of diet and dietary deficiencies in the past. This site had a long period of use, and the specifics of when in the long eighteenth century these two individuals lived and died is unknown. Analysis of dental calculus from named individuals buried at a site with a much shorter period of use, dating to the mid-seventeenth century, may be of greater value in establishing when the potato first began to be consumed in England.

In regards to dental calculus evidence for potato consumption and biomolecular evidence for occurrences of scurvy, it is challenging to meaningfully correlate the two types of data. If biomolecular evidence suggests that an individual had suffered from scurvy, we may be surprised to find potato starch in their dental calculus. However, the occurrence of scurvy and the consumption of the potato could have

happened at two different times in that individual's life; we currently have no way of ascertaining when for either. Furthermore, given that there is no set amount of food particles that will definitely become trapped in dental calculus following consumption, we have no way of knowing how frequently different foodstuffs were being eaten, or how to establish the relative quantities within an individual's diet from what is observed in their calculus, and in turn how this may relate to under- or malnutrition.

'Can we use a combination of human skeletal remains and historical documentary evidence to trace dietary and social change?'

This project has shown that we can - and should - use a combination of human skeletal remains and historical documentary evidence to better understand the hugely complex nature of dietary and social change during the long eighteenth century in England. Bioarchaeological data has the potential to take our understanding beyond what is known from documentary sources, but these sources also help to build a clearer picture of the social, political and cultural context for the biomolecular findings, ultimately improving the overall quality of inferences made as a result of the research process. However, as has been mentioned previously, there is a lot more work that needs to be done if we ever hope to fully understand the changes that took place during this period.

Aims and Objectives

'To connect environment, nutrition and health, through the combined approach of osteological, biomolecular and historical research methods' and 'To attempt to identify under- and malnutrition in 17th-19th century skeletal collections using this combined analytical approach.'

Research into the historical documentary evidence available for this period in England - particularly regarding London - has confirmed the

high level of scholarly interest in the ways in which environment and nutrition link to health, and the effects that this had on life in the long eighteenth century. However, through this project we have learned that, while under- and malnutrition are considered by many researchers to have been significant problems during this period, both archaeological and historical evidence to conclusively support this is hard to come by.

While it has not been possible to identify definitive evidence of under- or malnutrition in the five human skeletal populations studied here, a systematic, detailed approach was employed in trying to do so, and this may yet prove to be successful.

When undertaking research that focuses on health and disease in the past, we need to remember that we have our own modern-day cultural biases and opinions influencing our approach. While we may today understand that nutrition and health are linked, and that both of these can be affected by the physical and social environments in which we live, this may not have been the way that these themes were viewed in long eighteenth century England. As such, part of an effective research process is outlining our own cultural framing, and showing awareness of the ways in which that may differ to the cultural framing of the period of interest.

'To systematically test existing methods for isolating collagen peptides from archaeological bone, and to develop and test potential new methods for this purpose, ultimately with the aim of identifying the best minimally destructive method'

As part of this project, 24 methods – some being existing, already published methods, and some being variations on those – were systematically tested, with the aim of establishing which method is best for the purpose of collagen extraction from archaeological bone. No

systematic testing of the existing methods has been published to date, and the results of this will have important implications for others working in similar areas, making this a key finding from this project.

As was discussed in Chapter Two, the term ‘best’ is relative in this particular instance to what you want to study. It was decided to use Method N – a minimally destructive method involving a time-specific buffer soaking protocol that includes a sodium hydroxide rinse step - for the testing of the rib samples collected for this project. Method N was chosen as it gave a good result for peak count in Phase One of the systematic testing, the best result for peak count in Phase Two, and the best result for overall peak intensity in both.

‘To test a low-cost, minimally-destructive method for the biomolecular identification of scurvy in human skeletal remains, which will further our understanding of the biological processes behind how scurvy affects collagen’

The low-cost, minimally destructive method for the extraction of collagen from archaeological human skeletal remains (‘Method N’), established as part of this project, was then used in the search for a scurvy biomarker, along with the standard destructive method for collagen extraction (‘Method C’). As has already been discussed as part of this chapter, the results of the scurvy biomarker analysis suggest that the way in which we previously thought scurvy affected collagen appear to be wrong, and an alternative hypothesis has been proposed. If this alternative hypothesis regarding levels of hydroxylation is proved to be correct, the this will have significantly furthered our knowledge regarding the changes that happen to human collagen as a result of Vitamin C deficiency.

'To use the potential scurvy biomarker to take our understanding of the nutritional status of people living in England during the 17th-19th centuries beyond what documentary sources tell us'

Unfortunately, as the potential scurvy biomarkers identified in Chapter Six require further testing, it has not been possible to apply them in such a way that can confidently further our understanding of the nutritional status of people living in England from the 17th-19th centuries. A preliminary application of the biomarkers to the data from the five sites included in this project suggested that the Priory Yard and Cross Bones burial ground populations were generally more scorbutic than the Bow Baptists population. However, it should be remembered that the quality of data produced from the human skeletal remains collections housed by the Museum of London was surprisingly low. In relation to this, further work needs to be carried out on both the potential differences between eppendorf data and falcon tube data, and the effects that different post-excavation processing methods can have on collagen survival, before the potential biomarkers can reliably be applied to the data produced here.

Regarding post-excavation processing, on the basis of the results produced here, it is recommended that human skeletal remains only be dry brushed in future, and not cleaned using water. Ideally, accurate records of post-excavation processes should also be kept and should be available to researchers, as this may influence decisions on which samples or collections are appropriate to be sampled.

'To research the history of the areas in which the different skeletal populations to be analysed lived, in order to attempt to explain the osteological and biomolecular findings, and to provide a social, political and cultural context for the research.'

Research into the documentary evidence relevant to the five sites studied here has furthered our understanding on the various factors that affect the formation of a burial ground population, and has shown that not all individuals buried at a particular site will have lived their whole lives - if any time at all - in the immediate area. This subsequently makes the task of understanding the social, political and cultural context(s) relating to the burial ground populations studied here much more challenging than initially anticipated, particularly during a time period where there was so much change taking place. However the inclusion of historical research and analysis has elucidated important social, political and cultural factors relevant to life for the poorer classes in England during this period. Due to the currently inconclusive nature of the biomolecular findings within this project, it has not been possible to directly apply this information here, but it could help to explain future archaeological findings in similar areas of research.

Limitations of the Project

A continual problem faced by archaeologists is access to appropriate samples for the research questions that they want to investigate. Ideally, this project would have studied human skeletal remains from securely dated sites with short periods of use, whereby all individuals buried at those sites were unquestionably known to have been living in the immediate area and to have been poor or impoverished. However, it is highly unlikely that such a population ever existed, and if they did, they certainly were not available for inclusion here. Furthermore, as was the case for this project, there are often restrictions that result in only a subsection of the entire site population being available for sampling. While this is currently inevitable and understandable, in order to protect and ensure the survival of human skeletal remains collections, it means that we are only studying a portion of the total site population. Caution

should therefore always be shown when attempting to draw conclusions about a whole group from a sub-sample. It is hoped that with the development of non- or minimally-destructive techniques - such as the one developed as part of this project - this will increase the sampling access opportunities for archaeological researchers.

Two important limitations of this project were the classification of the site populations as ‘poor’ or having links with ‘poverty’, and the general assumption that the parish in which people were buried was the same as the parish in which they’d always lived. In regards to the classifications of ‘poor’ and ‘poverty’, perhaps the most important influence of historical research methodologies on this project is the emphasis on the fact that language use changes over time. As archaeologists we have a tendency to oversimplify the classification of a site population’s economic status, but this has important consequences for what we then go on to conclude about the lives of the individuals living there. Regarding the parish issue, we have already established that the way in which burial ground populations were formed during this period was influenced by a number of different factors, and it is very likely that at least some individuals buried at each site had not lived their whole lives in that parish. It is still possible to draw conclusions about life in England during the long eighteenth century from these burial ground populations, but this fact does need to be acknowledged in order to fully understand the historical context of the site. Crucially though, these factors do not affect the validity of the biomarker research undertaken here: people from any socioeconomic background, having lived anywhere in England during the long eighteenth century, could have suffered from scurvy.

A further limitation was the fact that some current debates relating to the themes of this work either haven’t yet or cannot be solved. For example, the aforementioned debates surrounding the McKeown Thesis and average calorie intake during our period of interest. These issues being

unresolved inevitably restricts the conclusions that can be drawn regarding the relationships between environment, nutrition and health. However having an awareness of them avoids incorrect inferences being made, and can be used to frame research questions and aims in such a way that enables meaningful conclusions to be drawn at an appropriate level.

The surprising results of the collagen extraction experiments meant that more time was spent on the method development aspect of the project than was initially anticipated. This had a knock-on effect on the time available for other aspects of the project, such as the scurvy biomarker research and dental calculus analysis. However the findings from this systematic testing and method development were considered to be of significant importance to justify this extra time.

In the case of the dental calculus analysis, this also took longer than anticipated, and it was deemed that the value of the data produced was outweighed by the time commitment required. While both the additional time applied to the collagen extraction method testing and development, and the decision to discontinue the dental calculus analyses undoubtedly changed the project and moved it away from the original conceptualisation of the work, they also both enabled us to learn more about bioarchaeological research techniques and the ways that these are, can be, and perhaps should be applied.

Directions for Future Work

This project has revealed areas of analysis that could benefit from further research, but which were beyond the scope of this project. For example, it is recommended that further work be carried out to establish the

reasoning for the differences in collagen extraction yields when carried out using eppendorf tubes versus falcon tubes.

There would potentially be value in carrying out light microscopy analysis of dental calculus from individuals buried in a short usage period site dated securely to the mid-seventeenth century. This could enable the identification of early consumption of the potato in England, which would help to solve this current unknown in the tuber's history in England. However, considering the time commitment required for this work, it would only be recommended if other research questions could also be answered using the same resulting data.

Further testing of the proposed new method for extracting collagen from archaeological bone – on a greater number of samples, but still in comparison with other similar methods - would be recommended. This would enable more certainty on which parts of the collagen chain Method N is able to routinely detect. Furthermore, if tested against the standard destructive method (here termed Method C), this could facilitate a greater understanding of what happens to collagen, and of what is extracted versus what is either destroyed or left behind, when employing these two different protocols.

The most important aspect of this project that has potential for further research relates to the three new potential scurvy biomarkers. The analysis work that was undertaken in order to identify these was carried out to a level of detail not previously seen in bioarchaeological research regarding dietary deficiency, but further testing - preferably on a human skeletal collection known to have been suffering from scurvy around the time of death - could help to establish whether any or all of these could be true biomarkers for the disease.

Final Comments

This thesis aimed to further our understanding of poverty in England during the long eighteenth century by taking an integrated approach to the study of the human skeletal remains from five post-medieval sites from the south of England. The angle taken to investigate this, using both archaeological and historical research techniques, was under- and malnutrition, with a particular focus on the Vitamin C deficiency disease, scurvy.

It was noted at the start of this thesis that, on a basic level, the project detailed here uses scurvy as a proxy for poverty. The literature-based research presented as part of this project has shown that archaeologists have traditionally viewed what we today refer to as scurvy in this way, but we have also considered the logical reasoning why this is a disease that could potentially have affected any individual from any socioeconomic background. We have also acknowledged that the issue of assigning the term 'poverty' is complex, which further complicates the possibility of linking a particular disease with socioeconomic status. Regardless, it is important to remember that this does not affect the validity of the biomolecular research that has been presented here.

As a result of this project, a new method for collagen extraction from human skeletal remains – Method N - has been established, and potential new scurvy biomarkers have been identified.

Method N is a minimally destructive method involving both a 168-hour ammonium bicarbonate buffer rinse and a sodium hydroxide rinse of the bone sample prior to extracting the collagen. The pre-extraction treatment of this sample using both a buffer rinse and a sodium hydroxide rinse seems to have created the best conditions for the extraction of collagen out of all those that were tested here. As was discussed in

Chapter Three, it could be the case that this method appears to perform better across the board than the commonly employed 'Method C' (involving acid demineralisation of the mineral component of the sample), because the demineralisation process is too aggressive, with the acid actually destroying some of the collagen at the same time as it destroys the mineral, but further testing would be required to know this for definite.

The data analysis work undertaken in an attempt to identify potential scurvy biomarkers was completed to a level of detail not routinely seen in archaeological investigations of collagen. This work concluded that a presence/absence approach to the biomolecular identification of scurvy – whereby a particular peptide being present or absent determines presence or absence of the disease – was not appropriate, however a ratio approach enabled the identification of three potential biomarkers worthy of further investigation.

When attempting to use data produced using Method N to study potential scurvy biomarkers in human skeletal remains from different locations, we need to be sure that the collagen extracted using that method is consistent across samples and therefore comparable. The preservation levels of archaeological collagen in bone samples is rarely, if ever, uniformly consistent due to a wide range of factors. The aim of developing Method N was to establish a cheap, fast, minimally destructive method for the extraction of human collagen, in order to both preserve archaeological human skeletal remains, and to obtain access to potential samples where access is usually very restricted. In developing this method, a number of different samples were tested that had been subjected to a variety of different environments and taphonomic processes due to their different burial locations, and statistical analysis of the data produced suggested that it was comparable. However further testing of Method N on a greater number of samples will reveal whether

or not this is truly the case. There will always be some samples that produce more data than others; this does not mean that they are not comparable at all, it just depends on what you are trying to compare. In terms of the potential scurvy biomarker, as the focus was on a ratio approach, this somewhat avoids this problem and helps to make the data from different sites safely comparable; if the peptides are not there, then nothing can be concluded, but if they are there then the hydroxylation ratio can be used to give a cautious interpretation.

The integrated approach taken to this research has produced a higher quality of work, and has enabled us to learn more about life in eighteenth century England, than would have been possible from a single-discipline approach. It is recommended that this approach continues to be applied in future research projects, as questions regarding life in eighteenth century England are of interest and relevance to both archaeologists and historians. We now know that the concepts of poor, poverty and disease rely strongly on cultural framing and are subject to change over time. While this makes the research process more challenging, being aware of this potential limitation ensures that the questions we ask are more carefully thought out, and the subsequent conclusions that we draw are more meaningful.

Appendix

The full library of data files produced as part of this thesis is accessible here:

https://doi.org/10.5281/zenodo.3575053

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